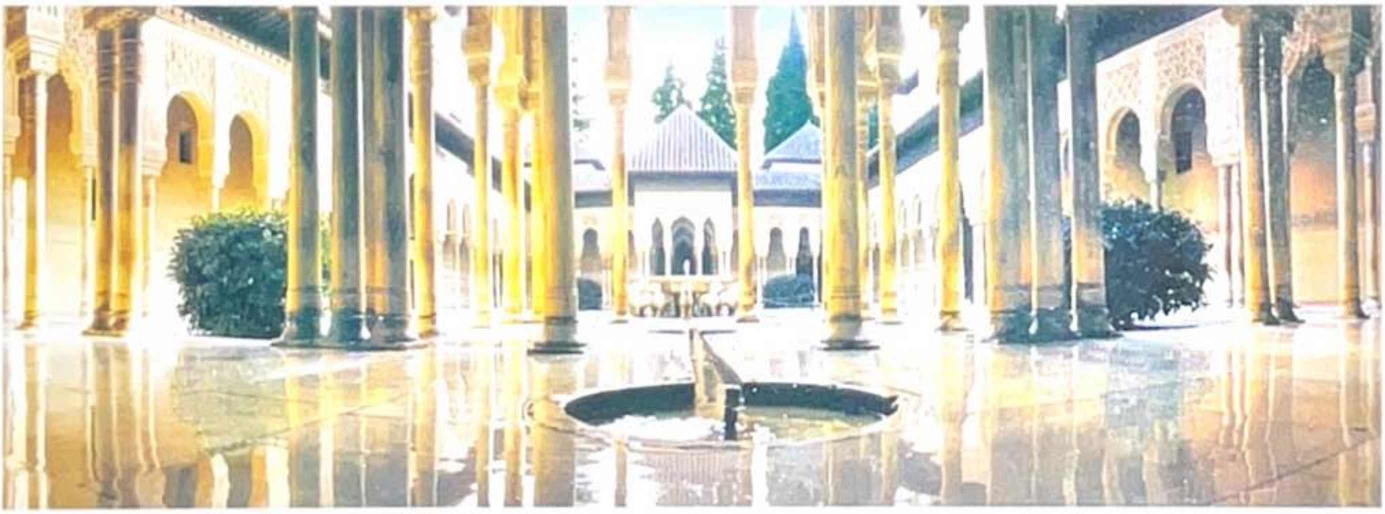
Final Programme

5th International Conference on Mycorrhiza

"Mycorrhiza for Science and Society"

23-27, July 2006 - Granada, Spain



Estación Experimental del Zaidín
CSIC

ICOM5

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Final Programme

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5th International Conference on Mycorrhiza

"Mycorrhiza for Science and Society"

23-27, July 2006 - Granada, Spain

ICOM
2006

WELCOME

Dear Friends and Colleagues:

The Organising Committees of ICOM5 have great pleasure in welcoming the almost 700 registrants (from 50 Countries) to the Conference and to Granada, Spain. We are pleased that you were able to attend. We are inviting all of you to enjoy the scientific and social programmes, that we have designed trying to do our best, and the beautiful City of Granada.

We hope that ICOM5 will serve as a discussion forum to analyze recent advances in mycorrhiza research and to assess how this is improving basic science knowledge and satisfy societal demands. Remember that ICOM5 theme is "***Micorrhiza for Science and Society***".

We are sure that ICOM5 will be a platform to make friends and to establish new scientific collaborations for implementing ICOM aims, as we will evaluate in ICOM6.

GENERAL INFORMATION

Venues

All scientific activities will take place at the Congress Centre of Granada. We are sure that the excellent characteristics of this building will make enjoyable our scientific activity. The Opening Ceremony and Plenary lectures will be in the Manuel de Falla Hall. The Oral presentations in the Scientific Sessions and Workshops will take place in parallel in the Andalucía Rooms and in the Manuel de Falla Hall. All of the Conference rooms, the Poster display Area, and the Sponsor stands/desks are on **Level + 1** of the Congress Centre (see floor plan).

Registration desk/Conference Secretariat

The registration desk will be situated close to the Manuel de Falla Hall, and will be open:

Sunday, July 23	17.00 to 20.45
Monday, July 24	8.00 to 14.00
Tuesday/Wednesday/Thursday	8.30 to 14.00

After Registration on Sunday 23 July a "get-together reception" will take place in the open air terrace and amphitheatre at the Congress Centre, as offered by ICOM5 organization. Accompanying persons are welcome.

Refreshments (coffee breaks)

Morning and afternoon refreshments will be served in the Poster and Exhibition Area, close to the Conference rooms.

Lunches

Lunches (included in the Registration fees) will be served in the Restaurant, **Level 0** of the Congress Centre at 14.30.

Wines of the World

The "Wines of the World" competition tasting will be on Monday 24 July, from 9.30 to 11.00 pm, in the "Carmen de los Martires". Accompanying persons are welcome. ***Do not forget to bring your bottle of wine.*** Prizes for the highest scoring- wines are envisaged. Heavy and light finger food and soft drinks will be provided free of charge.

Poster display

Posters will be displayed in the Exhibition Hall, on **Level +1** of the Congress Centre, close to the Conference rooms. Authors are asked to put up their poster preferentially on Sunday 23, between 18.00 and 21.00. Velcro strip will be provided to attach posters to the display board. Please do not use pins. Identification of the appropriate poster board will be by mean of a number corresponding to that given in the Book of Abstracts. The poster will be displayed until the end of the Conference.

Smoking

By law, the Congress Centre is a non-smoking venue, however very restricted smoking areas are provided.

Mobile phones

We are asking you for maintaining your mobile phone switched to a silent mode when in the Conference rooms.

E-mail

Eight computers and free lines to connect your own portable computer, with internet access, are provided free of charge. These will be situated in the Exhibition Hall of the Congress Centre (**Level +1**).

Telephones

Public telephones are available and very easy to be found in the Congress Centre. You can use either € coins or telephone cards. These cards are available in the Newspaper kiosks and in the Tobacconist shop ("*Estanco*" in Spanish), that you can find in your way from your Hotel to the Congress Centre.

Name badges and tickets

The name badge is the official pass and must be worn at all time to gain entry to the Congress Centre and to all ICOM5 activities (either scientific or social). The Security Personnel at the entrance of the Congress Centre will ask you for showing the ICOM5 badge to allow you to entry. Tickets must be shown at all social functions.

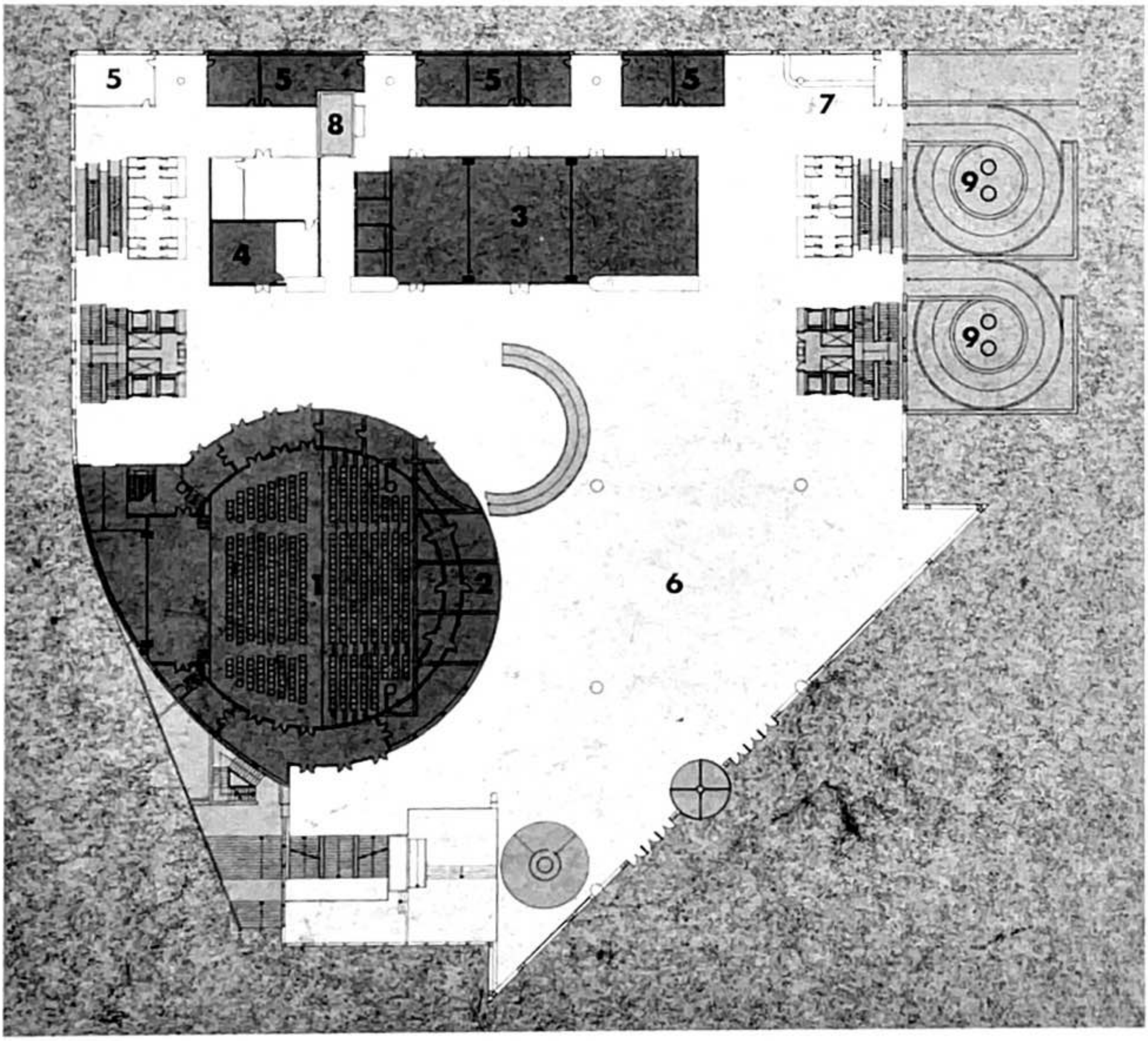
Notices and messages

A notice-board will be provided near the ICOM5 Registration Desk for Programme updating and personal message displaying. Logistic announcements will be made when appropriate at the oral sessions.

Speaker Support Centre
Will be in room 4 (Level+1)

Insurance

The ICOM5 Organizers are unable to accept responsibility for personal accidents and/or damages to private property by any participant, during the conference or at the social events.



Map of the *Level +1* of the Congress Centre

1.Manuel de Falla Hall
2. Registration Desk/Secretariat
3. Andalucía Room I, II and III
4. Speaker Support Centre
5. Not relevant for ICOM5
6. Poster Area and Sponsor exhibition stands/desks
7. Cafeteria (with spaces for small meetings)

Keynote lectures

(All presentations in the Manuel de Falla Hall)

Linking above and belowground multitrophic interactions across spatio-temporal scales. *W. van der Putten*, Netherlands Institut of Ecology, Heteren, Netherlands.

Macro- and microevolution in plant-microbe systems. *N.A Provorov,* All-Russia Research Institute for Agricultural Microbiology, St.-Petersburg, Russia.

Strategies for the development and release of improved microbial inoculants for food production. *F. O'Gara,* Univ. College Cork, Cork, Ireland.

Soil microbial resources and agricultural policies. *D. Atkinson,* SAC, Aberdeen, UK.

Sessions: Oral presentations

Session 1: Cell programmes: (1) genome to transcriptome

Coorganized by: *U. Nehls & N. Ferrol*
(Manuel de Falla Hall)

Transcriptomics of arbuscular mycorrhiza in the model legume *Medicago truncatula.* *H. Küster,* Institute for Genome Research, Bielefeld University, Germany.

Transcript patterns associated with ectomycorrhiza formation in poplar. *S. Duplessis,* INRA/UHP Nancy, France.

Transcript profiling and positional cloning of a *Medicago truncatula* mycorrhizal mutant. *Q. Zhang,* Boyce Thompson Institute for Plant Research, Ithaca, USA.

Lunch is not for free: Coordination of carbohydrate and nutrient exchange at the plant/fungus interface. *U. Nehls,* Eberhard Karls University, Tuebingen, Germany.

Analysis of mycorrhizal functioning by using transcriptomic approaches. *P. Franken,* Institute of Vegetable and Ornamental Crops, Grossbeeren, Germany.

Spatial patterns of gene expression in the extramatrical mycelium and ectomycorrhizal root tips. *T. Johansson,* Lund University, Sweden.

Session 2: Cell programmes: (2) proteome to metabolome/isoformome

***Coorganized by**: E. Dumas-Gaudot & F. Martin*

(Manuel de Falla Hall)

Functional genomics of early interactions in Ectomycorrhizal Symbiosis of poplar x *Laccaria bicolor*. *G.K. Podila,* University of Alabama, USA.

Functional genomics for a better understanding of nitrogen nutrition in ectomycorrhizal symbiosis. *M. Chalot,* Nancy-University and INRA, France.

Starvation stress responses in the mycorrhizal ascomycete *Tuber borchii*: regulatory and structural signatures of the symbiotic lifestyle? *S .Ottonello,* University of Parma, Italy.

Protein profiling analyses in arbuscular mycorrhizal symbiosis. *G. Recorbet,* INRA/CNRS/U. Bourgogne, Dijon, France.

Reorganization of plastid shape and metabolism in root cortical cells colonized by arbuscular mycorrhizal fungi. *T. Fester,* Leibniz-Institute of Plant Biochemistry, Halle, Germany.

Towards the fluxome - prospects for applying metabolic flux analysis to mycorrhizal systems. *Y. Shachar-Hill,* Michigan State University, USA.

Session 3: Genetics/genomics of mycorrhizal fungi

***Coorganized by**: G. Podila & P. Lammers*

(Andalucía Hall)

The genome sequence of the symbiotic fungus *Laccaria bicolour*. *F. Martin,* INRA-Nancy, France.

The genome project for *Glomus intraradices*, a model arbuscular mycorrhizal fungus. *P.J. Lammers,* New Mexico State University, Las Cruces, USA.

Meiosis and post-meiotic mitosis during basidiospore formation in the ectomycorrhizal fungus *Pisolithus* sp. *A.N. Campos,* Universidade Federal de Viçosa, MG, Brazil.

A genomic approach to the understanding of *Gigaspora margarita* and *Candidatus* Glomeribacter gigasporarum association. *S. Ghignone,* Istituto per la Protezione delle Piante, CNR, Torino, Italy.

Evolutionary genomics of the ectomycorrhizal fungus *Paxillus involutus*. *A. Tunlid,* Lund University, Sweden.

Exploiting *Glomus intraradices* sequencing data to dissect molecular mechanisms of plant genome control over fungal gene expression in mycorrhiza. *P. Seddas,* INRA/CNRS/U. Bourgogne, Dijon, France.

Session 4: Signals and perception

Coorganized by: ***G. Bécard & K. Akiyama***
(Manuel de Falla Hall)

Chemical identification of signalling molecules in symbiotic interactions between plants and arbuscular mycorrhizal fungi. *K. Akiyama,* Osaka Prefecture University, Japan.

A transient increase in cytosolic calcium is triggered in host plant cells by diffusible molecules released by AM fungi. *L. Navazio,* Università di Padova, Italy.

Fungal responses to strigolactones: from presymbiotic to symbiotic stage? *N. Séjalon-Delmas,*_
University Toulouse III, France.

Apocarotenoid accumulation and reorganization of plastids in arbuscular mycorrhizal roots. *D. Strack,* Institute of Plant Biochemistry, Halle, Germany.

Recognition events in AM fungi during symbiosis establishment. *N. Requena,* University of Karlsruhe, Germany.

Studies on cell-to-cell communication in the ectomycorrhizal fungus *Pisolithus tinctorius.* *B. Xoconostle-Cázares,* CINVESTAV, México.

Session 5: Evolution and phylogeny of mycorrhizal fungi

Coorganized by: *T. Bruns & S. Rosendahl*
(Andalucía Hall)

Implications of nr28S gene phylogeny for systematics of Glomeromycota. *J.B. Morton,* West Virginia University, USA.

New molecular marker genes for arbuscular mycorrhizal fungi and their applications. *D. Redecker,* University of Basel, Switzerland.

Serial speciation and morphological stasis in little brown boletes. *A.F. Taylor,* Swedish University of Agricultural Sciences, Uppsala, Sweden.

Phylogeny and recombination in the ectomycorrhizal fungus *Paxillus involutus* as revealed by gene genealogies of seven nuclear loci. *J. Hedh,* Lund University, Sweden.

Phylogeny and ecology of the Sebacinales: an overview. *M. Weiß,* Universität Tübingen, Germany.

Genetic exchange and experimental genetics in AMF: Evolutionary consequences. *I.R. Sanders,* University of Lausanne, Switzerland.

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Session 6: Population biology of mycorrhizal fungi

Coorganized by: *I. Sanders & R. Marmeisse*
(Andalucía Hall)

Population genetics and life cycle of the ectomycorrhizal fungus *Tuber magnatum.* *A. Rubini,*_ Plant Genetics Institute, CNR, Perugia, Italy.

Local environmental conditions affects diversity and genetic structure in the ectomycorrhizal fungi *Tricholoma populinum* and *Tricholoma scalpturatum.* *H. Gryta,* Lab. EDB (CNRS-UPS), Univ. Toulouse, France.

Prospects of parentage analysis in ectomycorrhizal fungi. *A.M. Kretzer,* SUNY College of Environmental Science and Forestry, USA.

Species concepts and the inference of cryptic species and recombination in the putative asexual fungus *Cenococcum geophilum.* *G.W. Douhan,* University of California, Riverside, USA.

Spore development and population structure in *Glomus*: is there a link? *J.L. Jany,* Cornell University, Ithaca, USA.

Maintenance of the arbuscular mycorrhizal mutualism. *J.D. Bever,* Indiana University, Bloomington, USA.

Session 7: Mechanisms in stress resistance/tolerance

Coorganized by: *J. M. Ruiz-Lozano & J. Colpaert*
(Manuel de Falla Hall)

Altered Zn fluxes and compartmentation in the ECM fungus *Suillus bovinus* as mechanisms involved in Zn tolerance. *K. Adriaensen,* Hasselt University, Diepenbeek, Belgium.

Heavy metal tolerance in ectomycorrhizal fungi: new insights. *D. Blaudez,* INRA/UHP, Nancy, France.

Hunting for genes involved in heavy metal tolerance in ericoid mycorrhizal fungi. *S. Perotto,*_Università di Torino and IPP-CNR, Torino, Italy.

Gene expression in tomato and *Glomus intraradices* under salt stress. *U. Hildebrandt,* University of Wuerzburg, Germany.

Ectomycorrhization improves host root hydraulic conductivity via aquaporin expression. *Z. Marjanovic,* Center for Multidisciplinary Studies, Belgrade, Serbia.

Priming plant defenses against pathogens by arbuscular mycorrhizal fungi. *M.J. Pozo,* Estación Experimental del Zaidín, CSIC, Granada, Spain.

Session 8: Functional diversity in mycorrhiza

Coorganized by: I. Jakobsen & H. Wallander
(Manuel de Falla Hall)

Structure and function of an ectomycorrhizal fungal community. *R.T. Koide,* The Pennsylvania State University, USA.

Linking functional and genetic diversity in the arbuscular mycorrhizal fungal symbiosis. *T.R. Scheublin,* Vrije Universiteit, Amsterdam, Netherlands.

Does carbon flux from ectomycorrhizal fungi drive soil biodiversity? *D. Johnson,* School of Biological Sciences, University of Aberdeen, UK.

A novel assembly of ericoid mycorrhizal fungi from arctic tundra varies in function and fungicide tolerance. *A. Jumpponen,* Kansas State University, Manhattan, USA.

Nitrogen stable isotope measurements link carbon and nitrogen fluxes in ectomycorrhizal symbioses. *E.A .Hobbie,* University of New Hampshire, USA.

Plant phosphorus status represses the symbiotic phosphate uptake pathway in the tomato mycorrhiza. *R. Nagy,* Federal Institute of Technology (ETH) Zurich, Lindau, Switzerland.

Session 9: Biodiversity and ecological impacts

Coorganized by: G. Cuenca & R. Koide
(Manuel de Falla Hall)

Fine scale distribution of pine ectomycorrhizal fungi. *I.C. Anderson,* The Macaulay Institute, Aberdeen, UK.

Fragmented landscapes – The role of dispersal, abiotic factors and competition for community assemblage of AMF. *Y. Lekberg,* Montana State University, USA.

Some trends in the global distribution of the genus *Scutellospora*: a preliminary study. *M. Lovera,* Centro de Ecologia, IVIC, Caracas, Venezuela.

Communities of AM fungi in agricultural habitats and landscapes of Central Europe. *F. Oehl,* University of Basel, Switzerland.

Role of mycorrhizal response diversity in forest succession following disturbance. *S.W. Simard,*
University of British Columbia, Canada.

Evidence for ectomycorrhizas in *Pakaraimaea dipterocarpacea* from South America and an ancient origin of ectomycorrhizas in Dipterocarpaceae. *B. Moyersoen,* Université de Liège, Belgium.

Session 10: Anthropogenic impacts on mycorrhiza diversity and functioning

Coorganized by: *A. F. S. Taylor & P. Jeffries*
(Andalucía Hall)

Do crop genotypes have residual effects on Glomeromycota communities in agro-ecosystems? *D. van Tuinen,* INRA/CNRS/U. Bourgogne, Dijon, France.

Anthropogenic influences on soil structure: glomalin and arbuscular mycorrhizae. *M.C. Rillig,* University of Montana, USA.

CO_2-enrichment and the ectomycorrhizal symbiosis: consequences for community structure, hyphal dynamics and nutrient transport gene regulation. *J.L. Parrent,* Duke University, USA.

The exotic, invasive hemlock woolly adelgid (*Adelges tsugae*) affects ectomycorrhizal fungal communities and soil conditions in eastern U.S. forests. *A.R. Tuininga,* Fordham University, Armonk, USA.

Influence of restoration practices on underground and aboveground biodiversity of heavy metal polluted areas. *K. Turnau,* Jagiellonian University, Krakow, Poland.

Can we develop a general predictive model of mycorrhizal fungal community response to changing atmospheric chemistry? *E.A. Lilleskov,* US Forest Service, Houghton, USA.

Session 11: Biome-related functional ecology

Coorganized by: *C. Gehring & M. van der Heijden*
(Andalucía Hall)

Unearthing plant-to-soil carbon flux through AM mycelia in grassland: Field 13CO2 pulsing, mesh cores, collembolans and isotope-ratio PLFA analysis. *J.R. Leake,* University of Sheffield, U.K..

Symbiont diversity as a driver of plant diversity and ecosystem functioning in grassland. *M. van der Heijden,* Vrije Universiteit, Amsterdam, The Netherlands.

Functional ecology of ectomycorrhizal symbiosis during early primary succession. *K. Nara,* The University of Tokyo, Japan.

Mycorrhiza in alpine erosion control and slope stabilisation. *F. Graf,* Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland.

How much do AMF communities contribute to the soil stability of arid ecosystems? *V.B. Chaudhary,* Northern Arizona University, USA.

Mycorrhizal functioning in restored seasonal tropical forest, Mexico, following fires and hurricanes. *R. Vargas,* University of California, Riverside, USA.

Session 12: Narrowing the gap between theory and practice in mycorrhizal management

Coorganized by: *N. Collins Johnson & M. Vosatka*
(Andalucía Hall)

Knowns' and 'unknowns' in mycorrhizal management. *L.K. Abbott,* The University of Western Australia, Australia.

Using models grounded in stoichiometry to predict plant-mycorrhizal ecology across scales. *J.D. Hoeksema,* University of California, Santa Cruz, USA.

Arbuscular Mycorrhizal Fungi as a key factor for restoration model of humid forest. *J. Álvarez-Sánchez,* Universidad Nacional Autónoma de México, México.

Function of arbuscular mycorrhizas in revegetation process in volcanic deposit-affected lands. *M. Saito,* National Institute for Agro-Environmental Sciences, Tsukuba, Japan.

Ecological risks *versus* benefits in AMF inoculum production for tropical agriculture. *G. Cuenca,* IVIC, Centro de Ecología, Caracas, Venezuela.

How to narrow gaps between scientific research and commercial application of mycorrhizal fungi products – a plea for support! *M. Vosatka,* Institute of Botany, Academy of Sciences of the Czech Republic, Pruhonice, Czech Republic.

Session 13: Mycorrhizal networking

Coorganized by: *J. Leake & M. Giovannetti*
(Andalucía Hall)

Vegetative compatibility, self recognition and nonself incompatibility in arbuscular mycorrhizal networks. *M. Giovannetti,* Department of Crop Plant Biology, University of Pisa, Italy.

Extraradical arbuscular mycorrhizal fungi development in different soil environments. *C. Hamel,* Semiarid Prairie Agricultural Research Centre, AAFC, Swift Current, Canada.

Spatial pattern and control of arbuscular mycorrhizal fungal community diversity in an old-field meadow. *J.N. Klironomos,* University of Guelph, Canada.

Partial mycoheterotrophy - a novel nutritional mode of forest ground vegetation. *G. Gebauer,* University of Bayreuth, Germany.

First evidence of mutualism in orchid mycorrhiza: Net carbon and nutrient fluxes between an orchid and its mycorrhizal mycelial network. *D.D. Cameron,* University of Sheffield, UK.

The role of ectomycorrhizal fungal communities in the success or failure of tree establishment. *I.A.Dickie,* Landcare Research, New Zealand.

Session 14: Mycorrhizosphere interactions/biology

Coorganized by: *S. Timonen & R. Sen*
(Manuel de Falla Hall)

Effects of mycelial exuduates from an arbuscular mycorrhizal fungus on soil bacterial community composition *in vitro*. *J.F. Toljander,* SLU, Uppsala, Sweden.

Influence of *A. niger* fermented sugar beet waste on AM hyphal growth and P uptake, and on microbial populations in soil. *A. Medina,* Estación Experimental del Zaidín, CSIC, Granada, Spain.

Contribution of arbuscular mycorrhizal fungi to the bioremediation of PAH in the mycorrhizosphere. *C. Leyval,* LIMOS, CNRS-UHP Nancy, France.

Mineral weathering - how important are ectomycorrhizal fungi? *M.M. Smits,* Wageningen University, Netherlands.

Mechanisms behind mycorrhiza helper effect exerted by *Streptomyces laceyi* AcH 505. *M.T. Tarkka,* University of Tuebingen, Germany.

Diversity and expression of nitrogenase genes (*nifH*) from ectomycorrhizas of Corsican pine (*Pinus nigra*). *H. Izumi,* University of Aberdeen and The Macaulay Institute, Aberdeen, UK.

Session 15: Mycorrhiza in alternative production systems

Coorganized by: ***X. Li & M. Honrubia***
(Manuel de Falla Hall)

Diversity of ectomycorrhizal fungi in wooded banks of different age in The Netherlands. *J. Baar,*
Wageningen University, The Netherlands.

Biotechnology and cultivation of desert truffles: an agricultural tool for degraded semiarid lands. *A. Morte,* Universidad de Murcia, Spain.

Practical application of endomycorrhizal fungi in China: retrospect and prospect. *J.L. Zhang,* China Agricultural University, China.

Alteration of the production of phytochemicals in sweet basil under the influence of the arbuscular mycorrhizal symbiosis. *J.P. Toussaint,* The University of Adelaide, Australia.

Farming systems and carbon sequestration. *P. McGee,* University of Sydney, Australia.

Mycorrhizae inoculated seedling production systems in organic farming under greenhouse and field conditions. *I. Ortas,* Çukurova University, Adana, Turkey.

Session 16: Improved inoculum technology and implementation

Coorganized by: *A. Adholeya & S. Gianinazzi*
(Andalucía Hall)

***In vitro* mass production technology for arbuscular mycorrhizal fungi: Scientific and industrial aspects.** *A. Adholeya,* The Energy and Resources Institute, New Delhi, India.

Different arbuscular mycorrhizal inoculant technologies used under tropical and subtropical conditions. A study case on a Cuban experience. *F. Fernandez-Martin,* Instituto Nacional de Ciencias Agricolas (INCA), Cuba.

Do commercial mycorrhizal inocula need to be tuned for target environments? *J. Albrechtová,* Charles University, Prague, Czech Republic.

Challenges for development of mycorrhizal inoculants adapted for specific markets. *S. Gagné,* Premier Tech Ltd, Québec, Canada.

Improved method for inoculation of *Populus tremula* with ectomycorrhizal fungi. *I. Langer,* University of Natural Resources and Applied Life Sciences, Vienna, Austria.

Field inoculation with AMF in agriculture and rehabilitation. Integration of technologies. *V. Estaún,* IRTA, Cabrils (Barcelona), Spain.

From	to	Sunday 23 July	Monday 24 July		Tuesday 25 July	
8:30	9:00		Opening ceremony			
9:00	9:30		**Session 1**: Cell programmes: (1) genome to transcriptome	**Session 5**: Evolution and phylogeny of mycorrhizal fungi	**Session 4**: Signals and perception	fu
9:30	10:00					
10:00	10:30					
10:30	11:00					
11:00	11:30					
11:30	12:00		Coffee break		Coffee brea	
12:00	12:30		**Session 2**: Cell programmes: (2) proteome to metabolome/isoformome	**Session 6**: Population biology of mycorrhizal fungi	**Session 3**: Genetics/genomics of mycorrhizal fungi	E ec
12:30	13:00					
13:00	13:30					
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14:00	14:30					
14:30	15:00		Lunch		Lunch	
15:00	15:30					
15:30	16:00					
16:00	16:30		**Workshops** Cellular aspects of AM fungi. C transfer: mechanisms and ecol. implications Mycorrhiza and heavy metal stress alleviation Ecological applications of ECM in forestry		**Workshop** Cross-talks between host, n Mycorrhizal N transpor Mycorrhiza and plant pathc Community structu	
16:30	17:00					
17:00	17:30	Registration and putting up posters				
17:30	17:45					
17:45	18:45		**Keynote lecture**: Linking above and belowground multitrophic interactions across spatio-temporal scales		**Keynote lect** Macro- and microevolution systems	
18:45	19:00		Posters		Posters	
19:00	19:30					
19:30	20:00					
20:00	20:30		IMS meeting			
20:30	21:00				Optional visit to the	
21:00	21:30	Welcome reception				
21:30	22:00		Wines of the World			
22:00	22:30					
22:30	23:00					
23:00	23:30					
23:30	0:00					

	Wednesday 26 July		Thursday 27 July	
sion 11: e-related nal ecology	**Session 7**: Mechanisms in stress resistance/tolerance	**Session 10**: Anthropogenic impacts on mycorrhiza diversity and functioning	**Session 13**: Mycorrhizal networking	**Session 15**: Mycorrhiza in alternative production systems
	Coffee break		Coffee break	
ssion 9: versity and cal impacts	**Session 8**: Functional diversity in mycorrhiza	**Session 12**: Narrowing the gap between theory and practice in mycorrhizal management	**Session 14**: Mycorrhizosphere interactions/biology	**Session 16**: Improved inoculum technology and implementation
	Lunch		Lunch	
ost and AMF abolism interactions CM)	**Workshops** Ericoid, orchid and DSE associations Mycorrhizal P transfer and discussion ... Multitrophic interactions Community structure (AM)		**Workshops** Mycorrhiza and abiotic stress alleviation Novel methodological approaches Mycorrhizas in threatened plant communities Mycorrhiza in land use and crop management	
ant-microbe	**Keynote lecture**: Strategies for the development and release of improved microbial inoculants for food production		**Keynote lecture**: Soil microbial resources and agricultural policies	
	Posters		Business meeting	
			Closing session	
nbra				
	Conference dinner			

Workshops

1 **Cellular aspects of AM fungi,** *chair N. Requena.*
(Room: Andalucia III)

Effect of activated carbon fiber on growth of arbuscular mycorrhizal fungi. *T. Ishii,* Kyoto Prefectural University, Kyoto, Japan.

A H⁺ ion flux signature for the hyphal asymbiotic development of the mycorrhizal fungus *Gigaspora margarita.* *A.C. Ramos,* Instituto Gulbenkian de Ciência, Oeiras, Portugal.

The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores. *H. Bothe,* The University of Cologne, Germany.

Hyphal healing mechanism in arbuscular mycorrhizal fungi. *I.E. de la Providencia,* Université catholique de Louvain, Belgium.

Mating type loci in the asexual arbuscular mycorrhizal fungi *Glomus intraradices.* *H. Niculita,* University of Lausanne, Switzerland.

2 **C transfer: mechanisms and ecological implications,** *chair S. Smith.*
(Manuel de Falla Hall)

The first glomeromycotan monosaccharide transporter is characterized. *D. Cohen,* University of Technology, Darmstadt, Germany.

Symbiotic phenotype of transgenic *Medicago truncatula* plants with reduced amounts of the sucrose synthase MtSucS1. *M.C. Baier,* Bielefeld University, Germany.

Localization of acid invertases in ectomycorrhizal roots of *Populus* sp. *W. Heyser,* University of Bremen, Germany.

Fungal specificity and phenology affects net carbon transfer through hyphal and soil pathways in the laboratory and in the field. *L.J. Philip,* University of British Columbia-Vancouver, Canada.

Can the ectomycorrhizal fungus *Lactarius quietus* supply oaks with carbon during spring reactivation? *J. Garbaye,* INRA-Nancy, France.

Carbon use, metabolic modeling and isotopic fractionation of ectomycorrhizal fungi. *F. Scandellari,* University of Bologna, Italy.

3

Mycorrhiza and heavy metal stress alleviation, *chairs C. Leyval & K. Turnau.*

(Room: Andalucia II)

Experimental and meta-analytical approaches in heavy metal phytoremediation. *P. Audet,* University of Ottawa, Canada.

Responses of two poplar species, inoculated with arbuscular mycorrhizal fungi, to zinc or copper. *G. Lingua,* Università del Piemonte Orientale "A. Avogadro", Alessandria, Italy.

Another *Thlaspi* story –hyperaccumulation of Cd in embryos of *Thlaspi praecox.* *M. Regvar,* University of Ljubljana, Ljubljana, Slovenia.

Heavy metal tolerance in mycorrhizal plants involves common features with other stress responses in plants. *A. Gollotte,* INRA/CNRS/U. Bourgogne, Dijon, France.

GintABC1* and *GintMT1* are involved in Cu and Cd homeostasis in *Glomus intraradices. *M. González-Guerrero,* Estación Experimental del Zaidín, CSIC, Granada, Spain.

Role of arbuscular mycorrhizal fungi in plant adaptation to arsenic contaminated soil. *B.D. Chen,* Research Center for Eco-Environmental Sciences, The Chinese Academy of Sciences, China.

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Session 7: Mechanisms in stress resistance/tolerance	**Session 8**: Functional diversity in mycorrhiza	**Session 9**: Biodiversity and ecological impacts
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The presenting authors of the poster are requested to be available the indicated day for the corresponding Session

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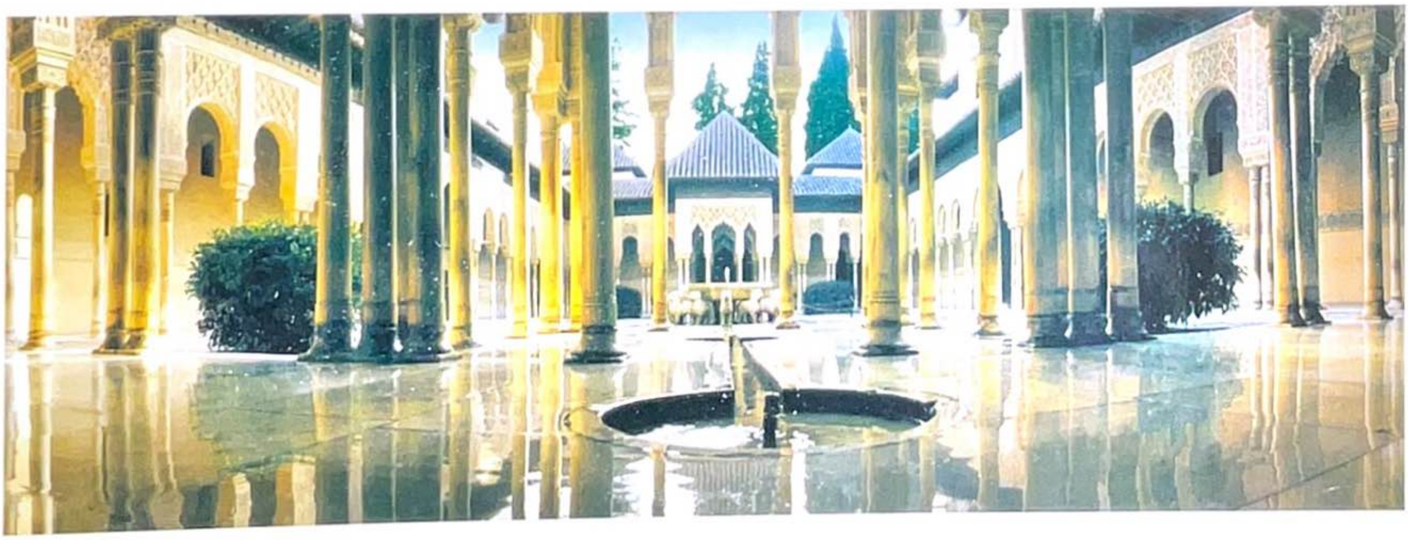
Abstracts

5th International Conference on Mycorrhiza

"Mycorrhiza for Science and Society"

23-27, July 2006 - Granada, Spain



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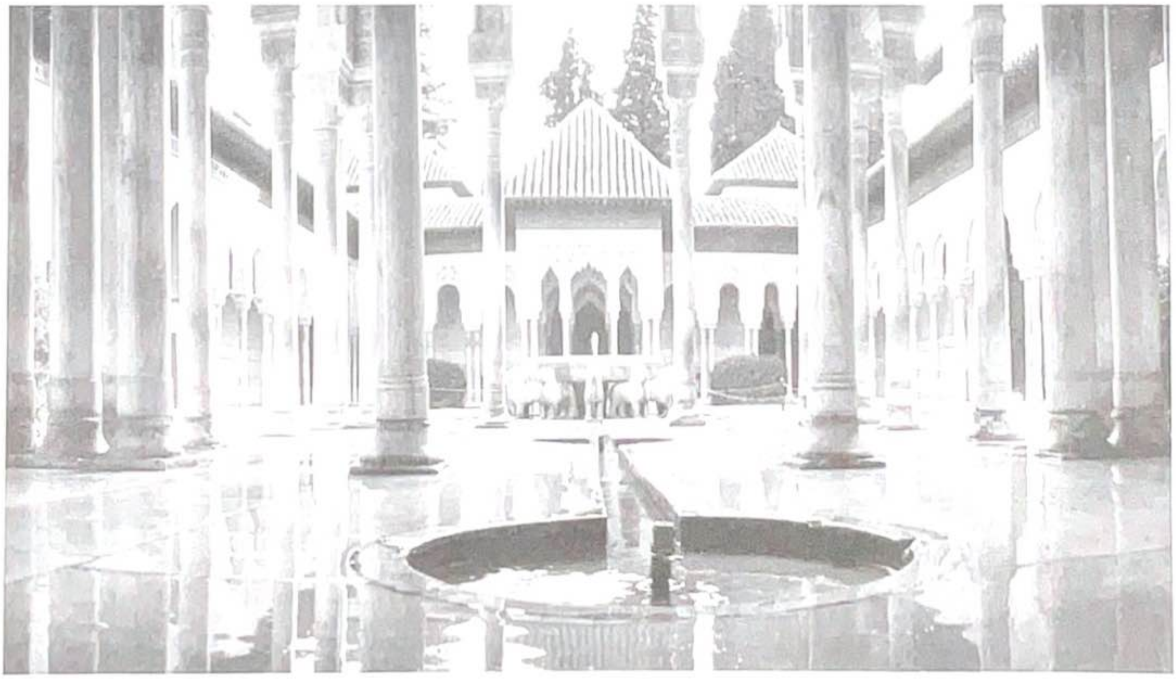

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Dedication

The Organizing Committees of ICOM5 would like to dedicate this Book of Abstracts to the memory of our colleague and friend Hannes Schüepp whose death in February 2006 surprised and saddened all of us. Hannes made an unforgetful contribution to mycorrhizal research by bringing researchers together in a European Network (COST Action 8.10) which focussed on arbuscular mycorrhizal fungi as components of the soil microbiota, and their importance for plant nutrition and plant health in sustainable soil–plant systems. This network, which expanded through subsequent COST Actions (8.21, 8.38) to include as many as 32 different countries, will be remembered as a driving force for cooperative research activities and especially for the friendly atmosphere under which it operated. It also provided the basis for the publication of two books (Birkhäuser Verlag, Basel), co-edited by Hannes, on the "Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems" and "Mycorrhizal Technology in Agriculture". Many of us have benefited from Hannes's wide knowledge and advice through personal discussions or at scientific meetings. He will be missed not only for his scientific merit but also for his friendliness and sincerity towards all those that have had the privilege to know him.

(Taken from a text by K. Haselwandter, 2006, *Mycorrhiza* 16:149-150)

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ICOM Student Awards

The organisers of ICOM5 wish to express their sincere gratitude to the donors of prizes that will be attributed on the occasion of ICOM5 to students presenting oral or poster presentations, as recognition of the quality of their contribution to mycorrhiza research:

The Harley Medal

Created by the International Mycorrhiza Society (www.mycorrhizas.org/) to highlight John Laker Harley's career as a model to young scientists, the 'Harley Medal' will be awarded to the student presenting an oral paper which best emulates the rigorous approaches to mycorrhizal research that J. L. Harley pioneered during his lifetime. It will be accompanied by a generous donation from Lindsay Harley.

The CSPI Award

In line with traditions initiated at previous ICOMs, the Centre for Soil-Plant Interactions (CSPI, www.ees.adelaide.edu.au/research/cspi/) is pleased to offer a student prize for an excellent poster presentation at ICOM5. The aim of the prize is to encourage posters of high quality and originality with respect to both content and presentation.

Biotisa Prize

The recently created company Biotisa wishes to award a prize for a student contribution (poster or oral) which reports research showing a particular potential for the promotion of practical aspects of mycorrhiza.

Acknowledgements

As Chairman of the ICOM5, I would like to express my special thanks to Persons, Institutions and Sponsors contributing to this Conference. My sincere gratitude to all members of the Conference and Local Organising Committees without whose help ICOM5 would not have happened.

My recognition also goes to participants of ICOM4 who approved the initiative from the European Network on arbuscular mycorrhiza (COST Action 838), implemented and presented by Silvio Gianinazzi, to choose Granada as the appropriate venue for ICOM5. I thank Mayra Gavito for representing our team in Montreal and for bringing the ICOM mascot safely to Andalucía.

More particularly, I wish to extend my heartfelt thanks to three persons for their excellent work in ensuring the success of ICOM5. Firstly, to **Vivienne Gianinazzi-Pearson**, *Chairperson of the Steering Committee*, who guided ICOM5 activities and greatly contributed to designing and promoting the quality of the Scientific Programme of the Conference. Secondly, to **Francisca Gonzalez (Paqui)** for her impressive work in establishing and maintaining ICOM5 databases. Finally, to **Conchi Azcón** for her timeless, intelligent, and efficient dedication to all aspects of ICOM5 organization, and in particular for the huge task of establishing and finalising the Scientific Sessions and Workshops presentations involving thousands of e-mail messages.

I am sure that all ICOM5 participants will recognize the excellent work of Vivienne, Paqui and Conchi.

I will never forget your impressive job. Many many thanks.

José-Miguel Barea

Preface

Substantial progress has been made over the last few years in the understanding of mycorrhizal symbioses. ICOM5 aims to discuss these recent advances and to outline future trends in mycorrhizal research. Recently acquired knowledge of this universal fungal/root association has lead to an increased understanding of: (i) the population biology of mycorrhizal fungi; (ii) many basic biological processes such as the development of compatibility and synergism between organisms, and the genetic and cell programmes involved; (iii) the influence of mycorrhiza on plant fitness and stress adaptation, and on soil quality, thus favouring sustainability; (iv) the role of mycorrhizal symbioses in the evolution of life; (v) microbial interactions in the mycorrhizosphere; (vi) successful applications of mycorrhizal technology to promote sustainable practices in plant production systems, as well as in conservation and ecosystem restoration schemes; and (vii) mycorrhizal symbioses as a model for understanding other types of plant-microbe associations.

ICOM5 thus provides a discussion forum to evaluate the impact of recent advances in mycorrhiza research in improving basic science knowledge or in satisfying societal demands (e.g. the sustainable production of safe foods and the promotion of environmental quality). Hence, the theme chosen for the Conference was **Mycorrhiza for Science and Society**.

This book records the abstracts of the papers which will be given during the Conference. The abstracts have been organized according to three types of ICOM5 activities: (i) Keynote addresses, (ii) Sessions (with oral and poster presentations) and (iii) Workshops (oral presentations).

Many thanks to the authors of these abstracts: it is an honour to present this book to you as a basic document for discussions during the Conference and as a record of the contribution of ICOM5 to future research and applications of mycorrhiza.

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Keynote lectures

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Keynote lectures

Keynote lectures

Linking above and belowground multitrophic interactions across spatio-temporal scales

van der Putten, W.
Netherlands Institut of Ecology, Heteren, Netherlands

Ecological concepts are usually developed for visible and easily manipulated models. The unfortunate fate of ones working in more complex systems, such as in soil, is then to test if these relatively simple concepts also hold beyond simple model systems. Considering the difficulty of experimental studies with soil organisms, or with species interactions in natural systems, it is not surprising that the concept of multitrophic interactions has been strongly developed for understanding how crop plants may deal with their aboveground invertebrate enemies. Belowground biological control research has been largely focusing on trial and error, rather than on developing and applying multitrophic interaction concepts. In soil, theory development mainly stems from soil food web research, and this has been mainly developed to deal with the enormous belowground biocomplexity in order to assess the contribution of decomposing soil organisms to the flow and stability of major ecosystem processes, such as the cycling of C, N and energy. A concept that has become powerful is that of feedback between plants and the soil in which the plants are rooted. This feedback can be due to plant-induced changes in soil nutrient status, organic matter, and pH, as well as to changes in the composition of the soil biota. Despite the enormous wealth of soil organisms to which plant roots are exposed to, only a subset of the soil community may respond to the presence of a specific plant species. These responding organisms usually increase in abundance and consequently influence plant performance. Some soil organisms, such as mycorrhizal fungi usually (but not always) enhance plant performance while others, for example pathogens and root feeders primarily reduce plant performance. However, beneficial and pathogenic soil organisms also have their natural enemies, so that the feedback effects of plants with their soil community may depend on how these belowground multitrophic interactions operate. I will highlight some of our work in coastal foredunes, where soil organisms contribute to plant succession. Plant performance and the rate of plant replacement along the successional gradient (from beach to inland) depend on the absence or presence of pathogens and root-feeding nematodes. The nematodes, however, appear to be controlled by a variety of factors. I will show some examples from the EU-EcoTrain project that investigated how the root-feeding nematodes can be controlled by the host plant (bottom-up), other nematodes (horizontal) or by soil microorganisms (top-down control). Interestingly, AM fungi appeared to control some, but not all root-feeding nematodes. The EcoTrain project was proposed to stand model for nematode control in the wild ancestors of agricultural crops and the results may ultimately lead to new strategies for biological control of root-feeding nematodes in agricultural systems. As above and belowground subsystems are intrinsically linked, our understanding of the ecology of species, their functioning in communities and the consequences for ecosystem processes may benefit from considering above- and belowground multitrophic interactions in conjunction. However, examples of studies on these multitrophic (tri- or more trophic interactions above and belowground) are scarce, although studies are in progress. Therefore, I will build up starting from cases where above- and belowground herbivores interact through a shared host plant. Then, I will increase complexity of the interactions in the examples moving from small-scale lab studies to field and, finally, continental scales. In my examples, I will show that the interactions between plants and their aboveground herbivores and their antagonists cannot be fully understood without considering interactions below ground and *vice versa*. This above-belowground approach of communities and ecosystem functioning is crucial in order to get a better understanding of what controls biodiversity, ecological succession, species abundance and which factors may explain biological invasions of organisms in novel habitats. The successes of understanding plant defense and how direct and indirect defense strategies may find their trade off in plant life histories leaves a number of unanswered questions. Is the multitrophic interactions concept generally applicable? Do multitrophic interactions apply equally well to pathogens as to invertebrates? Is recruitment of antagonists as reliable in the soil as above ground? Has the breeding of crop plants for fast growth, high yield and nice taste resulted in a trade-off with the capacity of direct defense? How do direct and indirect defense trade off in wild plants? How do multitrophic interactions relate to biodiversity and how does biodiversity relate to multitrophic interactions? How do mycorrhizal fungi fit in? These questions are part of the large research agenda that we are facing. Quite some of these issues need to be solved in order to better understand, predict and counteract effects of global human-induced changes in order to enhance sustainability of natural and production systems.

Macro- and microevolution in plant-microbe systems

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Plant-microbe symbioses provide exciting field for evolutionary research since novel structures, functions and gene networks are developed in partners to improve their adaptive potentials. The evolutionary genetics of symbioses assume analyses of tightly interconnected molecular and population processes in which the macro-evolutionary genome reorganizations (which have resulted in evolution of "symbiotic" gene networks) are elicited by micro-evolutionary pressures induced specifically in the host-microbe systems. The evolutionary approach to symbioses was introduced by A. de Bary who defined it as a "living together of differently named organisms" including beneficial (mutualistic) and pathogenic (antagonistic) interactions. Molecular data reveal genetic mechanisms that are common to mutualism and pathogenesis, suggesting an evolutionary continuum which includes both types of symbiosis. From the plant side, similarities are evident between the systems responsible for cooperation with beneficial microbes (mycorrhizal fungi, N_2-fixing rhizobia) and for defense against pathogens. The comparative analyses of developmental programs for different mutualistic interactions suggest that many genes controlling the extant plant-microbe symbioses were recruited from the systems encoding for arbuscular mycorrhiza (AM) - an ancestral form of interaction between terrestrial plants and their microbial allies. However, the commonalities between mutualism and pathogenesis are restricted to early partner interactions implemented at cellular/tissue levels. Genetic analyses have revealed regulatory differences between hosting beneficial microbes and suppressing pathogenic ones, e.g., legume mutants defective in AM or nodule development is usually not affected in disease resistance. Much more difference has been revealed between mutualism and pathogenesis at the level of systemic regulation (SAR circuits *vs* AON regulation of nodulation), of biochemical interactions (integration of partners' C/N metabolic pathways in nodules *vs* unilateral nutrient flow from hosts to pathogens) and of ecological impacts (improvement of host fitness in mutualism *vs* its decrease in pathogenesis). The dialogue of rhizobia with host defense reactions (mediated by bacterial cell envelope components) is basically restricted to the early (pathogen-like) symbiotic processes and is not required for the late (mutualistic) processes. The phylogenetic analyses of plant-associated fungi and bacteria suggest that the inter-conversion between pathogens and beneficial symbionts is restricted to horizontal transfer of some genes (e.g., encoding for Type Three Secretion Systems and for the symbiotically essential surface components) in the Rhizobiaceae bacteria and to the direct filiations between different forms of "defensive symbioses" in the Clavicepaticeae fungi. Mathematical simulation suggests that diverse selective mechanisms may be responsible for evolutionary isolation of different types of symbiotic microorganisms: individual (Darwinian, frequency-dependent) selection in pathogens, group (inter-deme, kin) selection in beneficial symbionts. These differences are related to the type of genetic feedback linking microbes and their hosts: negative in pathogenesis, positive in mutualism. Different mechanisms for maintaining genetic homeostasis is revealed in plant-microbe interactions since the individual selection for symbiotic traits from the host side may be balanced either by individual (pathogenesis) or by group (mutualism) selection pressures from the microbial side. For simulation of the evolution of mutualistic symbioses, methods for analyzing the biological altruism should be transferred from intra-species to inter-species interactions. In summarizing results from molecular (genomic) and population analyses, we suggest that plant-associated microbes undergo macroevolution directed by adaptation towards: (1) universal systems for hosting microbes within the inter- and intracellular compartments, (2) regular circulation of microbes within various habitats in the "host-environment" ecosystems. A universal scheme for operation of different micro-evolutionary pressures (natural selection, genetic drift, population waves) in plant-associated microbial populations is proposed to simulate the plant-microbe coevolution. Supported by RFBR grant 06-04-48800.

Strategies for the development and release of improved microbial inoculants for food production

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As the human population continues to increase demands are being placed on global food crop production. Excessive application of agricultural fertilizers has resulted in major concerns regarding soil and groundwater contamination and challenges exist to promote productivity of food crops in an environmentally sustainable manner. One such low input approach is the use of beneficial multifunctional microbial inoculant consortia. For example the soil-borne fluorescent pseudomonads and the arbuscular mycorrhiza. These synergistic microbes have been shown to improved plant growth *via* various mechanisms such as protection against pathogens and ability to increase the availability of nutrients such as phosphates to the plant. Production of the antimicrobial metabolite 2,4-diacetylphloroglucinol (Phl) is a central mechanism utilized by *Pseudomonas fluorescens* strains in biocontrol. Phl is a significant secondary metabolite as it has been shown to have broad activity e.g. antifungal, anti-rhizomania, antimicrobial and antihelminthic. It also has been reported to induce systemic plant defences and to alter plant root exudation profiles. Complex regulatory mechanisms govern Phl production and operate at both the transcriptional and posttranscriptional level. Exploitation of functional genomics has made it possible to utilize polyphasic strategies to generate improved microbial inoculants *via* genomic and physiological reprogramming of these regulatory systems. However, despite technological advances certain factors still need to be addressed to improve microbial inoculant technology for food crop production. Although many inoculants show good performance in specific trials, this is often not translated under diverse field situations. The first relates to efficacy of the products. Although many strains show good performance in specific trials, this is often not translated into consistent, effective biocontrol in diverse field situations. Some of this is due to external factors such as soil or climatic conditions, but a major part is due to intrinsic traits of the microbe, such as variable production of required metabolites and poor colonisation under certain conditions. Molecular signalling between microbes and their plant hosts plays a fundamental role in establishment of beneficial interactions in the rhizosphere. By profiling the complete microbial genetic response to plant root exudates, it is now possible to assemble a full picture of how gene expression, and thereby function, is modulated in the microbe after the perception of plant signals. This strategy has the potential to elucidate molecular microbe interactions that are important for biocontrol and plant productivity. Another factor is the need to satisfy specific regulatory requirements. In Europe, Directive 2001/36/EC (amending directive 91/914/EEC) sets out strict requirements in terms of biosafety and environmental assessment that need to be fulfilled in order to receive approval for commercial registration and release of an inoculant as a plant protection product in the EU. The EU Council Directive 2001/38/EC (repealing Council Directive 90/220/EEC) legislates for the deliberate release of genetically modified organisms into the environment. This has been addressed within the framework of the EU ECO-SAFE project, a multidisciplinary European collaborative network. Understanding molecular signalling processes and the functions they regulate is fundamental to enhancing beneficial plant-microbial interactions in the rhizosphere. Knowledge gained will facilitate in overcoming existing limitations and aid the subsequent registration and release of improved microbial inoculant consortia for use in food crop production.

Soil Microbial Resources and Agricultural Policies

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For any innovation in agriculture to be adopted it must have proven efficacy, there must be away of using it in practise and it must sit comfortably with societal norms and expectations. The range of effects which are occasioned by mycorrhizal colonization have been clear for some time. Current research is showing an increased number of instances where mycorrhizas have a positive effect. Systems of application and management exist. The major barrier to increased mycorrhizal use in agriculture has in the past been their incompatibility with the predominant farming systems of the West. Four recent developments suggest that this may now change. (1) Traditionally financial support for farmers, from Government, was linked to specific commodities and to production. Recent developments in the Common Agricultural Policy (CAP) have decoupled production and payment and have begun a process of moving funding into environmental and social activities (Pillar 2). This both restores the key link between producers and consumers and moves the target towards quality and away from quantity. It increases the prospect of the development of small scale farming. (2) The EU's Water Framework Directive will over the next decade have the effect of reducing the levels of use of agricultural inputs such as pesticides and fertilizers. It will promote alternative means of supplying nutrients and achieving crop health. (3) Concern over climate change will increase the importance of carbon budgeting and will pressure activities, such as the production of chemically fixed nitrogen, which use large amounts of hydrocarbon fuels. (4) The debate in the EU over the introduction of GMO crops and foods indicated that the public were becoming increasingly concerned about the consequences of high input agriculture on food quality, the environment and the fairness of trade. Much of the GM debate was a discussion of core values, especially in respect of effects on society and environment. A consequence of all of these changes has been an increase in the consumption of food from systems which demonstrably minimise the use of chemicals and maximise the use of natural resources. Organic Farming is an example. Organic Farming systems are explicitly systems designed so as not to require inputs of nutrients and energy demanding mean of maintaining unstable ecosystems. Here nitrogen is obtained by fixation using legumes and processed into a crop available form though the use of livestock. "By-products" such as straw and animal wastes become useful inputs rather than "wastes". In such systems mycorrhizal fungi have a key role in respect of the availability of soil nutrients, resistance to diseases, growth regulation and provision of environmental information. How mycorrhizas might contribute to the development of such systems is a key question. Unequivocally the potential now exists for mycorrhizas to aid in the development of alternative approaches to agriculture. These will permit the production of the quality foods which are needed for small scale farming enterprises to be successful in the less favoured farming areas of Europe, and in third world. These systems are key to sustainable rural development and fairer world trade. At the same time they should have a positive impact on carbon budgets and on the environment. These issues will be discussed in relation to the functioning of mycorrhizas and the further development of systems which optimise their activities.

Sessions

Sessions

SESSION 1

Cell programmes: (1) genome to transcriptome

Transcriptomics of Arbuscular Mycorrhiza in the Model Legume *Medicago truncatula*

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We developed 70mer oligonucleotide microarrays termed Mt16kOLI1Plus. These tools are based on 16.470 probes representing ~13.000 genes of the model legume *Medicago truncatula*. To identify genes consistently up-regulated in different AM interactions of *M. truncatula*, an approach based on the combination of EST-sequencing with *in silico* and microarray-based transcriptome profiling was pursued. These experiments identified a range of novel AM-induced genes including membrane transporters and transcriptional regulators. Comparisons of these transcriptome profiles to gene expression during nodule development indicated the common activation of different genes that can be associated with processes relevant for the maintenance and function of either symbiosis. Based on the relation of AM transcriptome profiles to gene expression during biotrophic pathogenic interactions of *M. truncatula*, a core genetic programme specifically activated in response to AM fungi can be defined. To obtain a complete overview of AM-related gene expression, transcriptomics experiments in arbuscular mycorrhiza will have to be extended to the concomitant genome-wide expression profiling of the two genomes that mastermind the symbiotic interaction. The genome sequence of the AM fungus *Glomus intraradices* is currently sequenced at the Joint Genome Institute (JGI). Subject to the status of this project, strategies to develop genome-wide expression profiling tools in the *Glomus* community will be discussed.

Transcript patterns associated with ectomycorrhiza formation in poplar

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Ectomycorrhiza development alters gene expression in the fungal and plant symbionts. The identification of a large number of genes expressed exclusively or predominantly in the symbiosis will contribute greatly to the understanding of the development of the symbiotic organ. We defined a research program that aims at understanding key processes governing ectomycorrhiza development and functioning in the model tree species *Populus*. Poplar genome sequence was made available by the Joint Genome Institute (DOE, USA) in 2004 and thousands of ESTs of this species have been sequenced. Poplar is amenable to genetic transformation, which makes this tree of particular interest for functional studies of the ectomycorrhizal symbiosis. A macroarray consisting of 4,6K root cDNAs and a 28K cDNA-microarray pooling sequences from leaf, root and wood tissues were designed to perform expression study of *Populus* genes during the symbiotic development. We analyzed RNA levels from symbiotic tissues of *Populus tremula x alba* and *Populus trichocarpa x deltoides*, associated with the basidiomycetes *Pisolithus microcarpus* or *Laccaria bicolor* progressing through ectomycorrhiza development. Transcript level of hundreds of genes was altered during the symbiotic event and several symbiosis-related genes previously identified in other ectomycorrhizal associations were also induced in this study. These genes may contribute to the functionning of the symbiotic organ and are key targets for further studies.

Transcript profiling and positional cloning of a *Medicago truncatula* mycorrhizal mutant

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A screen of an EMS mutagenized *Mediago truncatula* population resulted in the identification of a mycorrhizal mutant, *str* (stunted arbuscule), in which arbuscule development is arrested. The mutation impairs arbusucle development when associated with mycorrhizal fungus *Glomus versiforme*, *Glomus intraradices* and *Gigaspora gigantea*. Unlike other *M. truncatula* mycorrhizal mutants identified to date, *str* has a wildtype nodulation phenotype. *Str* was crossed to *M. truncatula* A20 ecotype and a mapping population of 3903 F2 and F3 plants was generated. Using SSR and CAPS markers *str* was mapped to the end of the south arm of chromosome 4. Microarray experiments were conducted to study the transcript profiles of *str* roots following inoculation with *G. intraradices*. A comparison of gene transcript profiles of *str* and A17 (wildtype) will provide information about molecular changes in str roots which give the mycorrhizal phenotype.

Lunch is not for free: Coordination of carbohydrate and nutrient exchange at the plant/fungus interface

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Monosaccharides are of special importance for ectomycorrhizal fungal growth and maintenance since forest ecosystems are poor in easy degradable carbohydrates. For ectomycorrhizal fungi only two carbohydrate sources are available: dead off cells (e.g. bacteria, fungi, nematodes, plant material) or the plant partner in symbiosis. Since the majority of available carbohydrates originate from the plant partner, the fungus has to generate a strong carbon sink to enable a continuous and sufficient support. With respect to the plant partner, fine roots are covered with fungal hyphae and thus isolated from the surrounding soil in ectomycorrhizal symbiosis. Since the majority of tree fine roots are mycorrhized under natural conditions, plant nutrition is dependent on the fungal partner. Due to the dependency of plant and fungus to the reciprocal exchange of carbohydrates and nutrients, control mechanisms have to be established to avoid parasitism of one of the partners. In this contribution a model will be presented, of how fungal carbohydrate support could be linked to plant nutrition in symbiosis. The model is based on analysis of plant and fungal monosaccharide transporters in the background of a poplar mutant defective in ectomycorrhiza formation and large scale expression analysis.

Analysis of mycorrhizal functioning by using transcriptomic approaches

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The arbuscular mycorrhizal (AM) symbiosis is mainly characterised by the nutrient exchange between the fungal and the plant partner. In order to study the molecular physiology of this process, *Medicago truncatula* and *Glomus intraradices* were chosen as model organisms. A large international collection of expressed sequence tags (ESTs) exist for the plant, while a fungal EST data base is currently being build up in frame of the German program MolMyk and will be presented. These ESTs are being used on the one hand to study the functional diversity of interactions between *M. truncatula* and different AM fungal isolates with the main focus on different plant phosphate uptake pathways. On the other hand the regulation of *G. intraradices* genes by the carbohydrate status of the plant has been analysed. The results reflected the tight mutual dependency of the two partners. Apart from nutrient exchange, bioprotection of plants against root pathogens plays an important role. The tripartite interaction between *M. truncatula*, *Glomus mosseae* and the root rot causing agent *Aphanomyces euteiches* has been established as model and was analysed using subtractive hybridisations and screening of microarrays. The results point to the involvement of certain phytohormones and secondary metabolites in bioprotection. Several genes are being studied in more detail using among others transgenic root organ cultures.

Spatial patterns of gene expression in the extramatrical mycelium and ectomycorrhizal root tips

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Functional compartmentation of the extramatrical mycelium of ectomycorrhizal (ECM) fungi is considered important for the operation of ECM associations, although the molecular basis is poorly characterised. Global gene expression profiles of mycelium colonizing an $(NH_4)_2SO_4$ nutrient patch, rhizomorphs and ECM root tips of the *Betula pendula / Paxillus involutus* association were compared by cDNA microarray analyses. Expression profiles of rhizomorphs and nutrient patch mycelium were similar to each other but distinctly different from mycorrhizal tips. Statistical analyses revealed 337 of 1075 fungal genes differentially regulated among these three tissues. Clusters of genes exhibiting distinct expression patterns within specific tissues were identified. Genes implicated in the GS/GOGAT and urea cycles, and the provision of carbon skeletons for ammonium assimilation via β-oxidation and the glyoxylate cycle were highly expressed in rhizomorph and nutrient patch mycelium. Genes implicated in vesicular transport, cytoskeleton organisation and morphogenesis and protein degradation were also differentially expressed. Differential expression of genes among the extramatrical mycelium and mycorrhizal tips indicates functional specialization of tissues forming ECM associations.

1.1 Expression of *Amanita muscaria* trehalose biosynthesis genes is strongly enhanced at the plant/fungus interface

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An important ectomycorrhizal feature is the fungal support with plant derived hexoses. To maintain a strong carbon sink in symbiosis, imported carbohydrates must be converted quickly into fungal metabolites. In *Amanita muscaria*, a remarkable amount of imported hexose is converted into the carbohydrate storage compound trehalose. To get insight in the impact of trehalose metabolism in symbiosis, we investigated the expression of genes encoding proteins for trehalose biosynthesis (trehalose-6-phosphate synthase, *AmTPS1* and trehalose-6-P-phospatase *AmTPS2*). When comparing gene expression in extraradical mycelium and mycorrhizas, the transcript levels of both genes weres enhanced in symbiosis. Separate analysis of Hartig net and fungal sheath revealed that *AmTPS1* and *AmTPS* expression is strongly enhanced at the Hartig net, indicating a preferential trehalose biosynthesis at the plant/fungus interface. To get some clues on potential signals regulating *AmTPS1* and *AmTPS* expression in symbiosis, trancript levels of both genes were investigated in hyphae grown at different carbon and nitrogen regimes. While nitrogen had only a little impact, continuous carbohydrate supply (typically found only a the Hartig net) resulted in enhaced *AmTPS1* and *AmTPS* expression. However, sugar-dependent enhancement of *AmTPS1* and *AmTPS2* expression in mycelium grown in liquid culture is smaller than that observed in the Hartig net, indicating the necessity of further signals in symbiosis.

1.2 Regulation of apocarotenoid production in arbuscular mycorrhizal roots

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Colonization by arbuscular mycorrhizal (AM) fungi leads to the accumulation of carotenoid cleavage products (apocarotenoids) in the colonized roots. In some cases the high levels of the accumulating compounds lead to a macroscopically visible yellow colouration of the roots. The corresponding activation of carotenoid biosynthesis appears to be a general response of plant roots to AM fungal colonization. The metabolic precursors for carotenoid biosynthesis (isopentenyldiphosphate and dimethylallyldiphosphate) are produced by the plastid located methylerythritol phosphate (MEP) pathway. We are investigating the regulation of this process by analysing the enzymes catalysing the first two steps of the MEP pathway, 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR). Immunolocalization of DXR in AM roots revealed that only plastids of a specific shape during the late stage of the symbiosis contain this protein. These observations suggest a correlation of apocarotenoid accumulation and arbuscule degradation which is in accordance with data regarding the kinetics and the location of the accumulating compounds. Transcriptional regulation is studied by analysing the promoter of a specific, mycorrhiza-responsive isoform of DXS (DXS2), using promoter and promoter deletion constructs.

1.3 Functional annotation of the *Laccaria bicolor* genome: the cytoskeleton

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Ectomycorrhizas are characterised by the differentiation of two fungal structures, namely the fungal mantle and the Hartig net that plays a key role in the trophic interaction between symbiotic partners. The ontogeny of both structures is characterised by a dramatic modification in hyphal morphogenesis. Upon contact with a compatible host root, hyphae become profusely branched, leading to a pseudoparenchymatous fungal structure at the origin of fungal mantle and Hartig net. Such branching probably results from a reorganisation of hyphal cystoskeleton. At the molecular level, cytoskeleton organisation and maintaining involves the co-operation of numerous proteins which are classified in the \"Cytoskeleton\" KOG category. In the framework of the *Laccaria bicolor* sequencing project, we identified the different genes belonging to this category.

1.4 Regulation of genes encoding enzymes involved in sucrose metabolism in tomato mycorrhizal roots

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Arbuscular mycorrhizal fungi, as obligate symbionts, depend for growth and activity on the supply of carbon compounds by their host plant. In fact, it has been shown that between 4 and 20% of total carbon fixed by the plant is allocated to the fungal partner and that carbon partitioning activity increases in mycorrhizal plants. To get insights into the regulation of host plant carbon metabolism by the development of the symbiosis, we have analysed the expression of a set of tomato genes encoding enzymes involved in carbon metabolism and determined the levels of soluble carbohydrates in non-mycorrhizal and mycorrhizal roots. Two of the five tomato genes encoding putative cell wall invertases, Lin6 and Lin10, were up-regulated in mycorrhizal roots. Development of the symbiosis also induced the expression of the vacuolar invertase TIV1 and the sucrose synthase TOMSSF. The levels of glucose and fructose were lower in mycorrhizal that in non-mycorrhizal roots, indicating consumption of sucrose breakdown products by the colonised roots. Functional analysis of Lin10 in yeast revealed that it does not encode a cell wall invertase but a fructan exohydrolase, an enzyme that has been proposed that might be involved in stabilising the symbiosis between plants and fructan-producing microbes. To get some clues about the mechanisms of the transcriptional activation of TIV1, Lin6, TOMSSF and Lin10 by the symbiosis we are currently analysing their promoter sequences.

1.5 *Medicago truncatula* gene expression specific to arbuscular mycorrhiza interactions

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We selected 14 genes that are exclusively present in cDNA libraries of mycorrhizal roots of *M. truncatula* to analyse their expression during mycorrhizal interactions. Plant origin of the genes was confirmed by PCR on genomic DNA of *M. truncatula* and their expression was monitored in roots inoculated or not with *G. intraradices* or *G. mosseae* in a wild-type plant genotype. None of the genes were expressed in non-inoculated roots. Transcripts of 9 genes were detected in roots inoculated with *G. intraradices* at the stage of appressorium formation and 6 showed common responses to inoculation with *G. mosseae*. At the stage of arbuscule formation, 13 of the *M. truncatula* genes were expressed uniquely in roots colonized by *G. intraradices*, of which 10 were also induced by *G. mosseae*. Most of the genes were inactive in inoculated roots of the *Mtsym13* (Myc-) mutant of *M. truncatula* and only 3 were activated in *Sinorhizobium meliloti*-nodulated roots. None were induced by phosphate fertilization or inoculation of roots with the pathogenic fungus *Fusarium acuminatum*. Gene expression profiles in interactions of wild-type *M. truncatula* roots colonized by five other arbuscular mycorrhizal fungi further confirmed the co-induction of plant genes, pointing to common events in the mycorrhiza-related genetic programme. Plant genes specifically expressed in roots colonized by arbuscular mycorrhizal fungi can provide molecular markers of successful symbiotic interactions.

1.6 The endo-and ecto mycorrhizal symbioses regulate Pi transporters in *Populus*

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Mycorrhizal fungi colonize root systems of most land plants and modulate plant growth by enhancing the availability of nutrients, including Pi by forming extraradical mycelia which operate as functional extensions of the plant root system. Our aim is to decipher the mechanisms that regulate the uptake and distribution of Pi in mycorrhized trees to understand the adaptation of plants (alone or during symbiosis) to Pi starvation. We have identified 12 homologs of high affinity Pi transporters (Pht1) in the genome of Populus trichocarpa, including endomycorrhiza-specific and ectomycorrhiza-induced Pi transporters. We have investigated the tissular expression of this Pht1 family, under different growth conditions, including Pi starvation, endo- and ectomycorrhizal associations. Pi starvation triggered the upregulation of the majority of the members of the Pht1 family; PtrPht1;9 and PtrPht1;11 shared the highest upregulation. We observed a striking upregulation of PtrPht1;9 and PtrPht1;12 in ectomycorhizas., whereas PtrPht1;10 was the only induced in endomycorhizal roots. Identification of fungal signals induced in the ecto and endomycorrhizae regulation of Pi transporters is currently underway.

1.7 Expression analysis of sugar transporters in tomato plants colonized by *Glomus mosseae* or *Glomus intraradices*

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Arbuscular mycorrhizal (AM) fungi, as obligate symbionts, rely on the plant host for the supply of carbon assimilates required for their growth, maintenance and functioning. There is evidence that photoassimilation and subsequent carbon supply to the root system are closely linked to the development of AM fungi in the roots. In order to investigate the effect of carbon fungal drain on photoassimilate partitioning, we have analysed how the expression of genes encoding sugar transporters, key players in carbon allocation in higher plants, is regulated by the development of the symbiosis. The host plant chosen for this study was tomato and the selected AM fungi were *Glomus mosseae* and *Glomus intraradices*, two AM fungi which present a different carbon-sink capacity. Expression of the three sucrose and four monosaccharide transporter genes identified in tomato has been analysed by real-time RT-PCR in roots and leaves of mycorrhizal and non-mycorrhizal plants. Gene expression data will be presented and discussed.

1.8 Genes encoding transcription factors in *Glomus intraradices* and their expression at the appressoria stage of arbuscular mycorrhiza interactions

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Molecular pathways governing the life cycle of arbuscular mycorrhizal (AM) fungi and their interactions with root tissues are not yet fully understood. Most studies of fungal responses to host plants have targeted developmental stages before root contact (germinating spores), or after root colonization (intraradical mycelium). We are focussing on the early cell events of appressoria contact with the root surface. Recent monitoring of *G. intraradices* gene expression at this stage of mycorrhiza interactions has revealed differential fungal responses to roots of host and non-host (Myc- mutants) *M. truncatula* (Seddas et al. submitted), suggesting a fine regulation of fungal genes by the host plant. Transcription factors are central regulators of gene expression which control many physiological and developmental processes but little is known of these elements in AM fungi. We have identified seven sequences in *G. intraradices* with similarity to fungal genes encoding transcription factors, from partial genome sequencing data generated by the *Glomus* sequencing project (DOE, Joint Genome Institute, USA). Transcript profiling (real-time RT-PCR) of the corresponding genes in appressoria formed on roots of wild-type or Myc- mutants of *M. truncatula* points to their differential expression when symbiosis-related plant genes are inactivated, suggesting that the encoded transcription factors could play a role in regulating early events leading to successful mycorrhizal interactions.

1.9 The impact of ectomycorrhiza formation on transcription factor gene expression

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Transcription factors are key elements for the regulation of plant physiology in ectomycorrhizal symbiosis. Transcriptional control could occur at different levels, e.g. protein localization (cytosol versus nucleus) or gene expression. We have focused on the identification of poplar transcription factors that are differentially expressed in fully developed *Populus/Amanita muscaria* ectomycorrhizas. 145 potential transcription factor genes belonging to different gene families (MYB, bZIP, MADS-box, GATA, HMG) were chosen from the annotated genome of *Populus trichocarpa* and investigated for their expression levels in poplar organs and ectomycorrhizas by quantitative RT-PCR. 69 genes were detectable in vegetative poplar organs. 26 transcription factor genes showed similar expression in all tissues, 18 were expressed equally in leaves and stem, 13 were mainly expressed in leaves, 10 reveled highest transcript levels in the stem, and only two genes showed highest expression in roots. The impact of mycorrhiza formation was investigated for those transcription factors that reveled similar transcript levels in all vegetative organs or root-specific gene expression. Two of the genes were down regulated in functional mycorrhizas, while the others were up regulated and ten of these transcription factor genes showed highest expression in mycorrhizas. The impact of ectomycorrhiza formation, plant nutrition and phytohormone exposure on the expression of these genes will be discussed.

SESSION 2

Cell programmes: (2) proteome to metabolome/isoformome

Functional genomics of early interactions in Ectomycorrhizal Symbiosis of poplar x *Laccaria bicolor*

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Analysis of the molecular pathways governing ectomycorrhizal symbiosis have been limited, due to lack of basic knowledge of ECM fungal genomes and difficulty in the appliation of functional genomic tools. *Laccaria bicolor* is a common ectomycorrhizal fungus (ECM) that is a mutualist with many northern temperate forest trees including both hardwood trees such as poplars and softwood trees such as pines and is an important component of the forest ecosystem and forest health. *L. bicolor* is also an important part of mesocosm of *Populus* species, a model tree species. The U.S. DoE Joint Genome Institute and an international consortium has undertaken and completed the sequencing, assembly and primiary annotation the entire 60 Mb genome of the ectomycorrhizal basidiomycete *L. bicolor*. The *L. bicolor* genome contains about 20,000 intron-rich gene structures-more than twice as many as other known fungal genomes. With the availability of genome resources, our lab has been developing and applying functional genomic tools including yeast two-hybrid techniques to study the early interactions in the development of ECM symbiosis. These studies along with others are anticipated to provide further insights into the molecular bases of symbiosis and adaptation and opportunities toward moving mycorrhizal research into the mainstream biotechnology and ecosystem science. The current status of the functional analyses pertaining to ECM symbiosis, will be presented.

Functional genomics for a better understanding of nitrogen nutrition in ectomycorrhizal symbiosis

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Under natural conditions the majority of plants are believed to have mycorrhizal (MYC) associations (intimate symbiotic association between fungi and roots), either with arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) fungi, which can have a significant role in the worldwide nitrogen cycle. This mycorrhizal status relies 1) on efficient uptake processes by the fungal partner 2) on the bidirectional transfer of nutrients between the two symbionts, which was first demonstrated by Melin and Nilsson >50 years ago. However, the identity of the N solutes (organic versus inorganic) taken up and translocated by MYC fungi and the question of whether or not fungal symbionts translocate a significant part of the newly absorbed or formed ammonia has remained unanswered until recently. The sequencing of the *Laccaria bicolor* genome, together with data from other fungal models (*Hebeloma*, *Tuber*, *Paxillus*, *Amanita*) and the use of appropriate functional tools offers an unprecedented opportunity to decipher between the different scenarios. Most particularly, the isolation and characterization of complete sets of genes encoding transporters for inorganic / organic N, and for assimilating enzymes will bring fundamental knowledge that will be used to tackle changes in capacities for N uptake and assimilation under natural conditions.

Starvation stress responses in the mycorrhizal ascomycete *Tuber borchii*: regulatory and structural signatures of the symbiotic lifestyle?

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Symbiosis-specific genes as well as distinctive molecular features of otherwise common gene products are thought to be the two main determinants of the symbiotic lifestyle. Evidence for the co-occurrence of these two evolutionary mechanisms has been obtained from microarray gene expression profiling and follow-up comparative studies in the mycorrhizal ascomycete *Tuber borchii* subjected to N deprivation, an environmental cue that promotes mycorrhization. About one third of the N status-regulated genes have not been described previously and might be unique of symbiotic fungi or mycorrhizal Pezizales. Only two N assimilation components were found among N status-regulated genes. One of them, coding for a high-affinity nitrate transporter, preferentially accumulates in root-contacting hyphae and displays a nitrate-independent mode of regulation that is so far unique of mycorrhizal fungi. The top upregulated and downregulated genes, coding for a phospholipase A2 and for a polypeptide resembling the cyanobacterial lectin cyanovirin-N, are restricted to filamentous ascomycetes, respond to both N and C deprivation and also display remarkably distinctive regulatory (and structural) features compared to their non-symbiotic counterparts. It thus appears that taxon-specific mechanisms based on the implementation of novel molecular traits onto preexisting scaffolds have strongly contributed to the evolution of the symbiotic lifestyle.

Protein profiling analyses in arbuscular mycorrhizal symbiosis

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Because proteins are well known as key effectors of plant responses to environmental cues including recognition, signalling, transport and defence reactions, main interest has been paid to characterize those involved in the establishment and functioning of arbuscular mycorrhizal (AM) symbiosis. The recent development of high throughput techniques has enabled a large-scale analysis of symbiosis-related proteins. Depending on the symbiotic stage targeted and on the abundance of mycorrhizal material, different proteomic strategies have been set up. In mature mycorrhiza, sub-cellular proteomic approaches have been developed in the model legume *Medicago truncatula* to target symbiosis-related membrane proteins eligible as involved in nutrient transport and signalling between symbionts upon arbuscule formation. Modification of the *M. truncatula* root proteome during the early stage of AM symbiosis was also investigated by comparing the protein patterns obtained from non-inoculated roots and roots synchronized for appressorium formation in wild-type (J5), penetration-defective (TRV25, *dmi3*) and autoregulation-defective (TR122, *sunn*) genotypes. Concomitantly, proteomic approaches were developed on *in vitro*-grown mycorrhiza to identify extraradicular fungal proteins along with genuine endomycorrhizins. The future release of the genome sequencing programs launched for *M. truncatula* and *Glomus intraradices* is likely to provide additional knowledge about AM symbiosis-related proteins.

Reorganization of plastid shape and metabolism in root cortical cells colonized by arbuscular mycorrhizal fungi

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Plastids are reacting to the colonization of root cortical cells by arbuscular mycorrhizal (AM) fungi by (i) a marked proliferation leading to network-like organelle structures and (ii) the biosynthesis of AM-specific apocarotenoids of unknown functions. The combination of metabolite and transcript profiling revealed that apart from apocarotenoids, plastids produce higher amounts of fatty acids and amino acids in AM roots. These compounds are apparently necessary for formation of the symbiotic interface, explaining the marked multiplication of lens-shaped plastids close to young arbuscules. Apocarotenoid formation, in contrast, is activated during the later phase of arbuscule degradation. The tubular plastids formed then are in part decorated by rings of the plastid division protein FtsZ and thus possibly involved in repeated fusion/division events. Plastid metabolism, in this phase, is possibly involved in recycling degraded fungal material. Formation of apocarotenoids during arbuscule degradation might be connected to the concomitant accumulation of hydrogen peroxide.

Towards the fluxome - prospects for applying metabolic flux analysis to mycorrhizal systems

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High throughput methods for identifying and quantifying DNA, mRNA, proteins and metabolites give us system-wide information on the cellular infrastructure of life. Experience in other fields shows that this omic revolution generates many important hypotheses and challenges us to test them . For this we must go beyond "parts list" biology and analyze how these parts function. For metabolism this requires quantitative mapping of fluxes through metabolic networks. Advances in experimental, analytical and software tools for metabolic flux analysis now allow maps of the fluxes through central metabolism to be obtained from the results of stable isotope labeling experiments. This has led to notable successes in understanding and engineering metabolic function in bacteria and current studies in plants are showing that these methods are applicable to eukaryotic systems also. The roles of known pathways, and the operation of new ones are revealed and general phenomena such as metabolic efficiency, futile cycles, and compartmentation can be explored. In mycorrhizal studies we are on the threshold of obtaining genomes and other omic datasets are beginning to appear. What then is the value and what are the prospects for metabolic flux analysis in mycorrhizas ? To answer these question I shall present the prerequisites, the tools, and some examples of what we have learned from our current work in plant systems and discuss the path towards applying such analyses to mycorrhizal studies.

2.1 Proteomic approach for evaluating copper stress in *Cannabis sativa* roots mycorrhized and non-mycorrhized by *Glomus intraradices*

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Cannabis sativa has a role, improved by arbuscular-mycorrhizal (AM) symbiosis, in phytoremediation of metal polluted soils, due to its fast growth and capability to accumulate high amounts of metals. A metal which is often found in polluted soils is copper, a plant micronutrient toxic at high concentrations, whose effects have not yet been investigated in hemp. The aim of this work was to investigate the response mechanisms to Cu in *C. sativa* plants grown in absence and in presence of 150 ppm of CuSO4 and of the AM fungus *Glomus intraradices*. To evaluate protein expression pattern, analytical two-dimensional electrophoresis was performed on IPG strips 3-10 and by vertical slab 12% SDS-PAGE; 2-DE gels were Coomassie stained and analysed with the image analysis PD-Quest software, for qualitative and quantitative evaluation of differentially expressed proteins. Since the genome of *C. sativa* is still unsequenced, spots identifications have been carried out by nano-LC (ESI Q-TOF) MS/MS peptide sequencing. Copper stress induced the down-regulation of enolase, thioredoxin peroxidase and glycine rich RNA binding protein; the disappearance of elicitor-inducible protein and 60S ribosomal protein L12; and the up regulation of aldo/keto reductase, putative auxin induced protein and 40S ribosomal protein S20 in copper treated plants. *G. intraradices* symbiosis modulated the response to copper of *C. sativa* proteome, reducing the number of proteins involved in detoxification processes.

2.2 Total lipid and fatty acid accumulation during basidiospore formation in the ectomycorrhizal fungus *Pisolithus* sp

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Basidiospores of *Pisolithus* sp. contain large amounts of lipids, indicating the provision for future germination in the host's rhizosphere. However, the accumulation, composition, and mobilization of lipids during spore formation and germination have remained largely unknown. In this work, the storage of lipids and the compositional analysis of fatty acids during basidiosporogenesis were analyzed in fresh basidiocarps using bright field microscopy and gas chromatography. Fungal basidiomata present abundant lipid bodies in the hyphae, basidia, and basidiospores. This evidences a considerable transport of carbon in the basidiome to meet the carbon demand during basidiospore formation. Fatty acid composition analysis revealed the presence of 24 compounds with chains of 9 to 18 carbon atoms, either saturated or containing one to two insaturations. The composition and content of fatty acids varied according to the developmental stage of the peridioles. In free basidiospores, the predominant compounds were 16:0, 16:1w5c, 18:1w9c, and 18:2w6,9c/18:0ante, at concentrations of 76, 46, 192, and 51 $\mu g\ g^{-1}$ of dry matter, respectively. These results indicate that oleic acid is the major constituent of the lipid reserves in the basidiospores. Further work is being conducted to determine how the mobilization of lipids can be triggered during spore germination. Financial support: FAPEMIG, project CAG 775/03, CAPES, and CNPq.

2.3 Subcellular proteomics of root plastids in response to *Glomus intraradices* colonization of *Medicago truncatula*

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Arbuscular mycorrhizal (AM) symbiosis is a mutualistic association between soil-borne fungi and roots of most plant species. Involving bilateral exchanges between the two partners, this symbiosis is connected to drastic changes in root cell organization such as the formation of an extended plastid network covering the arbuscules. These networks are dynamic structures formed and degraded concomitantly with the formation and degradation of arbuscules. The plastid metabolism is also affected by the AM colonization. For example they start to synthesize secondary (apo)carotenoïds (mycorradicin). Here we will provide data showing that the accumulation of mycorradicin is correlated to the level of colonization of *M. truncatula* root by *Glomus intraradices* . Our results confirm that mycorradicin accumulation begins 4-5 weeks after inoculation i.e., when arbuscules starts to senesce. The highest amount (1.8 nmol g-1 FW) of mycorradicin was reached 6 weeks after inoculation. In order to get further insights into the metabolic changes occuring in the pastids, comparative subcellular proteomic analyses between *G. intraradices*-colonized and non-inoculated roots of *M. truncatula* are undergoing.

2.4 Unravelling a function for the yellow pigment and other apocarotenoids in arbuscular mycorrhizal roots through suppression of biosynthesis

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The colonization of plant roots by AM fungi frequently involves the appearance of a yellow coloration known as the „yellow pigment". The linear C_{14} apocarotenoid (carotenoid cleavage product) chromophor termed mycorradicin accumulates together with other cyclic C_{13} apocarotenoids (glycosylated cyclohexenone derivatives). The function of this phenomenon is still unknown. We employ a knock-down approach (RNA*i*) on biosynthetic enzymes in *Medicago truncatula* to specifically reduce apocarotenoid levels in transgenic roots. Our current target is the mycorrhiza-induced *1-deoxy-D-xylulose 5-phosphate synthase2* isogene (*MtDXS2*). Using an *Agrobacterium rhizogenes*-mediated transformation system of *M. truncatula* seedlings we generated plants with wild type-shoots and transgenic roots. A significant reduction of apocarotenoid levels was achieved in several experiments correlated with suppressed *MtDXS2* transcript levels as compared to empty vector controls. In a nine weeks colonization experiment this reduction correlated with significantly reduced levels of mycorrhization and dramatically reduced levels of functional arbuscules as deduced from *MtPT4* (phosphate transporter) transcript levels. This effect implicating a role of apocarotenoids in maintaining functional arbuscules is dependent on the growth and colonization stage of the plant and on environmental conditions. A new model on apocarotenoid action will be presented.

2.5 Endocellulase activity in pea symbiotic mutants colonized by arbuscular mycorrhizal fungi

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Plant cell wall hydrolytic enzymes seem to be important to root penetration by Arbuscular Mycorrhiza (AM) fungi and development of AM symbiosis. In this study we test the possibility that variations in fungal colonization due to different plant capacities to form AM can be a good experimental system to identify hydrolytic enzymes important to root colonization. Quantitative and qualitative endocellulase activity in roots of different pea mutants altered in their symbiotic abilities were analysed by the viscosity method and polyacrylamide gel electrophoresis enzyme assays respectively. There were differences in root colonization among plant mutants according to their symbiotic features and a positive relationship between fungal colonization and endocellulase activity in roots was also found. The electrophoretic analysis of enzyme activity in roots showed qualitative and quantitative changes in the pattern of bands with endocellulase activity between colonized roots of the different plant mutants and non colonized roots. Results suggest that changes in endocellulase activity in colonized roots is associated with fungal spread within the cortex and arbuscule formation.

2.6 Selection and characterisation of two *Mtsym 13 Medicago truncatula* mutant sub-lines differing in specific coumestans root content

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As flavonoid/isoflavonoid compounds are often involved in plant-microbes interactions, *Medicago truncatula* symbiotic mutant TRV25 (Myc$^-$; Nod$^-$) was assessed for root isoflavonoid content, in comparison to the wild-type J5. We found that some TRV25 plants contained several specific compounds (Cx) with typical UV spectra of coumestans. Cx were absent from the wild-type J5 and from other plants of the genotype TRV25. A secondary mutation, not previously selected, appears to be present on some plants of the TRV25 genotype. In producing descendants of TRV25 plants containing or not Cx we found that all descendants were identical to their parents concerning the root content of Cx. So we have selected two sub-lines of TRV25 genotype, one called TRV25-C with plants containing Cx, the other called TRV25-D with plants in which Cx are absent. Cx were not detected in the shoots. Mycorrhizal inoculation and some abioic elicitors (Cu SO4, UV light, nitrogen nutrition) influence the Cx level. Another interesting feature is that mycorrhizal inoculation has a significant depressive effect on shoot growth of these two sub-lines of TRV25, in culture conditions where the wild-type J5 shoot growth was significantly increased by AM colonisation. The biological role of the specific coumestans and the influence of the mycorrizal inoculation on the growth of two sub-lines TRV25-C and TRV25-D will be discussed.

2.7 Metabolite profiling of arbuscular mycorrhizal roots of *Medicago truncatula/Glomus intraradices*

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Metabolite profiling of primary and secondary metabolites of developing arbuscular mycorrhizal (AM) roots (2-8 weeks) was performed using GC/TOF-MS, LC/ESI-MS and RP-HPLC-DAD to determine relevant changes in the metabolite patterns in comparison to nonmycorrhizal controls with low and high phosphate supply. The time course of mycorrhization determined by staining differs from those of *MtPT4* and *Glomus* rRNA expression. Mycorrhization and high phosphate supply lead to a similar shoot biomass increase, but show different metabolite patterns. Trehalose, palmitvaccenic and vaccenic acids as well as campesterol occur only in AM roots due to its fungal origin. An increase in some amino acids and fatty acids (palmitic, oleic) indicate a general activation of plastid metabolism. Whereas levels of some constitutive isoflavonoids increase in AM roots at later stages, the exclusively fungus-induced apocarotenoids (derivatives of cyclohexenones and mycorradicin, including the "yellow pigment") exhibit a striking time-dependent accumulation pattern. Analyses of cell wall-bound components show the presence of typical phenolics in addition to AM-induced tyrosol. Principal Component Analyses of unpolar primary metabolites (GC/MS) and secondary metabolites (HPLC) separate clearly AM roots from those of controls, an additional grouping in harvest days is given by secondary metabolites. These results are confirmed by Hierarchical Cluster Analysis.

2.8 Membrane proteomics of arbuscular mycorrhiza

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Two sub-cellular proteomic strategies have been developed to extend the identification of symbiosis-related proteins in *Medicago truncatula* roots (1,2) during arbuscular mycorrhizal (AM) symbiosis. The first strategy, directed to extrinsic membrane proteins of M. truncatularoots inoculated or not with *Glomus intraradices*, revealed that 36 spots proteins were differentially-displayed in response to the fungal colonization including 15 proteins newly induced, 3 up-regulated and 18 down-regulated. The vast majority of those identified by mass spectrometry was previously unknown in AM symbiosis (3). The second one was targeted to plasma membrane (PM) root proteins. PM-enriched fractions of control and *G. intraradices*-inoculated roots were submitted to: (i) two-dimensional liquid chromatography coupled to tandem mass spectrometry (2D-LC-MS/MS), (ii) SDS-PAGE separation combined to LC-MS/MS analysis. This allowed to identify 78 proteins, including hydrophobic ones, common to control and AM roots. Interestingly, 2 proteins were only detected in AM roots (4). They corresponded to an H+-ATPase (Mtha1) and a blue copper binding protein (bcp). The expression of the bcp transcripts is investigated during the AM colonization process and under various stress situations References 1) Bestel-Corre et al., 2002. Electrophoresis, 23, 122-137 2) Bestel-Corre et al., 2004. Phytochem, 65, 1651-1659 3) Valot et al., 2005. Plant Mol Biol, 59, 565-580 4) Valot et al., 2006. PROTEOMICS, 6, in press.

SESSION 3

Genetics/genomics of mycorrhizal fungi

The genome sequence of the symbiotic fungus *Laccaria bicolour*

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Ectomycorrhizas have a beneficial impact on plant growth in natural and agroforestry ecosystems. Central to the success of these mutualistic symbioses is the exchange of nutrients between the partners. To elucidate the genetic basis of this ecologically important behavior, the US DOE Joint Genome Institute (JGI) has sequenced the 65-megabase genome of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Agaricales, Tricholomataceae) to high draft using a whole genome shotgun method. This is the first symbiotic fungus genome to be sequenced. It contains about 20,000 intron-rich gene structures-more than twice as many as *Neurospora crassa* and *Phanerochaete chrysosporium*. Analysis of the gene set yields insights into unexpected aspects of *Laccaria* biology including the identification of genes potentially associated with wood decay and soil organic matter. This fungus also possesses an expanded family of G-protein-coupled receptors, several virulence-associated genes and large suites of enzymes involved in transduction pathways, secondary metabolism and cell wall synthesis. Alternatively spliced and altered transcripts are abundant. The genome is rich in transposons belonging to various class I and II families. Comparison of the genomes of the different pathogenic and saprobic fungi with the *Laccaria* genome will be of interest to a wide range of genome and evolutionary scientists. It will provide critical insights into the genetic makeup of plant-fungus interactions.

The genome project for *Glomus intraradices*, a model arbuscular mycorrhizal fungus

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Arbuscular mycorrhizal (AM) fungi colonize the majority of plants modifying nutrient uptake, disease susceptibility, water management and species diversity. The Joint Genome Institute (JGI) is sequencing the genome of *Glomus intraradices*, a representative AM fungus with an estimated genome size of ~16 Mbp. Analysis of preliminary genomic and EST data from New Mexico and JGI reveal a compact, gene-rich genome with short introns and a GC content near ~30%. Simple sequence repeats (SSRs) were identified for use as potential polymorphic markers. Sequence polymorphism was assessed by clustering of single pass ESTs into 410 composite cDNAs. No sequence polymorphism was observed for 75% of the clusters. Sequence analysis identified putative genes acting primary and secondary metabolism, transcription factors, signaling proteins, splicing factors, mitochondrial functions, and transporters. There is no evidence for a sexual cycle in AM fungi, yet putative orthologs to meiosis-specific fungal genes were present in the *G. intraradices* sequence data. Expression patterns for general and meiotic recombination pathways were determined by Q-PCR using putative *G. intraradices* orthologs to the meiosis-specific recombinase, Dmc1, and Rad51 from the general recombination pathway. While both genes were expressed, a very large increase in GiDmc1 expression was observed during early spore germination, providing a developmental window for further analysis of the pathway.

Meiosis and post-meiotic mitosis during basidiospore formation in the ectomycorrhizal fungus *Pisolithus* sp.

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A complete understanding of the main stages of basidiospore formation in *Pisolithus* sp. is fundamental for the elucidation of the factors that influence spore germination. In this work, the events involved in basidiosporogenesis, meiosis, and post-meiotic mitosis were analyzed using fluorescence and scanning electron microscopy. The basidia are formed by the differentiation of cells along the hyphae of peridioles. *Pisolithus* sp. produces eight basidiospores per basidium imbibed in a gelatinous matrix that constitutes the basidiome. Karyogamy can take place in the basidia as well as in sub-basidial or sub-apical cells, followed by the typical events of meiotic division. After the formation of the meiotic tetrad, one round of mitotic division occurs, resulting in the production of eight nuclei inside the basidia. The newly-formed nuclei migrate into basidiospores asynchronously, resulting in the production of eight nucleate spores per basidium. These results indicate that the low germination percentages reported in the literature for *Pisolithus* sp. spores is not related to a lack of nuclei in the basidiospores. During the migration events, the haploid nucleus acquires a fusiforme shape, suggesting the participation of microtubules in the transport of this organelle into the spore. This work is the first report on the nuclear division during basidiosporogenesis in *Pisolithus* spp. Financial support: FAPEMIG, project CAG 775/03, CAPES, and CNPq.

A genomic approach to the understanding of *Gigaspora margarita* and *Candidatus* Glomeribacter gigasporarum association

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Symbiotic associations between endocellular bacteria and eukaryotic cells are widespread among animals and plants, but only a few examples have been described in the fungal kingdom. Arbuscular mycorrhizal species, belonging to the family Gigasporaceae, represent a specialized niche for rod shaped bacteria, recently named *Candidatus* Glomeribacter gigasporarum. Morphological and molecular studies on Gigaspora margarita BEG34 have shown that *Ca.* G. gigasporarum is an homogeneous population closely related to the ,-proteobacterial genus *Burkholderia*. A genomic library was constructed from *G. margarita* BEG34 spores into the fosmid vector pCC1Fos. The library contains ~36K clones with an average insert size of 37 kb. Fungal (GC 53.8%) and bacterial (GC 56.7%) clones were sorted using database sequence homology searches and then validated by PCR experiments. Analysis of 140 clones has shown that the endosymbiont genome comprises ~50% of the library. Nineteen clones have been validated by PCR and only ~40 non-overlapping inserts are needed to cover the 1.4 MB small genome of *Ca.* Glomeribacter gigasporarum. Sequencing efforts are now focused on clones showing high similarity with Burkholderiaceae genomic sequences. Early annotation of some bacterial clones has led to the identification of the complete division cell wall (*dcw*) cluster and other genes involved in the Vitamin B12 synthesis pathway.

Evolutionary genomics of the ectomycorrhizal fungus *Paxillus involutus*

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It is well known that ectomycorrhizal (ECM) fungi can differ markedly in their ability to form mycorrhizae and to promote the growth of the host plant. Generally such phenotypic differences could be the result of variations in gene content, quantitative differences in gene expression, and structural differences in gene products. The DNA microarray technology has open up new possibilities of comparing transcript abundance, and the presence of divergent and conservative genes in closely related organisms. We used such approach to compare the trancriptome and genomes of strains of *Paxillus involutus*. The analyses included Nau, that is not compatible with birch and poplar, and the two compatible strains Maj and ATCC200175. The array contained reporters for 1075 putative unique genes in *P. involutus*, derived from a collection of expressed sequence tags (ESTs). On genomic level, Nau and Maj were very similar. Only 16 out of 1,075 genes analyzed by microarray-based hybridizations had signals indicating differences in gene copy numbers. In contrast, 66 out of the 1,075 genes were differentially expressed in Maj compared to Nau after contact with birch roots. Thirty-seven of these symbiosis-regulated genes were also differentially expressed in the ATCC strain. Comparative analysis of DNA sequences of the symbiosis-regulated genes showed that two of them have evolved at an enhanced rate in Nau due to relaxed or positive selection.

Exploiting *Glomus intraradices* sequencing data to dissect molecular mechanisms of plant genome control over fungal gene expression in mycorrhiza

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Plant-fungus compatibility in arbuscular mycorrhiza must result from finely tuned interactions between genomes of the symbiotic partners. To gain insight into the role of the plant genome in mycorrhiza establishment, we are investigating whether symbiosis-related plant genes regulate fungal gene expression at the appressorium stage of root interactions. *Glomus intraradices* genes potentially active in mycorrhiza were identified in EST libraries by *in silico* expression analyses. Transcripts of a sub-set of these genes, implicated in transcription, protein synthesis, primary/secondary metabolism or of unknown function, were monitored in appressoria formed on roots of wild-type and mycorrhiza-defective (Myc^{-}) *Medicago truncatula*. Inactivation of plant genes in the Myc^{-} mutants altered *G. intraradices* gene expression, providing first evidence that cell processes modified by symbiosis-related plant genes impact on root interactions by directly modulating AM fungal activity. To further analyse the molecular basis of this phenomenon, initial partial data generated by the *Glomus* sequencing project (DOE, Joint Genome Institute, USA) was exploited to identify fungal genes encoding proteins with key functions in gene regulation and signal recognition pathways (transcription factors, protein kinases). Candidates will be presented that could play a role in regulating molecular mechanisms by which arbuscular mycorrhizal fungi differentiate functional appressoria on contact with host roots.

3.1 The genome sequence of the symbiotic fungus *Laccaria bicolor*: complete sets of genes for mobilization, transport and assimilation of nitrogen

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Under natural conditions the majority of plants are believed to have mycorrhizal (MYC) associations (intimate symbiotic association between fungi and roots), either with arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) fungi, which can have a significant role in the worldwide nitrogen cycle. This mycorrhizal status relies 1) on efficient uptake processes by the fungal partner 2) on the bidirectional transfer of nutrients between the two symbionts, which was first demonstrated by Melin and Nilsson >50 years ago. However, the identity of the N solutes (organic versus inorganic) taken up and translocated by MYC fungi and the question of whether or not fungal symbionts translocate a significant part of the newly absorbed or formed ammonia has remained unanswered until recently. The sequencing of the *Laccaria bicolor* genome and the use of appropriate functional tools offers an unprecedented opportunity to decipher between the different scenarios. Most particularly, the isolation and characterization of complete sets of genes encoding enzymes involved in N mobilization, for transporters for inorganic / organic N, and for assimilating enzymes will bring fundamental knowledge that will be used to tackle changes in capacities for N uptake and assimilation under natural conditions. In addition, comparative genomics using genome data from saprophytic or pathogenic fungi will allow us to identify genetic potential of the different fungal life styles.

3.2 Do AMF harbour meiosis-specific genes in their genome?

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The mutualistic arbuscular mycorrhizal fungi (AMF) are the only fungal phylum thought to be asexual. The genes involved in meiosis, mitosis and recombination are well known in other fungal organisms but no sequence information has been provided so far for AMF. By surveying EST and gene databases of the AMF *Glomus intraradices* we identified short sequences of several genes that that could play a role in meiosis, mitosis and recombination. We increased the sampling of these genes in Glomus intraradices by designing several sets of degenerate primers for these genes. Consequently, we successfully isolated several genes of putative high importance in meiosis, mitosis and recombination from *Glomus intraradices*. This approach allowed us to identify the presence of gene families and events of gene duplications, as well as to identify the nature of selective forces that act on these genes in this putatively ancient asexual organism.

3.3 Potassium transporter and potassium channel of the ectomycorrhizal fungus *Hebeloma cylindrosporum* are candidates for symbiotic nutrient exchange

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The basidiomycete *Hebeloma cylindrosporum* has been chosen as a model species for developing molecular analyses of solute transport in ectomycorrhizal symbiosis. A cDNA library was prepared in a yeast expression vector allowing gene cloning by complementation of yeast mutants. About 4000 ESTs were obtained, enabling the identification of a set of membrane transporters for *e.g.* Pi, K^+, sugar, amino acids or micronutrients. This library provides a valuable tool for research on ectomycorrhizal fungi and symbiotic interactions (Lambilliotte *et al.*, 2004, *New Phytol.* 164, 505-513; http://urgi.infobiogen.fr///Projects/GPiDB/Interface/). Interested in plant K^+ nutrition, we have identified a K^+ transporter, homologous to the yeast TRK transporter, *HcTRK*, and an ion channel from the Shaker family of voltage-dependent K^+ channels, *HcSKC*. Analyses of the expression patterns (Northern blot and RT-PCR analyses) will be presented as well as characterization of functional properties (in heterologous expression systems) in order to progress in the elucidation of the roles of these fungal transport systems. The identification of these two types of K^+ transport systems is promising concerning the functions of the two specialized fungal membranes, the hyphal membrane in contact with the soil mediating nutrient uptake and the mycorrhizal membrane in contact with root cortical cells allowing exchange of nutrients.

3.4 Characterization and expression of *heh*, the *Tuber melanosporum* homolog of the *het-e* heterokaryon incompatibility gene of *Podospora anserina*

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Tuber melanosporum is a black truffle of high economic value. Very little is know on its life cycle as a result of the lack of procedures to produce fruiting bodies in controlled conditions. Nothing is know about the sexual and vegetative compatibility mechanisms responsible for nuclear exchange between the different populations. Sequencing of randomly amplified genomic fragments of *T. melanosporum* identified a 600 bp-fragment showing a significant sequence similarity with *Podospora anserina* HET-E proteins with are involved in vegetative incompatibility system in this species. Using genome walking and RACE techniques we have cloned the full-length sequence of the homologous gene (*heh*) of *T. melanosporum*. Sequence similarity among *heh* and *het-e* protein products was higher in the N-terminal part of the protein and decrease toward the C-terminal where WD40 repeat domains are located. In this region, *heh* contains different putative introns of 66-78 bp. Analyses of the related cDNA clones obtained from mycelium of a *T. melanosporum* strain Tme4 (isolate collection of CMI-UNIBO) showed different splicing of these introns. The transcripts of the *heh* gene were detected in fruiting bodies, but not in mycorrhizas obtained with the same fungal strain. The heh locus was found not to be polymorphic in 20 fruitbodies of *T. melanosporum* collected in different areas of France and Italy.

3.5 Development of microsatellite markers in mycorrhizal fungi and applications in molecular ecology

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Simple sequence repeat (SSR) markers were developed in several mycorrhizal fungi using two strategies (database searches and cloning techniques). Because, very little DNA sequence information is available in molecular databases, we mostly used enriched genomic libraries to develop SSR markers. EST-SSR markers were developed for *Hebeloma* spp.from data mining and anonymous SSR markers were developed from SSR-enriched genome libraries for seven other mycorrhizal fungi (*Laccaria bicolor*, *P. involutus*, *P. tinctorius*, *R. vinicolor*, *S. tomentosus*, *A. byssoides* and *G. intraradices*). In particular, six SSR markers were found to be both unambiguously scorable and polymorphic among 12 *H. cylindrosporum* isolates and two SSR markers were transferable to other *Hebeloma* species. Seven SSR markers developed from SSR-enriched genome libraries for the basidiomycete *Laccaria bicolor* were further characterized using an array of 15 *L. bicolor* strains representative of diverse origins worldwide. The seven SSR loci could be amplified from DNA extracted from root tips of *L. bicolor* inoculated conifer seedlings. All the *L. bicolor* ectomycorrhizas analysed exhibited the same SSR multi-locus profile as that detected for the UAMH8232 inoculant strain. The set of markers were used to monitor the persistence of the *L. bicolor* strain UAMH8232 introduced into two clearcut sites in northern Alberta, Canada, 5 years after outplanting. Primers are being designed and tested for other mycorrhizal fungi.

3.6 Studying the behavior of nuclei in the arbuscular mycorrhizal fungus *Glomus intraradices*

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The coenocytic mycelium and chlamydospores of arbuscular mycorrhizal fungi are populated by hundreds of nuclei. The behavior of these nuclei during a life cycle is not very well understood. Models which aim at describing the genomic organisation of arbuscular mycorrhizal fungi lack data about nuclear flow. In the present study, we described the development of an *in vitro* system which allows live observation of nuclei during the life cycle of an arbuscular mycorrhizal fungus *Glomus intraradices*. This system will be a potent tool to describe the nuclear events during the spore formation and germination, as well as to study the nuclear behavior during anastomoses between hyphae emanating from different spores of *G. intraradices*.

3.7 Variation in the ITS region of rDNA assessed by PCR/RFLP in a collection of ectomycorrhizal fungi from Iberian Peninsula

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Variability of internal transcribed spacer (ITS) of ribosomal DNA (rDNA) assessed by restriction fragment length polymorphism (RFLP) analysis has been widely used as a species molecular marker in ectomycorrhizal communities. For identification of ectomycorrhizas this approach depends on the availability of a database with endonuclease restriction patterns of identified mycelial cultures or sporocarps. This work aims to develop a database of ITS/RFLP patterns of ectomycorrhizal symbionts in the Iberian Peninsula. In total 138 species from 34 genera were studied. ITS region was PCR amplified with the primers ITS1 and ITS4 and RFLP analysis was performed with Cfo I, Hinf I and Mbo I endonucleases. These enzymes were able to discriminate most of the fungal species, but not forms or varieties of the same species. Intraspecific variability of the ITS region was low (3%) and may represent the existence of cryptic species (eg. *Paxillus involutus* and *Amanita gemmata*). A database of ITS-RFLP patterns of described ectomycorrhizas was also constructed. This database has actually molecular data of 108 ectomycorrhizal morphotypes of Castanea sativa (35), Quercus suber (48) and Pinus pinaster (25), from which 82 remains unidentified. By comparison of results with other published data, different restriction patterns were obtained in some species with one or more endonucleases, providing further evidence that these databases must be constructed at a regional scale.

3.8 A fungal gene encoding a protease is essential for the establishment of the ectomycorrhizal symbiosis *Hebeloma cylindrosporum* /*Pinus pinaster*

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Ectomycorrhizal fungi live in symbiotic association with the roots of numerous tree species. This symbiosis plays a key role in forest ecosystems as it improves host plant growth, nutrition and fitness. In the aim to elucidate the molecular basis underlying the establishment of ectomycorrhizas, we transformed haploid mycelium of *Hebeloma cylindrosporum* via *Agrobacterium tumefaciens*. A library of 2,000 hygromycin-resistant monokaryotic transformants was generated and screened for symbiosis defects, using *Pinus pinaster* seedlings as host plants. Eighteen transformants displaying a dramatically reduced mycorrhizal ability were identified. Among them, 3 were found non-mycorrhizal (myc-). The molecular and genetical analyses of these myc- mutants is underway. One of them, 551C1, shows single T-DNA integration 375 bp upstream a 3,7 kb long Open Reading Frame encoding a protease. As a consequence, no transcript could be detected in the mutant strain grown in pure culture using RT-PCR analysis. The N-terminal sequence of the deduced protein contains a signal peptide that targets the protein to the extracellular matrix. This extracellular location suggests that the enzyme may be an important feature in the interaction between *H. cylindrosporum* and the plant. Current efforts are made to elucidate the role of this protein in the molecular cross talk between the partners of the ectomyccorhizal symbiosis.

3.9 The effects of sterol biosynthesis inhibitors fungicides on gene expression in arbuscular mycorrhizal fungi

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Fungicides belonging to sterol biosynthesis inhibitors (SBI) are the major class used in agriculture for the control of fungal diseases. Soil accumulation of fungicides could have detrimental effects on beneficial soil fungi such as arbuscular mycorrhizal fungi (AMF). Unfortunately, little is known about sterol metabolism in AMF and the impact of SBI fungicides on this group of fungi. As partner of an European project, our goal is to investigate the influence of SBI on a specific aspect of AMF biology, that is regulation of gene expression. It is well known from yeast transcriptome analyses that SBI have a deep impact on expression profiles not only of genes related to the sterol pathway but also of genes associated to several functional categories. As a targeted approach we have been focusing on the identification of genes involved in the sterol biosynthetic pathway (ERG genes). No homologs have been found searching Glomeromycota databases. Taking advantage of the availability of several fungal ERG sequences, degenerate oligonucleotides have been designed and used in PCR amplifications on *Glomus intraradices* DNA. PCR products of the expected size have been cloned and recombinant clones are currently under study. In parallel, genes whose transcripts are modulated by SBI exposure will be characterized by SSH cDNA libraries from *G. intraradices* grown on root organ cultures. The research is funded by the EU project FUNGIMYC (MEST-CT-2004-514213).

3.10 The genome sequence of the symbiotic fungus *Laccaria bicolor*: Lipid metabolism

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Tree species dominating forest ecosystems in boreal, temperate and montane regions develop symbiotic associations with soil fungi, so-called ectomycorrhizas. Central to the success of these mutualistic symbioses is the exchange of nutrients between the partners. To elucidate the genetic basis of this ecologically important behavior, the US Department of Energy Joint Genome Institute (JGI) has sequenced the 65-megabase genome of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Agaricales, Tricholomataceae) to high draft using a whole genome shotgun method. This is the first symbiotic fungus genome to be sequenced. It contains about 20,000 intron-rich gene structure. Analysis of the gene by the JGI and Genome Annotation Consortium yields insights into unexpected aspects of *Laccaria* biology. We have annotated genes involved in lipid metabolism and assessed the changes in fatty acid profiles in *Laccaria* mycelium. We found striking differences between *L. bicolor* and other fungi, including gene structure, clustering and compartmentation of gene coding for fatty acid synthesis and degradation of fatty acids via the β-oxidation cycle. The expression of these lipid metabolism genes in different *Laccaria* tissues will be discussed.

3.11 Discrimination of *Tuber aestivum*, *T. borchii* and *T. melanosporum* by multiplex PCR

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Different *Tuber* species are present in Sardinia but some of them are unmarketable. A molecular method based on multiplex PCR was developed for distinguish three marketable *Tuber* species, *T. aestivum*, *T. borchii* and *T. melanosporum*, from the unmarketable ones, *T. asa*, *T. excavatum*, *T. panniferum*, *T. puberulum*, *T. rufum* fo. *lucidum* and *T. rufum* fo. *nitidum*, simultaneously. For this purpose four specific primers were designed on the basis of the beta-tubulin gene sequence: three of them specific for *T. aestivum*, *T. melanosporum* and *T. borchii* and one common to the same three species. Characteristic fragments were obtained for *T. aestivum* (537 bp), *T. melanosporum* (419 bp) and *T. borchii* (288 bp), whereas no amplification was obtained for the unmarketable species. Moreover, this method allowed us to discriminate the marketable species from the unmarketable ones independently from the group they belong to (black truffles; white truffles). The use of the β-tubulin gene was due to the fact that the ITS regions, already used by other authors, did not allow us to find primers suitable for distinguish *T. borchii* from *T. asa*. Further experiments should be carried out in order to apply this rapid and reliable method to food products and so developing a useful tool to ascertain food quality and detect any fraud.

3.12 Identification of *Glomus intraradices* strains in commercial inocula substrates by using molecular biological methods

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Arbuscular mycorrhizal fungi (AM fungi) influence positively the development of many plants, and plant physiology in general. Over the past years, in many countries some AM fungal species are reproduced and included as the microbiological component in commercial inocula. These contain either one or several fungal species in different sources of substrates. Such inocula are currently used and applied in horticulture, agriculture and for re-cultivation. One of the most commonly used AM fungal species is *Glomus intraradices*. It was assumed for this study that not all commercial inocula started with the same isolate of *G. intraradices*, and thus, there are differences between the strains. Very little molecular data are available about AM fungal species present in commercial inocula. For this reason *G. intraradices* strains isolated from different commercial substrates were assayed using molecular biology methods like PCR, RFLP and sequencing of rDNA fragments. DNA was isolated from spores and intra-radical root structures of some commercial *G. intraradices* and characterized control strains. Approximately 1000 bp fragments were amplified by using *G. intraradices* specific primers. The RFLP analysis and sequence-data of these PCR fragments allowed the characterization of the strains and an identification of strain specific parameters. Thus, the used methods may enable to perform characterisation and identification of commercial *G. intraradices* strains from various inocula substrates.

3.13 Genetic and nutritional control of the arbuscular mycorrhizal symbiosis

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The establishment of the arbuscular mycorrhizal (AM) symbiosis requires mutual recognition of the symbiotic partners, and later the coordination of their development. Recently, several genes have been cloned which are involved in recognition and signal transduction in the AM symbiosis, as well as in the nodule symbiosis. However, the AM-specific upstream and downstream events remain elusive. We have identified several petunia mutants with defects in the development of the symbiosis. The Cathy mutant is affected at an early point of the interaction, possibly initial recognition. The arbuscule1 (arb1) mutant is affected in the initiation and morphogenesis of the arbuscules. For detailed phenotypic analysis, these mutants are stabilized by crossing with a line that harbors an inactice copy of the master transposon (act-). Besides the genetic makeup of the plant, the nutrient status of the plant influences the outcome of the interaction. Under high levels of phosphate (when the plant does not profit from the fungus), fungal colonization is suppressed. This suppression is observed only if plants are not limited in other nutrients, especially macronutrients. We further explore the influence of different nutrients on the development of the interaction.

SESSION 4

Signals and perception

Chemical identification of signalling molecules in symbiotic interactions between plants and arbuscular mycorrhizal fungi

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The plant-AM fungus interaction is initiated by mutual signal exchange between the two partners during preinfection stages. Host roots release signalling molecules called "branching factors (BFs)" that induce extensive hyphal branching in AM fungi. AM fungi have long been postulated to produce signalling molecules called "Myc factors (MFs)" that induce the molecular and cellular responses leading to successful root colonization by AM fungi. Neither of these signals had been isolated and chemically identified. Recently, we isolated a BF from the root exudates of the model legume *Lotus japonicus*, and identified it as a strigolactone, 5-deoxy-strigol. Strigolactones are a group of sesquiterpene lactones, previously isolated as seed germination stimulants for the root parasitic weeds *Striga* and *Orobanche*. Natural strigolactones, 5-deoxy-strigol, sorgolactone, strigol, and orobanchol as well as a synthetic analogue GR24 induced extensive hyphal branching in germinating spores of *Gigaspora margarita*. It now turns out that the same compounds are detected by beneficial fungal symbionts and devastating parasitic weeds as host-derived signals. Some solid evidence has been presented for AM fungal production of a long-hypothesized symbiotic signal, the MF. Having finished to identify the plant signals, we are now trying to isolate and identify MFs by using the transgenic *L. japonicus* T90B plants carrying AM-inducible $\mathit{Pro}_{\mathit{LjCbp1}}\mathit{:GUS}$.

A transient increase in cytosolic calcium is triggered in host plant cells by diffusible molecules released by AM fungi

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The implication of calcium as intracellular messenger in the arbuscular mycorrhizal (AM) symbiosis has not yet been directly demonstrated, although often envisaged. We used soybean cell cultures stably expressing the Ca^{2+} bioluminescent indicator aequorin to detect intracellular Ca^{2+} changes in response to the culture media of spores of *Gigaspora margarita* and other AM fungi germinating in the absence of the plant partner. Rapid and transient elevations in cytosolic free Ca^{2+} were recorded, indicating that diffusible molecules released by endomycorrhizal fungi are perceived by host plant cells through a Ca^{2+}-mediated signalling. The fungal molecules active in generating the Ca^{2+} transient were constitutively released in the medium, and the induced Ca^{2+} signature was not modified by the co-culture of germinating spores with plant cells. Even ungerminated spores were able to generate the signalling molecules, as proven when the germination was blocked by a low temperature. The lack of induction of a Ca^{2+} response by the fungal culture medium in aequorin-transformed cells of the non-host plant *Arabidopsis thaliana* suggests the specificity of such an early signal to the AM symbiosis.

Fungal responses to strigolactones: from presymbiotic to symbiotic stage?

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During establishment of endomycorrhizal symbiosis, AM fungi and their host plant exchange chemical signals. The chemical nature of a family of plant root signals has been recently revealed (Akiyama et al. 2005). These molecules are called strigolactones. They were known for a long time for their role in seed germination of parasitic plants such as witchweed and broomrape. The biosynthetic pathway of these molecules is still not deciphered, but there are good evidences that strigolactones derive from the carotenoid pathway (Matusova et al. 2005). The fungal response to strigolactones during the presymbiotic stage is characterized by a stimulation of spore germination and hyphal proliferation (Akiyama et al. 2005, Besserer et al. 2006). This response is preceded by an increase of respiration accompanied by a change of mitochondrial density, shape and motility (Besserer et al. 2006). First transcriptomic analysis on *Gigaspora rosea* spores stimulated by a strigolactone analogue (GR24) reveal that not only mitochondrial biogenesis is induced, but also lipid catabolism. These early responses indicate that during the pre-symbiotic stage, metabolism of germinating spores is switched to engage the fungus toward further growth and root infection. An important question remains to know the role that strigolactones play during the symbiotic stage.

Apocarotenoid accumulation and reorganization of plastids in arbuscular mycorrhizal roots

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Plant root-colonizing arbuscular mycorrhizal (AM) fungi activate the methylerythritol phosphate (MEP) pathway, carotenoid biosynthesis and oxidative carotenoid cleavage in roots, leading to C13 and C14 apocarotenoids, i.e. cyclohexenone and mycorradicin derivatives. Mycorradicin causes the characteristic yellow coloration of AM roots accumulating within a complex mixture of unknown components in vacuolar droplets. The cyclohexenone derivatives differ in ring substitutions and in the nature and degree of glycosylation. In molecular studies it was found that the transcript levels of two enzymes of the MEP pathway (1-deoxy-D-xylulose 5-phosphate synthase 2, DXS2, and 1-deoxy-D-xylulose 5-phosphate reductoisomerase, DXR), and of the carotenoid pathway (phytoene desaturase and zeta-carotene desaturase), are markedly increased in AM roots, along with that of a carotenoid cleaving dioxygenase. AM-induced expression of these genes correlates with proliferation and reorganization of root plastids, accompanied by the formation of FtsZ division rings. Most of these plastids are connected to each other, developing large networks based on plastid tubular extensions covering the arbuscules. Expression of DXS2-RNAi constructs in transgenic roots results in a dramatic decrease of the amount of accumulating apocarotenoids, which correlates with a marked decrease of transcripts of the phosphate transporter MtPT4 and reduction of functional arbuscules in certain stages of plant development.

Recognition events in AM fungi during symbiosis establishment

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Gin1 a unique two-domain protein from arbuscular mycorrhizal fungi was found in a differential screening for transcripts induced during early symbiosis. The protein has a self-splicing domain at the C-terminus homologue to the C-terminal domain (Hint) of animal hedgehog proteins. The Hint domain of metazoan proteins releases the mature N-terminal domain after attaching a cholesterol moiety to the carboxy end. This lipid modification determines the range of action of the protein and the interaction with its receptor at the cell membrane. In vitro analyses have showed that Gin1-C is also able to undergo splicing. To determine which molecule provokes the splicing in vivo, splicing assays with cholesterol and lipid extracts from spores, external mycelium and mycorrhizal roots were performed. Only sterol extracts from mycorrhizal roots were able to induce splicing. The N-terminus of GIN1 shares similarity with the GTP binding protein family (IAN) evolutionary conserved from plants to humans. They are related to the control of cell growth upon pathogen interaction. In vitro experiments with recombinant Gin1-N showed that the protein has ATPase activity rather than GTPase, and immunolocalization experiments have shown that Gin1 is at the plasma membrane. Our hypothesis is that GmGIN1-N covalently modified by a plant sterol controls fungal cell growth through its ATPase activity possibly by interacting with other proteins at the plasma membrane.

Studies on cell-to-cell communication in the ectomycorrhizal fungus *Pisolithus tinctorius*

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Plants and mycorrhyzal fungi have developed a successful symbiosis due in part to their highly coordinated gene expression. In order to coordinate in space and time the expression of the set of genes involved in the progression and establishment of such interaction, an active biochemical signaling mechanism would be necessary. In this sense, not only water, minerals and nitrogen would be transported in the symbiotic interface; but also low and high molecular weight signalling molecules could be also exchanged. Trangenic *Pisolithus tinctorius* was obtained by means of biolistic and *A. tumefaciens*-mediated genetic transformation. Genetic evidences will be presented on the stable genetic transformation as well on the expression of reporter genes such as GFP and BADH. GFP was observed to be expressed in a stable manner in saprophytic cells, mainly in aerial hyphae. Immunodetection of the expressed proteins in mycorrhyzed *Pinus*, theoretically synthesized in the transgenic fungus, suggests a limited movement to cortical cells. In parallel studies, a fungal two-component system was found to mediate the sensing of plant secondary metabolites. This gene encodes a hybrid protein and is turned on in the early stages of interaction between the symbionts. Rodriguez-Tovar et al., 2005. Stable genetic transformation of Pisolithus tinctorius. Journal of Microb. Methods 63:45-54. Tagu D. et al. 2002. The ectomycorrhizal symbiosis: genetics and development. Plant Soil 2002 244, 424-429.

4.1 *MtENOD11* gene regulation: a valuable tool to study mechanisms of infection-related endosymbiotic signalling

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The *Medicago truncatula ENOD11* gene, encoding an atypical repetitive Pro-rich extracellular protein, is expressed during early infection stages of both the AM and rhizobial endosymbiotic associations (Journet et al. 2001 MPMI 14, 737-48). Promoter analysis has recently revealed that the same promoter region (containing a critical AT-rich motif) is sufficient for tissue-specific gene expression during AM root colonisation and rhizobial infection, thus providing strong evidence that gene regulatory mechanisms have been conserved (Boisson-Dernier et al. 2005 MPMI 18, 1269-76). By making use of an *in vivo* fluorescent (GFP) fusion we have further shown that *MtENOD11* gene expression both precedes and accompanies the formation of the Pre-Penetration Apparatus (PPA). The PPA is a novel cytoskeletal/ER structure that is formed in the *M. truncatula* epidermis after appressorium formation and prior to fungal infection (Genre et al. 2005 Plant Cell 17, 3489-99). Furthermore, *MtENOD11* gene activation and PPA formation are abolished in *DMI* mutants (Chabaud et al. 2002 New Phytol. 156, 265-73; Genre et al. 2005), indicating that host signal transduction *via* a pathway involving DMI proteins (and probably also Ca2+ signalling) must occur after appressorium formation but before *ENOD11* activation and PPA formation in the epidermis. These combined findings will be discussed in relation to current theories of endosymbiotic signalling mechanisms.

4.2 Strigolactones-induced genes in *Gigaspora rosea*

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A branching factor activating presymbiotic growth of AM fungi was previously isolated from root exudates. It was found to induce mitochondrial activity of the fungus (Tamasloukht et al. 2003 Plant Physiol. 131: 1468-78). Strigolactones are root-exuded compounds, recently found to stimulate germination and presymbiotic stage of AM fungi (Akiyama et al. 2005, Nature 435: 824-827; Besserer et al. 2006, PLOS Biology in press). GR24, a strigolactone analogue, rapidly induces (within one hour) mitochondrial biogenesis in the fungus at concentration as low as 10-11 M. We are currently using a transcriptomic approach in order to further investigate the mechanisms by which AM fungi respond to strigolactones. A SSH library was obtained from stimulated spores of *Gigaspora rosea*. The differentially expressed clones obtained were spotted on macroarrays and hybridized with labelled complex probe. After sequencing, several putative unigenes were pooled by functional category. The most interesting candidates will be selected for validation of expression by QRT-PCR experiments. We expect several genes involved in energetic metabolism to appear overexpressed, in agreement with the previous data on mitochondria activation. We plan to examine whether the expression pattern of some of these fungal genes occurs also in planta during the symbiotic stage.

4.3 Arbuscular mycorrhizal fungi are able to colonize the roots of an abscisic acid-deficient tomato mutant

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Increased levels of endogenous abscisic acid (ABA) have been reported from various plant species in response to infection with fungi, including arbuscular mycorrhizal fungi (AMF). Low endogenous levels of this phytohormone have been associated with enhanced resistance to pathogens. However, the involvement of ABA in the regulation of the arbuscular mycorrhizal symbiosis is still not studied. Previous works in our laboratory have shown that the ABA-deficient tomato mutant *sitiens* displays more resistance to different foliar pathogenic fungi as compared to its wild type cv. Moneymaker. In the present work, we used *sitiens* and its wild type to investigate the interactions with 2 species of AMF: *Glomus intraradices* and *Glomus mosseae*. Five weeks after seedlings were transplanted into pots containing inoculum, root colonization was evaluated. In both *sitiens* and wild type roots, *G. intraradices* and *G. mosseae* formed apparently normal arbuscules and vesicles and no differences in the percentage of colonization were observed. The results indicate that no strong defence response capable of inhibiting intraradical colonization is activated in *sitiens*. In addition, it seems that increased levels of endogenous ABA in tomato plants may not be necessary for the development of the arbuscular mycorrhizal symbiosis.

4.4 How essential are strigolactones in the arbuscular mycorrhizal symbiosis?

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Strigolactones are molecules derived from the carotenoid biosynthesis found in plant root exudates and known to be seed germination stimulants of parasitic plants such as Striga and Orobanche. It was recently shown that these molecules are also extremely active on arbuscular mycorrhizal (AM) fungi during the germination stage (Akiyama et al. 2005, Nature 435: 824-827; Besserer et al. 2006, PLOS Biology in press). In order to determine whether these molecules are essential signal molecules for host recognition in the AM symbiosis, we carried out mycorrhizal experiments with maize, a plant known to produce two strigolactones (strigol and sorgolactone), treated or not with fluridone, an inhibitor of the carotenoid biosynthesis. We showed that the fluridone-treated plants grown in pot culture and inoculated with *Gigaspora rosea* were 66% less colonized than the control plants and that the addition of the strigolactone analogue GR24 in the fluridone-treated plants could restore normal mycorrhization. These data are presently being confirmed using maize mutants affected in the carotenoid metabolism. They provide the first experimental evidence that strigolactones play an important role in the establishment of the AM symbiosis.

4.5 Two-component signal transduction in ectomycorrhizal fungus *Pisolithus tinctorius*

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Little is known about the molecules regulating the interaction between plants and ectomycorrhizal fungi during root colonization. Symbiosis-related genes have been identified in various mycorrhizal associations (1). Like other plant-microbe symbiosis, ectomycorrhizal association must involve exchange of signals between the partners in the earliest step. The study of mutants obtained by genetic transformation will help to understand the role of specific gene products (2). Plant root exudates could be sensed by the fungus in pre-colonization process during mycorrhiza formation. We propose the participation of the two-component system as transduction patway in the pre-contact step. We obtained a fragment (2.0 kb) of a novel *P. tinctorius* gene encoding a two-component signaling protein (*Pthik1*). The histidine kinase domain and a response regulator domain are similar to those found in bacterial and fungi members of the two-component family of proteins. RT-PCR technique was used to detect the over expression of *HKPt* gene in the presence of the low weight plant phenolic compound, glycerol and zeatine. In contrast, the gene is not induced with NaCl and Sorbitol; this analysis indicates the possibility that the two-component system pathway might be operating during mycorrhiza formation, sensing plant compounds, and no in osmosensing response. 1 Tagu D. et al. 2002. PS 244, 424-429 2 Rodríguez-Tovar A. et al. 2005. JMM 63 (1), 45-54.

4.6 Activation of sucrose efflux by constitutive expression of *PRms* gene in tobacco plants improves mycorrhizal infection

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We study Arbuscular Mycorrhizal formation (AM) in transgenic tobacco plants transformed with the *PRms* gene of maize. Overexpression of *PRms* in tobacco promotes growth and enhanced resistance to fungal pathogens. Furthermore, activation of sucrose efflux and sucrose accumulation in leaf tissue together with constitutive expression of endogenous tobacco PR genes are characteristic features of PRms tobacco plants. Using two independent transgenic tobacco lines in two different experiments with *Glomus intraradices*, we clearly show that overexpression of *PRms* gene enhanced mycorrhizal colonization in roots. The increase in mycorrhizal colonization was correlated with enhancement in both arbuscules intensity and frequency of arbuscules with alkaline phosphatase activity in roots. No significant changes in plant regulator defence molecules that affect mycorrhization, such as Ethylene of Salicylic acid were found between mycorrhizal wild type and transgenic PRms plants. *PRms* alters partitioning and distribution of assimilates of a plant and these effects have profound consequences in arbuscular mycorrhizal formation.

4.7 Tryptophan dimer as a signal for arbuscular mycorrhizal fungi in bahiagrass roots under water stress conditions

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We made an attempt on the identification of peptides as growth stimulants for arbuscular mycorrhizal (AM) fungi in bahiagrass roots by using a flash chromatograph and an isoelectric focusing apparatus (Rotofor, Bio–Rad Laboratories). Some of the peptides which stimulated the growth of *Gigaspora margarita* and *Glomus* R–10 were identified. In particular, tryptophan dimer was abundantly accumulated in bahiagrass roots and released from the roots under water stress conditions, although it was not detected in the roots under normal conditions. This peptide strongly attracted the hyphae of *Gigaspora margarita* and promoted its hyphal growth. The other peptides had no effects on this attraction, but significantly stimulated the hyphal growth. Therefore, these results suggest that low molecular peptides such as tryptophan dimer would play important roles as signals for AM fungi in stressed roots.

4.8 Regulation and interaction of signaling genes in *Laccaria bicolor x Populus tremuloides* ectomycorrhizal symbiosis

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Understanding the early events of ectomycorrhizal (ECM) symbiosis is one of the least explored events. The regulation of signaling genes, which are activated during early interaction, is critical for recognition between the fungus and the plant roots and successful formation of mycorrhiza. Previous studies from our lab on ectomycorrhizal fungus *Laccaria bicolor* indicated that small proteins like RAS are regulated by symbiotic process and can play an important role by interacting with other signaling molecules. We used ECM model system *L. bicolor* (S238N) and *Populus tremuloides* to better understand the early process of ECM symbiosis. Using yeast two-hybrid system we have identified several genes belonging to G-proteins and their interactions as well as other proteins that interact with signaling gene proteins and are symbiosis-regulated. qRT-PCR expression studies confirmed the symbiosis-regulated temporal expression of these signaling genes during interaction of *L. bicolor* with roots of *P. tremuloides*. Our results suggested differential expression of various signaling genes, which could be categorized into early (6–48 hrs of interaction) and late expressing (48-96 hrs of interaction) genes. Their expression pattern at different time points suggest possible intracellular signaling cascade, which may regulate the process of mycorrhization. Keywords: Laccaria bicolor, Populus tremuloides, symbiosis, G-proteins RAS, qRT- PCR.

4.9 Expression pattern of *SCARECROW* gene in *Pinus sylvestris* mycorrhizal and NPA-treated short roots

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Pinus sylvestris has a root system consisting of three morphologically and anatomically different root types. The primary root has an undetermined capacity for continuous growth, whereas the lateral roots have a somewhat limited and the short roots a limited ability to elongate. For staying alive they have to be colonized by a symbiotic fungus. In *Arabidopsis* several genes encoding transcription factors regulating the differentiation of the root radial patterning have been isolated. Among the most central ones is *SCARECROW (SCR)* gene encoding a transcription factor of the GRAS family. SCR is necessary for the periclinal division of a cell close to the quiescent center (QC) of the root. After the periclinal division one of the daughter cells gives origin to endodermis and the other to cortex. The expression pattern of *SCR* has not yet been very extensively investigated in other plants than *Arabidopsis*. In the present work we cloned *PsySCR1* gene from *P. sylvestris* and followed its expression in different root types by *in situ hybridization* also when short root morphology was modified by the treatment with auxin transport inhibitor NPA. Visualization of the *PsySCR* transcripts in endodermis and in QC of different root types contributed for understanding of the differences in growth patterns of main, lateral and short roots. It also illuminated the structural features of short roots that make them preferential hosts for ectomycorrhizal fungi.

4.10 Soybean shoot suppresses the arbuscule formation of arbuscular mycorrhizal fungi

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Roots of soybean establish symbiosis with nodule-inducing rhizobia and AM fungi. The existing nodules systemically suppress subsequent nodule formation, a phenomenon termed autoregulation. Grafting experiment revealed that some forms of autoregulation are controlled by the shoot. We have studied the AM colonization of hypernodulating soybean mutants, which are defective in the autoregulation and found that the mutants had higher degree of arbuscule abundance than the wild types. This study examined the involvement of autoregulation in the arbuscule formation of AM fungi in the roots of soybean by means of reciprocal grafting technique. Soybean cv. Enrei (WT) and its hypernodulating mutant, En6500 were used in this study. Ten-day-old seedlings were cut at the hypocotyls and the shoots were grafted to self or reciprocal roots. Grafted seedlings were inoculated with *Bradyrhizobium japonicum* and *Gigaspora rosea* and grown in a glass house. Soybean grafts were harvested 60 days later and determined their development of AM colonization. The arbuscule abundance of En6500/En6500 graft was 1.5 times higher than that of WT/WT graft. In grafts between WT and En6500, an increased arbuscule abundance was expressed only when the latter was used as the shoot. The arbuscule abundance of WT/En6500 graft was comparable to that of WT/WT. From the results obtained, we suggest that soybean shoot contributes to the suppression of arbuscule formation.

4.11 Maize mutants affected at distinct stages of the arbuscular mycorrhizal symbiosis

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Macroscopic screening of maize lines carrying a high number of Mutator (*Mu*) transposable elements has lead to the isolation a number of mutants affected in the formation of arbuscular mycorrhizal (AM) symbiosis. A total of seven lines were isolated that demonstrated either reduced or enhanced colonization by the fungus *Glomus mosseae*. Three of these lines, representing three broad phenotypic classes, have been characterized in more detail (Paszkowski, Jakovleva and Boller, 2006). A program of genetic crossing has been initiated to stabilize the phenotypes of all seven mutants with the aim of providing material for subsequent cloning efforts. Initial attempts to isolate *Mu* flanking sequences from mutant lines have been based on MuTAIL-PCR. In this technique, a nested asymmetric PCR protocol is used to amplify sequences (~200-4000bp) adjacent to the point of *Mu* element insertion. Given the high copy number of *Mu* elements present in lines used for mutant identification, MuTAIL-PCR generates a population of flanking sequences. Depending on the nature of the material involved, in silico analyses of flanking sequences can narrow down the list of potential causative candidates. Ultimately, analysis of a segregating mutant population is required to determine the linkage of a given insertion event to a mutant phenotype. The efficiency of MuTAIL-PCR with this material will be discussed and the nature of candidate flanking sequences reported.

4.12 Arbuscular mycorrhizal fungal growth in presence of apigenin and apigenin glycoside

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The effect of apigenin and its glycosidated form 5,7,4´-hydroxy flavone glycoside on different arbuscular mycorrhizal (AM) fungal growth parameters e.g. spore germination, hyphal growth, hyphal branching, and root colonization of the AM fungi *Gigaspora rosea*, *Gi. margarita*, *G. mosseae* and *G. intraradices* was tested. Depending on the concentration apigenin exhibited a stimulatory or no effect on different fungal parameters, whereas in none of the treatments with the apigenin- glycoside any effect on AM fungi could be observed. Our data show that apigenin exhibits an AM fungal genus and even species activity and we provide strong evidence that glycosidation results in a loss of its activity towards AM fungi.

4.13 Chemotropism of *Gigaspora rosea* hyphae by *Rhodotorula mucilaginosa* exudates

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Chemotropism has been shown to be important in fungal contact with plant root. Exudates from roots and microorganisms play an important role in the attraction of arbuscular mycorrhizal (AM) fungi to the plant. Soil yeasts benefits AM fungi mainly through its exudates. Exudates of *Rhodotorula mucilaginosa* increased the hyphal length of *Gi. rosea* spores. The highest hyphal growth of *Gigaspora rosea* was observed in presence of exudates of *R. mucilaginosa* from 24 to 42 h of yeast culture. Some of these beneficial substances can be attributed to the rhodoturolic acid (RA) produced by the yeast. RA was detected only in culture of *R. mucilaginosa* after 24 h of culture. Higher attraction and growth of *Gi. rosea* hyphae in presence of RA or exudates from *R. mucilaginosa* was observed. The hyphal length of *Gi. rosea* spores in presence of exudates from *R. mucilaginosa* was strongly correlated with the hyphal length of the spores in presence of RA. Our results indicate that RA can be involved in the chemotropism of AM fungi.

4.14 Suppressive subtractive hybridization to detect plant genes expression during early stages of arbuscular mycorrhizal (AM) symbiosis

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The transcriptome profile of arbuscular mycorrhizal (AM) roots has been recently investigated in several plants, thank to the availability of a large number of EST collections and sequence data. However, informations concerning the early stages of the AM symbiosis are still limited, mainly because a strict timing control and a phenotipic marker are needed for a precise sampling, in order to avoid the dilution of specific transcripts. To investigate the gene expression of host cells during the initial contact with AM fungi, we used *in vitro* cultures of *Medicago truncatula* GFP:HDEL-transformed roots inoculated with *Gigaspora margarita*. The system allowed the direct confocal microscopy observation of cell responses and the formation of the Pre-Penetration Apparatus. We built a SSH library subtracting cDNA from *M. truncatula* root pieces contacted by *G. margarita* with cDNA from the corresponding control roots. ESTs of up-regulated genes were cloned and sequenced leading to the identification to 114 putative symbiosis-induced genes. A sequence subset, selected by dot-blot reverse northern screening, was real-time RT PCR analyzed in order to verify their relation to early events of symbiotic interactions compared to later stage (48 hrs post appressorium formation) of fungal infection. Transcript accumulation of selected genes in the mycorrhiza-defective *dmi3* mutant of *M. truncatula*, allowed us to position their expression in the signal transduction pathway leading to the symbiosis.

4.15 The systemin–jasmonic acid pathway modulates mycorrhizal colonization in tomato plants (*Lycopersicon esculentum*) inoculated with *Glomus intraradices*

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The role of systemin (S)-jasmonic acid (JA) pathway in the modulation of defense responses has been thoroughly documented. In contrast, the mechanisms trough which JA regulates mycorrhizal symbiosis are largely unknown. In this study, we have observed that roots of prosystemin over-expressing tomato plants (PS) inoculated with *Glomus intraradices* (Gi) consistently have more arbuscules than wild type (WT) and mutant spr-1 plants. The latter is unable to generate a systemin-related signal acting upstream JA synthesis. On the other hand, the number of arbuscules that develop in the roots of spr-2 mutant plants, which don't produce JA, is significantly reduced. Our results support the notion that JA production in tomato is essential for arbuscule abundance. Considering, that arbuscule number was not different in spr-1 and WT plants, it may be possible that JA generated from S-independent signals could be sufficient to promote arbuscule proliferation. Next, analyses were performed in order to test whether the S-JA pathway could influence carbohydrate metabolism in the mycorrhizal symbiosis. Our results showed that the expression of a wound inducible invertase (Wiv-1) was expressed exclusively in leaves of PS plants, although its expression was not altered by Gi colonization. These results suggest that carbohydrate metabolism is modified in plants where the pathway is constitutively activated, which could increase the sink strength and, indirectly, contribute to arbuscule formation.

SESSION 5

Evolution and phylogeny of mycorrhizal fungi

Implications of nr28S gene phylogeny for systematics of Glomeromycota

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The most recent classification of fungi in Glomeromycota is based solely on nr18S DNA sequences. The nr28S gene, which contains significantly more nucleotide variation, was used to test the validity of this classification. A 720-780bp long 5' region of the gene was sequenced from 68 species of the INVAM collection. Sequences of 12 of these were compared to those from Genbank to test for polymorphisms. Six additional species were included from Genbank sequences. Trees were generated by neighbor-joining, maximum parsimony, and Bayesian analyses. Three well-supported clades are delimited in *Glomus*, with one (Clade B) more ancestral than previously recognized. *Entrophospora* appears to be polyphyletic, and morphological explanations support this conclusion. Morphological and 28S sequence data are mostly congruent at the species level, facilitating interpretations of species boundaries. The clade classified as Diversisporales is poorly supported. Morphological, biochemical, and ecological traits also contradict validity of this order and these conflicts will be discussed. The lack of congruence suggests that nrRNA genes do not contain sufficient phylogenetic information to elucidate evolutionary patterns at the family and order levels. Efforts in our lab and elsewhere to explore single copy protein-encoding genes undoubtedly will provide further clarification.

New molecular marker genes for arbuscular mycorrhizal fungi and their applications

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Molecular markers are an indispensable tool to analyze phylogeny and evolutionary ecology of arbuscular mycorrhizal fungi (AMF). Besides nuclear-encoded ribosomal RNA, which still offers the broadest taxon sample, other genes have become available. Many of them show considerable intra-isolate and intra-spore variability which complicates using them to distinguish closely-related AMF taxa. In contrast to the nuclear rRNA genes, the mitochondrial large RNA subunit is homogeneous within AMF isolates tested so far. Nevertheless, the variable regions of this gene, in particular the introns, allow to efficiently distinguish morphospecies of *Glomus*. Some isolates of *Glomus intraradices* differ in several base positions of the exons, as well as in the number of introns and their nucleotide sequence. As specific PCR primers are available to amplify these gene regions from colonized roots, direct studies of the fine-scale genetic structure of symbiotically-active field communities and populations of *Glomus* have become feasible. The possible use of other new molecular markers like the rpb1 gene for ecological studies and phylogenetic analyses will be discussed.

Serial speciation and morphological stasis in little brown boletes

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Species delimitation within *Xerocomus* (Boletales) has been vague due to wide phenotypic variability. We have compiled ITS data from numerous representatives of putative taxa and recognised ITS-species using phylogenetic methods. Morphological and ecological characters were then sought that delimit these groupings. This approach has proved very successful and, in general, the observed ITS-species correspond very well with published morphological entities. Having established that the ITS is an excellent specific marker, we have pursued questions of geographic origin, host specificity and conspecificity between continents. Within Europe, *X. subtomentosus* harbours striking heterogeneity within the ITS, even within single individuals, and there is some evidence of geographic races. In contrast, the sister taxon *X. ferrugineus* is characterised by extreme homogeneity of the ITS. In spite of this, there is clear morphological support for host-related ecotypes. European names have been applied to fungal taxa worldwide, including *Xerocomus* that bear a superficial resemblance to the described European taxa. An analysis of the *X. subtomentosus* complex in Europe and N. America has identified 4 and 6 species, respectively, but with no overlap. The majority of these taxa are morphologically indistinguishable, suggesting that morphological stasis has occurred. Overall, ITS variation demonstrates serial speciation within *Xerocomus* at different levels related to geological history and ecology.

Phylogeny and recombination in the ectomycorrhizal fungus *Paxillus involutus* as revealed by gene genealogies of seven nuclear loci

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Paxillus involutus expresses an unusually broad host range, including both conifers and broad leaf plants, with variation between individual fungal strains. It is therefore a frequently used model organism in ectomycorrhizal studies on host specificity. The molecular phylogeny of *P. involutus*, using sequencing of the ITS-region of the r-DNA, shows a separation into two subgroups. One group is mainly found in parks and gardens and the other is mainly found in forests. To further characterize the genetic separation between these two groups, we have analyzed the sequence variation in regions of seven nuclear loci including *actA, betatubA, hydA, rabA, gpiA, cchA* and *lecA*. Previous studies using microarray-based comparative genomic hybridizations have shown that these genes display different levels of variation within the clade of *P. involutus*. Possible barriers of gene flow and recombination between the "Park" and "Forest" strains will be identified by comparing the gene genealogies of the seven nuclear genes.

Phylogeny and ecology of the Sebacinales: an overview

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The only recently described heterobasidiomycetous order Sebacinales has gained particular interest during the past years since molecular and ultrastructural studies demonstrated that members of this group are involved in a uniquely wide spectrum of mycorrhizal types. It has also been shown in *in vitro* experiments using the sebacinalean anamorphic strain *Piriformospora indica* that the interaction of Sebacinales with plant roots can significantly enhance growth, seed production and resistance against fungal pathogens in a phylogenetically wide range of host plants. Molecular phylogenetic studies suggest that Sebacinales branched off early within the Hymenomycetidae; more basal basidiomycetous groups contain plant parasites and mycoparasites but lack mycorrhizal taxa according to present knowledge. Sebacinales are divided in two distinct subgroups (informally designated as subgroups A and B) that correlate with the mycorrhizal types in which their members are involved. Basidiome-forming members are known only from group A. Teleomorphic individuals of group B have all been assigned to the *Sebacina vermifera* species complex. Molecular data suggest that the biodiversity within the Sebacinales is huge and that the known sequence types in this group are the very tip of the iceberg. Since there are only few morphological characters to clearly distinguish species within the Sebacinales, the percentage of cryptic species in this group may be very high.

Genetic exchange and experimental genetics in AMF: Evolutionary consequences

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Given the importance of arbuscular mycorrhizal (AM) fungi for the growth and ecology of plants, we know remarkably little about their basic genetics and genome organization. Furthermore, AM fungi form a phylum that diverged from other fungi a long time ago (400-1000 million years ago), allowing many possibilities for the evolution of a different genome organization and different genetic system to that seen in other fungi. AMF are thought to be entirely asexual and not exchange DNA with other AMF individuals. Recent studies on genome organization in AM fungi, coupled with studies of AM fungal population genetics and vegetative compatibility provide us with some important clues about the genetics of AM fungi. I will present how a population genetic approach can help us to understand genetic exchange processes among genetically different AMF individuals and how our recent findings on genetic exchange among AMF, and its consequences on the AMF phenotype, lays a foundation for experimentally studying AM fungal genetics. Finally, I will give an evolutionary ecology interpretation of why such unusual genome organization should be stable in underground hyphal networks of these important symbiotic fungi.

5.1 Sampling the P-Type II ATPase gene family in the *Glomeromycota* reveals the presence of macromolecular changes in a population of *Glomus intraradices*

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The P-type II ATPase gene family encodes proteins with an important role in adaptation of the cell to variation in external K+, Ca2+ and Na+ concentrations. Members of this gene family have been sampled in all of the fungal phyla except the arbuscular mycorrhizal fungi (AMF; *Glomeromycota*), which are known to play a key-role in terrestrial ecosystems and to be genetically highly variable within populations. Here we used highly degenerate primers on AMF genomic DNA to increase the sampling of fungal P-Type II ATPases. In parallel, homologous sequences of the P-type II ATPases have been used to determine the amount of polymorphism that is present at these loci among isolates of *Glomus intraradices* harvested from the same field. Here we show that, contrary to previous predictions, P-type IIC ATPases are present in all basal fungal taxa and that P-Type IIE ATPases should no longer be considered as exclusive to higher fungi. A comparison of homologous sequences encoding P-type IID ATPases showed unexpectedly that indel mutations among coding regions, as well as specific gene duplications occur among AMF individuals within the same field. On the basis of these results we suggest that the diversification of P-Type IIC and E ATPases followed the diversification of the extant fungal phyla with independent events of gene gains and losses. The consequences of macromolecular changes for the ecology and evolution of AMF are discussed.

5.2 Out of Africa? – Investigating the relationship between and among northern hemispheric and African *Lactarius* species

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Based on current ideas on the character evolution within the Russulaceae, the russulaceous flora of sub-Saharan tropical Africa is considered to be particularly rich in taxa that have retained primitive characters. Accordingly, the hypothesis was put forward that the Russulaceae developed together with the ancestors of the dipterocarps and caesalpinoids in Gondwana, possibly in the palaeotropics, and became only secondarily associated with the Fagales and Pinaceae in the northern hemisphere. Molecular phylogenetic analyses of ITS, LSU and *RBP2* data from *Lactarius* spp. (mostly of European and tropical African origin), were carried out to investigate the infrageneric relationships within the genus and to test the above-mentioned ideas on the origin of the Russulaceae with view to the genus *Lactarius*. The answer to the question of the origin of the genus is not straightforward. The results largely support the major entities of the currently accepted classification, which is entirely based on morphology. Support for the monophyly of the genus is very weak, though a single origin of the majority of European species (subgenera *Piperites* and *Russularia*) is strongly suggested. In general, relationships between tropical African species and groups of northern hemispheric taxa tend to be distant, indicating long separation times.

5.3 Cryptic species within the Desert truffle *T. boudieri* complex

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The ITS region as well as the 5' end of the 28S rRNA gene of hypogeous *Pezizaceae* and *Tuberaceae* underwent phylogenetic analyses using the neighbor-joining (NJ) and maximal parsimony (MP) methods. This analysis has corroborated the earlier discovery of three internal transcribed spacer (ITS) types in *Terfezia boudieri* isolates and emphasized the divergence of type 2 from types 1 and 3. Additional study describes the application of molecular tools to further investigate genetic polymorphism in this species, assisted by an examination of physiological traits. The markers used include sequences taken from, a *chitin synthase* partial sequence, a *α-tubulin* partial sequence and amplified fragment length polymorphism (AFLP)-based markers. The markers divide the isolates according to their ITS types. Over 100 fruit bodies, among which each ITS type was represented by at least 30 specimens, were tested with each of the markers. Two physiological traits examined, mycelial outgrowth and mycorrhiza formation also supported the division. The results presented support the division of *T. boudieri* into at least two cryptic-species.

5.4 Ultrastructure of spore development in *Scutellospora heterogama*

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The ultrastructural detail of spore wall development in *Scutellospora heterogama* is described. Although the main ontogenetic events are similar to those described from light microscopy, the complexity of wall layering is more complex than shown by light microscopy. The basic concept of an outer rigid spore wall enclosing two inner flexible walls still holds true, but there are additional zones within these three layers distinguishable at the ultrastructural level. For example, the outer spore wall has three layers rather than the two reported previously: an outer, thin ornamented layer and an inner, thicker layer both derived from the hyphal wall and present at all stages of development. A third inner layer develops during spore swelling and is the first true new spore wall layer. The two inner flexible wall layers are also complex and the ultrastructural observations reveal a distinct, membranous, striate layer within the outer layer of the inner spore wall. The second inner layer develops at least three sub-layers, one of which has an appearance similar to that of the expanding layer of the outer spore wall. The details of germination shield formation show that this occurs at an early stage and is an integral part of the formation of the inner wall groups. Keywords *Scutellospora*, spore ultrastructure, arbuscular mycorrhizal fungi, germination shield, Glomales.

5.5 Molecular variations of the genus *Astraeus*

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The purpose of this work was to present a preliminary assessment of the phylogenetic relationships in the genus *Astraeus* by means of sequencing the ITS1-5.8S- ITS2 region and combining morphological and molecular data. Collections of *Astraeus* basidiomes were made from different geographical areas in Thailand and compared with existing collections made worldwide. The marriage of observations on morphological features, including basidiospore ornamentation and molecular data indicated the probability of the existence of several species. Sequences for 41 *Astraeus* collections were compared and the phylogenetic analysis separated Thai *Astraeus* collections into 2 distinct groups. One contained the recently described, *A. odoratus* (Syn. *A. thailandicus*) and an Asian species called herein *A. asiaticus*. The existence of 2 additional species:- *A. pteridis* subgroup, and one so far un-named were demonstrated from North America. Evidence supported that molecular data can be used in combination with traditional morphological characteristics to resolve taxonomic uncertainties in the genus *Astraeus*. It is now apparent that the genus is far more complex than originally expected.

5.6 A special type of ectendomycorrhiza with Sebacinales as the dominant mycorrhizal fungi formed by members of the Andean clade of Ericaceae

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A hotspot of Ericaceae is located in the tropical, montane regions of Ecuador between 1800 and 3300 m a.s.l. Ericads of the monophyletic 'Andean clade' occur exclusively in the Andes and in some parts of Central America. Most species of the Andean clade grow as epiphytes or hemiepiphytes in the primary and secondary forest, whereas most other ericaceous species are terrestrial and grow in areas exposed to sunlight. Ericads form various specific mycorrhizal types: ericoid, arbutoid and monotropoid mycorrhizas. We studied the mycorrhizas of *Cavendishia nobilis* var. *capitata*, a member of the Andean clade, in the tropical mountain rain forest of southern Ecuador. Our microscopical and ultrastructural investigations showed the formation of a hyphal sheath, intercellular penetration of fine hyphae and intracellular colonization of the cortical cells by swollen hyphae of the same fungus. Direct sequencing of the associated fungi revealed members of the Sebacinales (Basidiomycota) as the most frequent associated fungi. Molecular phylogenetic analysis detected seven new clades of Sebacinales subgroup B. This type of mycorrhiza was found by us in 15 other species of the Andean clade and appears to be restricted to members of this clade. We suggest to name the new type „cavendishoid mycorrhiza" in order to separate it from the arbutoid type which is another, distinct ectendomycorrhiza.

5.7 Finding a mycorrhizal fungus for reintroductions of the threatened terrestrial orchid *Diuris fragrantissima*

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Australian terrestrial orchids rely on associations with suitable mycorrhizal fungi for in situ seed germination and establishment, an important prerequisite for self sustaining populations. Finding an appropriate mycorrhizal fungus is therefore imperative to successful reintroductions. Reintroductions have been planned to conserve the terrestrial orchid *Diuris fragrantissima*, which is Critically Endangered in Victoria, Australia, having been reduced to less than 25 plants at a single site. This study investigated the presence of a suitable mycorrhizal partner for *Diuris fragrantissima in situ, ex situ* and from closely related species, for use in reintroductions. Six hundred seed baits were placed at three original sites of *D. fragrantissima* but did not recover a single germinant. Twenty-two fungi isolated from *D. punctata, D. dendrobioides* and *D. chryseopsis*, and ten fungi isolated from *D. fragrantissima* in *ex situ* collection were used in germination trials with seed of *D. fragrantissima*. Three isolates initiated germination, including fungi isolated from 'asymbiotic' *ex situ D. fragrantissima*. Germination rates were always below 30%. Fungal isolates were identified by direct sequencing of the nuclear internal transcribed spacer and large subunit regions of DNA. All isolates were closely related to *Tulasnella calospora*. Evolutionary relationships between fungi and their orchid hosts across Victoria are discussed.

SESSION 6

Population biology of mycorrhizal fungi

Population genetics and life cycle of the ectomycorrhizal fungus *Tuber magnatum*

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Thanks to their organoleptic properties, truffles are fungi appreciated worldwide. Their quality and market price depend on the species and, traditionally, the place of origin. Genetic variability is generally thought to be very limited in the finest truffle species *T. melanosporum* and *T. magnatum*. Also, the current model for the truffle life cycle has a very closed mating system, with homothallism or even exclusive selfing as precursor to sexual reproduction. However, by using highly informative SSR (simple sequence repeats) markers and an extensive sampling we have recently demonstrated for the first time the existence in *T. magnatum* of a phylogeographic structure and of an extensive gene flow within local populations. Additionally, we have compared the SSR allelic configurations of samples from vegetative (gleba) and sexual (ascospores) portions of single ascocarps with those of the resulting ectomycorrhizae to provide direct evidence that *T. magnatum* outcrosses and that its life cycle is predominantly haploid. Our results led us to deeply reinterpret the life cycle of *T. magnatum* and all the other truffle spp. and to urge a significant reconsideration of existing data on truffle population genetic studies as well as of strategies concerning truffle growth management and marketing.

Local environmental conditions affects diversity and genetic structure in the ectomycorrhizal fungi *Tricholoma populinum* and *Tricholoma scalpturatum*

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The balance between clonal propagation (driven by mycelial growth) and sexual reproduction (driven by spore dispersal) affects establishment dynamics and colonization patterns of ectomycorrhizal fungi, and ultimately the evolutionary potential of populations. In this study, we analyze the genetic diversity and population structure of two species of *Tricholoma*, that were fruiting during the same period in two black poplar stands with different level of abiotic disturbance. We asked whether there are differences in diversity and colonization patterns of *T. populinum* (a poplar specialist) and *T. scalpturatum* (a generalist species), and how population structure and dynamics are affected by local environmental conditions. Sporophores of both species were sampled over several years. Genets were identified using RAPDs, ISSRs and rDNA IGS-RFLPs. Populations of *T. populinum* had small short-lived genets and a few large perennial genets represented by numerous sporophores, particularly in the non-disturbed site. In contrast, a large proportion of the observed genets in *T. scalpturatum* were represented by a unique sporophore, suggesting colonization by spores. Genet numbers and sizes strongly differ between the two study sites for each species, perhaps as a result of abiotic disturbance on mycelial establishment.

Prospects of parentage analysis in ectomycorrhizal fungi

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Parentage analysis has become a popular tool for many plant and animal studies, but has so far been rarely used in fungi. One of the powerful promises that parentage analysis holds for fungi is that it can trace paths of spore dispersal and establishment, even if the direction cannot readily be determined in most basidiomycetes. I have used 6 and 7 medium polymorphic microsatellite markers (average expected heterozygosities ~0.4) and Goodnight & Queller\'s likelihood ratio test to identify probable parent/offspring pairs in *Rhizopogon vinicolor* and *Rhizopogon vesiculosus* (Boletales, Basidiomycota) collected from three intensively sampled plots in the Oregon Coast Range. Across both species, 1012 and 568 genet pairs could not be excluded as possible parent/offspring pairs, but only 7 and 4 had a significantly (alpha = 0.001) higher likelihood of being related as parent and offspring than being unrelated. Of these, only two *R. vinicolor* genets were still supported as parent and offspring (alpha = 0.05) when tested against the alternative hypothesis of being full siblings; they were located approx. 45 m and 28 m apart from each other. Methods and challenges of parentage analysis in basidiomycetes will be discussed.

Species concepts and the inference of cryptic species and recombination in the putative asexual fungus *Cenococcum geophilum*

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Recent studies of the putative sexual fungus *Cenococcum geophilum (Cg)* have found that three divergent lineages, or cryptic species, of *Cg* may co-occur on the spatial scale of a single soil sample. Potential recombination in this fungus was analyzed by sequencing 10 loci from 44 isolates within one lineage that could represent two phylogenetic species. Recombination was investigated using phylogenetic and multilocus disequilibrium analyses (MDA). Phylogenetic incongruence between the loci was consistent with recombination. However, virtually all of the incongruence was caused by an apparent recombination event within the actin locus. Additional sequence data from this locus were obtained from potential parental and recombinant sequence types. Recombination analyses using six different methods detected probable intragenic recombination only in the actin locus. However, extreme actin sequence divergence suggests either; i) an ancient recombination event between ancestral *Cg* lineages or, ii) more recent horizontal inheritance during a parasexual event within the *Cg* species complex or another unrelated species. MDA was consistent with recombination only when isolates from each subclade were treated as distinct species, whether the actin locus was included in the data set or not. These results demonstrate that one's species concept and type of analysis have a significant impact on the interpretation of population genetic data.

A population genetic approach to uncover the reproductive mode of *Glomus etunicatum*

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Population genetic approaches have been proven in the past to be powerful tools to uncover the reproductive mode of various (mostly pathogenic) fungi of which the sexual stage was otherwise unknown. Based on the absence of obvious sexual reproductive structures both in contemporary and fossil arbuscular mycorrhizal fungi, these fungi are assumed to represent a group of ancient asexual organisms. We take a sequence-based approach to test the hypothesis that the mode of reproduction of *Glomus etunicatum* is clonal. We are exploring the population structure of globally distributed isolates of *G. etunicatum* with a particular focus on two intensely sampled populations from California, USA. We used 11 anonymous markers, various protein coding loci and ribosomal markers. Our preliminary results indicate that, while the detectable variation contains phylogeographic signal, most loci are extremely conserved on a global scale. We will discuss how these observations contribute to the understanding of the *G. etunicatum* reproductive mode.

Maintenance of the arbuscular mycorrhizal mutualism

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The processes that maintain mutualism within the plant-mycorrhizal fungal interaction has remained obscure. The degradation of the plant-mycorrhizal mutualism would be predicted as a consequence of costs the fungus endures in delivering phosphorus to its host. Consistent with this expectation, we have found that the benefit that plants receive from their mycorrhizal fungal community decreases over time, indicating that the best growth promoting fungi are the poorest competitors. Partner choice has been suggested as a mechanism to prevent this degradation of mutualism, however the potential for partner choice within AM fungi seems unlikely given the low specificity of association between plants and fungi. We used a carbon labelling experiment to demonstrate preferential allocation by the plant of carbon resources to the better AM fungal mutualist. Theory predicts that this preferential allocation could offset the competitive advantage of poorer mutualists. We demonstrate that this preferential allocation can prevent the degradation of the mycorrhizal mutualism provided there is an appropriate level of spatial structure within the soil.

6.1 Genetic diversity within and among European populations of the ectomycorrhizal fungal species *Tricholoma scalpturatum*

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Tricholoma scalpturatum is an ubiquitous ectomycorrhizal fungus associated with deciduous and conifer trees. A previous population study revealed the presence of two genetically distinct groups that occur sympatrically within a single site (Gryta et al. 2006). Here, our main objectives were to (i) investigate the occurrence of these two groups using a larger sample of this outcrossing species, and (ii) document the level of differentiation of 30 populations of T. scalpturatum across Europe. A total of 164 sporophores were collected from populations of Scandinavia and Southern France. Genetic characterization was done using ISSR markers and ITS-RFLPs. Results confirm our earlier observations of two distinct groups, which are probably cryptic species. They were present on a broad geographical scale, and produced sporophores during the same period in at least five sites. The genetic analyses also revealed a high level of population differentiation in each group ($\phi st = 0.31$ and 0.49 in group 1 and 2, respectively). Every population appeared to contain a small percentage of the overall genetic variation observed. Factors explaining the observed patterns could include genetic drift and effective barriers to gene flow. In the present study, genetic distances between populations were poorly related to geographical distances, suggesting that isolation by distance is unlikely to explain the patterns of genetic differentiation in this broadly distributed mushroom.

6.2 Dynamic of the ectomycorrhizal community in black truffle plantations in Spain

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The belowground diversity of ECM community in established *Tuber melanosporum* Vitt. inoculated seedlings plantations in Spain has been studied along twelve years. Root samples have been taken in 24 black truffle plantations in different Spanish regions. ECM tips have been characterized and identified morphological and anatomically. More of 50 morphotypes have been characterized, 22 of them were identified to species, genus or order level. These morphotypes belong to the genus *Boletus, Cenococcum, Cortinarius, Genea, Hebeloma, Hymenogaster, Lactarius, Pisolithus, Russula, Scleroderma, Tomentella, Tuber* and *Xerocomus*. The most abundant morphotypes are *Tuber melanosporum, T. aestivum, T. brumale*, Type AD, and *Quercirhiza squamosa*. Mycorrhizal diversity has increased with the age of the stands. It seems relevant the occurance of 20 morphotypes with rhizomorphs as a colonization strategy, like: *Cortinarius, Hebeloma, Pisolithus, Scleroderma, Tomentella* and other telephoroid morphotypes. The occurance of 29 thelephoroid morphotypes is also important. *Tomentella galzinii, T. subtestacea, Quercirhiza cumulosa, Q. squamosa, Q. stellata* , type Thelephoroid, type AD, type SB and other morphotypes characterized but not identified yet have been found. It seems that both, types with rhizomorphs and telephoroid types, have an important role in the dynamic of the ECM community on truffle plantations.

6.3 The impact of ethoprophos, an organic-pesticide, on arbuscular mycorrhizal fungus

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We used a newly developed compartmented growth-system to investigate the effect of ethoprophos, a phosphorus containing pesticide, on the growth of AM fungus under the same host-growing condition. Comparing to control, addition of 0.5, 1.5 and 3.5 mg/kg ethoprophos increased the total hyphal length and the hyphal length with succinate dehydrogenase activity of *Glomus mosseae* significantly. With increasing of ethoprophos concentrations in soil, the relative growth rates of total hyphae were slowly decreased, but the values were still higher than those of control; however, the relative growth rates of hyphae with succinate dehydrogenase activity were lower than those of control and became almost zero under the higher doses of ethoprophos (1.5 and 3.5 mg/kg). It is concluded that low dose of ethoprophos (0.5 mg/kg) stimulated the growth and metabolic activity of AM fungus, and the increase of mycelium biomass and acceleration of turnover under high dose of ethoprophos (1.5 and 3.5 mg/kg) might be attributed to an emergency responding to the toxicity stress.

6.4 Arbuscular mycorrhizae associated with subtropical fruit crops in Central Chile

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A regional survey was done in Central Chile by analyzing samples from two subtropical fruit crops: citrus (*Citrus macrophylla*, Carrizo Citrange) and avocado (*Persea americana*). Both species showed associations with arbuscular mycorrhizae. Twelve species of mycorrhizae were identified, most belonging to the genus *Glomus*. Differences were also found in the number of propagules per sample, most likely due to the condition of the host and the horticultural practices in the different orchards.

6.5 Genetic relationships among *Rhizopogon roseolus* (= *R. rubescens*) isolates from Japan based on rDNA-ITS sequences and AFLP markers

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Rhizopogon roseolus (= *R. rubescens*), an ectomycorrhizal fungus in pine forests, has been highly prized as an edible fungus in Japan and is received one's attention in relation to conservation of seashore Japanese black pine (*Pinus thunbergii*) forests. To understand the genetic structure of Japanese *R. roseolus* population, we investigated the genetic relationships among the *R. roseolus* isolates from 15 different regions of Japan using rDNA-ITS sequences and AFLP markers. The rDNA-ITS sequences of 56 isolates matched closely (>99.7% similarity) each other. Those sequences from Japanese *R.roseolus* also showed more than 95% similarities with sequences of the same species in the DNA database. On the other hand, by AFLP analysis using three primer pair combinations, a total of 223 DNA fragments were detected reproducibly, 74.4% of which were polymorphic. Similarity of AFLP patterns between isolates ranged from 77 to 100%. Phenetic analysis by the UPGMA method based on the AFLP data showed the clustering of isolates distributed close together geographically. Results of the AFLP analysis suggest that the Japanese population of *R. roseolus* is accumulating genetic variations corresponded to the geographical distribution, although sequence divergence of the rDNA-ITS region was very low.

6.6 Genetic structure of arbuscular mycorrhizal fungi in fallow and cultivated fields

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The impact of fallow on the genetic structure of AMF was studied by hierarchical sampling of spores from four plots in fallow and cultivated fields. A nested multiplex PCR was used to assign the spores to genotypes. Variable introns of the two protein coding genes GmFOX2 and GmTOR2 were applied as codominant genetic markers together with the LSU rDNA. Gene diversity and genetic structure of *Glomus mosseae*, *Glomus geosporum* and *Glomus caledonium* were compared within and between the fields. Spores of *G. caledonium* and *G. geosporum* were more abundant in the cultivated field; whereas *G. mosseae* was more frequent in the fallow field. Similarly, the cultivated field had more genotypes of *G. caledonium* and *G. geosporum* compared to the fallow field. Heterogeneity was most pronounced in the fallow field. Analysis of gene diversity indicated that a larger part of the heterogeneity could be attributed to variation between plots in the fallow field, suggesting that the population was constituted by larger mycelia than in the cultivated field. Keywords: arbuscular mycorrhiza, gene diversity, Glomus spp., multilocus genotyping, population structure, hierarchical sampling, nested multiplex PCR.

6.10 Spore Dispersion of *Tricholoma matsutake* at a *Pinus densiflora* Stand in Korea

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Pine mushroom, *Tricholoma matsutake*, is the most profitable non-timber forest product in far-east Asian countries. However, the mushroom cannot be cultivated yet because the life cycle of the fungus is not fully understood. In this study, we tried to find how far the spores disperse from a fruiting body of the fungus. We established 16 slide glasses spread with glycerin around a fruiting body in four directions with four kinds of intervals at three points within the mushroom productive stand after removing other fruiting bodies. The numbers of spores on each slide were counted by line-transect method under the microscope with an image analyzer. The number of spores increased as time flew from the first day (475 spores/cm^2) to the fourth day (836 spores/cm^2) after the pileus opened. The number of spores dispersed down the slope was about 1.5 times of that dispersed up the slope. By the way, the number of spores dispersed was decreased exponentially from the viewpoint of distances from each fruiting body. More than 95% of the spores were dropped within a meter from the fruiting body with about 70% of the spores drop within 0.5 meters. However, the number of spores dispersed over 5 meters from the fruiting body reached more than 50 millions when we considered the number of spores produced by a fruiting body (about 5 billions).

6.7 Abundance of *Corallorhiza odontorhiza* (Orchidaceae) reflects abundance of ectomycorrhizal root tips and fungal community composition

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The essential relationship between the distribution of appropriate mycorrhizal fungi and orchids has long been a mystery. Adult orchids can indicate places where appropriate fungi are present, but lack of plants does not necessarily indicate a lack of fungi. We collected soil samples from plots established within a population of the mycoheterotrophic orchid *Corallorhiza odontorhiza* in which all plants had been mapped for the previous 6 years. Soil samples were distributed among areas with consistently abundant orchids and those with few or no orchids. In each soil sample, we quantified ectomycorrhizal (ECM) root tips, separated them into morphotypes, and used DNA sequencing to identify root tips that hosted the *Tomentella* spp. fungi needed by *C. odontorhiza*. We found that *Tomentella* spp. were distributed throughout the site, including areas with no orchids. This corresponds to the pattern of germination in seed packets. However, there were fewer ECM root tips and root tips hosting *Tomentella* spp. in sparse than in dense areas of the population. The Tomentelloid fungal communities in the soil (as identified using length heterogeneity-PCR of the ITS region with Tomentelloid-specific PCR primers) were also substantially different among dense and sparse areas of the population. This suggests that abundance of adult *C. odontorhiza* reflects abundance of *Tomentella* spp. rather than simply their presence.

6.8 Change of ectomycorrhizal vertical distribution with humus horizons in high mountain spruce forests

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The composition of ectomycorrhizal community requires special attention because of its functional significance in forest trees. Its differences within species and sites have been attributed primarily to edaphic factors, such as litter type and soil quality. Furthermore, vertical distribution of ECM species is known to change with both humus horizons and their physical and chemical features. The research was performed in 4 coeval (160±10 -yr-old), spruce stands in the Trento Province (northern Italy), differing in soil type (Cambisol and Podzol) and exposure (North and South), to verify how the vertical distribution of the ECM species changes with these parameters. Quantitative and qualitative analyses allowed us to relate ECM species to OF, OH, A and B horizons and their physical-chemical features. Results demonstrated that, depending on soil type and exposure, a few ECM specifically characterize the humus horizons, showing also different distribution patterns (uniform, random, aggregated). Ecological and functional aspects related to the obtained results will be discussed.

6.9 Different phenotypes of arbuscular mycorrhizal fungi from field roots of Argentina

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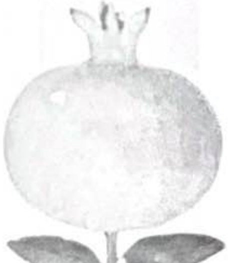
We obtained arbuscular mycorrhizal fungi in transformed roots from field colonized roots, sampled from the provinces of Buenos Aires, Salta and Cordoba (Argentina). Different continuous pure cultures of *Glomus intraradices* were obtained and hyphal growth rate and spore production was measured in each isolate. Hyphal length was measured by counting the number of hyphal intersects on an L-shaped line at four randomly chosen positions on the plate. Spore density was measured by counting the number of spores in four randomly chosen squares. Isolates differed significantly in their final hyphal length and spore number. The hyphal length ranged from 50 to 650m/plate and spore number ranged from 76 to 246 spores/cm-2. Also, differences in number of spores per cluster (1 to 25), spores diameter (31 to 85μm) and morphology of branched absorbing structures (BAS) and mycelium were observed. Because the isolates were in a constant environment, phenotypic variation depended on genotypic variation. This variation could be created by selection of different environments in the field and could explain differences in the plant ecological behavior. Cultures were deposited in the bank of glomeromycota in vitro (http://www.si.fcen.uba.ar).

SESSION 7

Mechanisms in stress resistance/tolerance



Altered Zn fluxes and compartmentation in the ECM fungus *Suillus bovinus* as mechanisms involved in Zn tolerance

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Zinc tolerance is exhibited by the ectomycorrhizal fungus *Suillus bovinus* thriving on Zn-contaminated sites. To investigate the mechanism of zinc tolerance, zinc fluxes and subcellular compartmentation in both Zn-tolerant and Zn-sensitive isolates were investigated. Zn accumulation was followed over time at 200 µM and 1 mM $[Zn]_{ext}$. We observed a significantly higher net Zn uptake rate in sensitive isolates compared to tolerant isolates. However, at 1 mM $[Zn]_{ext}$ membrane integrity of the sensitive isolates was severely disturbed after 2 days of Zn exposure, resulting in ion leakage and reduced uptake of Zn. These results did not allow us to define whether the difference measured in net Zn uptake is because of reduced influx or enhanced efflux of Zn. In a second set of experiments, radiotracer flux analyses were performed to define these fluxes and the Zn compartmentation in a tolerant compared to a sensitive fungal isolate. Zn compartmentation in the mycelial cell wall and cytoplasm was comparable in both isolates. However, in the sensitive isolate we measured a significant influx of excess Zn into the vacuole. No difference was found in the concentration-dependent kinetics of Zn influx between the isolates. Therefore we conclude that an active Zn efflux is most likely involved in the adaptive Zn tolerance of the Zn-tolerant genotypes. These data represent a first step towards the understanding of metal-tolerance mechanisms in the ectomycorrhizal *S. bovinus* isolates.

Heavy metal tolerance in ectomycorrhizal fungi: new insights

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Ectomycorrhizal (ECM) fungi usually protect their host plants against the deleterious effects of heavy metals (HM). However, the molecular and cellular mechanisms underlying the response of ECM fungi to HM remain poorly understood. We therefore used different approaches to identify genes involved in HM tolerance in ECM fungi. An improved HPLC method for the simultaneous measurement of thiol-containing compounds from cysteine and its derivatives (γ-glutamylcysteine, glutathione) to HMW compounds (phytochelatins) was used. We found that glutathione and γ-glutamylcysteine contents increased when *Paxillus involutus* was exposed to Cd. An additional compound (3 kDa), most probably related to a metallothionein, increased drastically in mycelia exposed to Cd. Moreover the screening of cDNA arrays allowed the identification of a gene (*PiMT1*) coding a metallothionein. *PiMT1* is strongly regulated by Cd and Cu and its overexpression was shown to increase Cu tolerance in both yeast and fungi. To identify other genes involved in HM tolerance, the functional complementation of a metal-sensitive yeast mutant with an expression library from the ECM fungus *Hebeloma cylindrosporum* was also performed. Functional characterisation of two metal transporters will be presented. Our Unit is co-heading the sequencing and annotation of the genome of *Laccaria bicolor*. Gene annotation allowed us to identify putative candidates involved in HM tolerance. These candidate genes will be further characterised.

Hunting for genes involved in heavy metal tolerance in ericoid mycorrhizal fungi

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Ericoid mycorrhizal plants can tolerate high heavy metal concentrations in soils thanks to the symbiotic association with endomycorrhizal fungi. Our work aims at unravelling the molecular mechanisms that allow ericoid fungi to survive and tolerate toxic concentrations of heavy metals, and to protect the host plant from heavy metal toxicity. We have investigated the genetic diversity of ericoid fungal isolates from polluted and unpolluted sites with genome-wide approaches, such as AFLP and SSH, in order to identify regions related to metal tolerance, but we have also focused on individual genes. In particular, we have used a metal tolerant strain of the ericoid species *Oidiodendron maius* to identify genes possibly involved in the tolerance mechanisms. Gene expression of this fungal strain in the presence of two different pollutants, such as zinc and cadmium, was monitored with the construction and screening of cDNA libraries. Variation in the gene expression level on high concentrations of zinc was monitored on cDNA clones by reverse northern blot hybridisation. In addition, genes involved in cadmium tolerance were identified by functional complementation of a cadmium sensitive yeast mutant of *S. cerevisiae* (Δyap1) with a cDNA library constructed from ericoid fungal mycelia growing on the same metal. A gene coding for a cystein-rich protein showing high sequence similarity with a plant efflux system for cadmium was identified with this latter approach.

Gene expression in tomato and *Glomus intraradices* under salt stress

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Under diverse stress conditions, most plants are colonized by AMF. Any alleviation of salt stress on crop plants via AMF would be of particular interest due to the enormous potentialities in applications. Among the proteins functioning in plant salt tolerance, Na+/H+ transporters and aquaporins appear to be of paramount importance. The present study compares expression of Na+/H+ transporter and aquaporin genes in tomato colonized by AMF and in non-colonized controls under NaCl stress. As revealed by Northern analyses and in situ hybridizations, expression of two Na+/H+ transporter genes is not significantly affected by salt stress or by colonization with AMF. In contrast, transcript levels of a tonoplast and a plasmalemma aquaporin gene are reduced by salt stress. This effect is distinctly enhanced by colonization of the tomato roots with AMF. In leaves, colonization by AMF results in a drastic increase of the mRNA of all three aquaporin genes assayed under salt stress [1]. With the aim of understanding mechanisms of AMF salt stress tolerance, a cDNA library was constructed using a suppressive subtractive hybridization (SSH) technique. The isolated cDNAs correspond to *G. intraradices* genes expressed and up-regulated in the extraradical mycelium exposed to 0.7% NaCl for 48 hours versus controls without NaCl. Results issued from these experiments will be presented and discussed. [1] Ouziad et al., 2005 (doi:10.1016/j.envexpbot.2005.05.011).

Ectomycorrhization improves host root hydraulic conductivity via aquaporin expression

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Ectomycorrhiza establishment has positive effects on plant water status and its capability to withstand and recover from drought. The reason could be simply an increase in the absorptive surface of the root system, or a mycorrhiza-regulated modulation of plant physiology. To determine the impact of mycorrhization on aspen water uptake, aquaporin genes were isolated from a mycorrhizal cDNA library. Seven putative plasma-membrane aquaporins (PIP) where identified. Four of these genes were preferentially expressed in roots. Mycorrhiza formation resulted in an increased transcript level for three genes, two of which (e.g. *PttPIP2.5*) are the most prominently expressed aquaporins in roots. The impact of drought stress on PIP gene expression was followed in fine roots of mycorrhized and non-mycorrhized aspen. Drought stress was detected by a decreased photosynthetic yield. For three out of five investigated PIP genes expression did not differ between mycorrhized and non-mycorrhized plants. However, expression of the two other aquaporin genes (e.g. *PttPIP2.5*) was more pronounced in fine roots of mycorrhized plants, indicating that the root-fungus-symbiosis may improve the plant water transport capacity. When PttPIP2.5 was expressed in *Xenopus laevis* oocytes, the corresponding protein enabled strong water transport. The physiological impact of the observed expression pattern will be discussed with respect to mycorrhiza formation and plant survival under water stress conditions.

Priming plant defenses against pathogens by arbuscular mycorrhizal fungi

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The key to effective resistance against deleterious organisms is the timing and magnitude of the defense reactions triggered in response to their attack. Upon appropriate stimulation, animals and plants can develop an enhanced capacity to mobilize infection-induced cellular defence responses, a phenomenon known as priming. This boost of basal defenses is a common feature of the plant's immune system, and it has important ecological fitness benefits. Different stimuli can trigger such primed state of the plant, including some chemicals and certain beneficial microorganisms as arbuscular mycorrhizal (AM) fungi. Plant defense mechanisms are tightly regulated through an interconnected network of signalling pathways in which jasmonic acid, ethylene and salicylic acid play major roles. Priming is often manifested as a sensitization of the tissues to one or some of these signalling molecules and results in a more efficient reaction to stress conditions. Using biochemical and molecular methods we show that root colonization by Glomus mosseae primes tomato plants for a stronger activation of JA dependent defenses. Genomic approaches coupled to bioinformatic tools will allow us to identify key regulatory elements in the priming process associated to the AM symbiosis.

7.1 Cadmium stress alleviation by arbuscular mycorrhizal symbiosis: proteomic studies of the mechanisms involved

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Ecosystems are submitted to various abiotic stresses, among which heavy metals (HMs) represent major industrial pollutants. Cadmium (Cd) accumulates in agricultural environments through industrial and human activities, including phosphate fertiliser and sewage sludge applications. It has damaging effects on plant metabolism. Metal availability to plants can be modulated by soil microorganisms, including arbuscular mycorrhizal (AM) fungi. In the present work, the effects of Cd toxicity on *Medicago truncatula* inoculated or not with the AM fungus *Glomus intraradices* are being studied at three levels: (1) plant biomass production together with green part chlorophyll quantification, (2) development of *G. intraradices* inside the roots and (3) root and shoot profiles of total proteins. A Cd concentration of 2 ppm caused growth reduction of both roots and shoots that was alleviated in colonized plants. Global changes of protein accumulation in roots and shoots of Cd-stressed *M. truncatula* during the symbiotic interaction with *G. intraradices* were monitored by differential protein display (2-dimensional gel electrophoresis). Cd provoked changes in protein accumulation in both tissues, some of them being reverted by AM symbiosis. These protein modifications are being identified by mass spectrometry. Results are discussed in relation to a possible role of AM symbiosis in detoxification and/or resistance mechanisms towards Cd in *M. truncatula* plants.

7.2 Dry matter and root colonization of plants by *G. deserticola* in presence of physical fractions of dry olive residue

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Olive production is a significant agricultural activity in the Mediterranean countries. The extraction of olive oil generates large amounts of olive mill residues (DOR) which may be used as fertilizer due to the large amount of organic matter and plant nutrients. However, DOR has phytotoxic effects due to the presence of water soluble substances. The physical extractions of DOR gave an aqueous (ADOR) and an exhausted (SDOR) fraction with less phytotoxicity on tomato and alfalfa than the original samples. Arbuscular mycorrhizal (AM) fungi increased the sensitivity of plants to the phytotoxicity caused by the application of DOR. However, *G. deserticola* not only decreased the sensitivity of plant to the phytotoxicity of the physical fractions (ADOR and SDOR) of this residue but also considerably improved the growth of tomato and alfalfa in presence of low doses of these fractions. The percentage of root length colonized by *G. deserticola* strongly decreased in presence of DOR However, the ADOR and SDOR fractions decreased the AM root length colonization of tomato but not of alfalfa colonized by *G. deserticola*. Our results suggest that the phytotoxicity of the olive residues can be eliminate by the combination of physical extraction and by AM fungal inoculation and the use of this agrowaste as organic amendment in agricultural soil can be possible.

7.3 *Scleroderma bermudense* improves water status and limits sodium and chloride in seagrape (*Coccoloba uvifera* L.) seedlings

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Coccoloba uvifera (L.) L. (Polygonaceae), also named seagrape, is a small tree widely distributed along Altlantic, Caribbean and Pacific coasts of the American tropics and subtropics. It is an important tree for edible fruits, ornemental plantings and coastal windbreaks along Caribbean's Beach and Roadside. The purpose of this study was to test the hypothesis that the ectomycorrhizal (ECM) fungus, *Scleroderma bermudense*, have a high capacity to alleviate the saline stress in *Coccoloba uvifera* (L.) L. seedlings. Plants were grown over a range (0, 200, 350 and 500 mM) of NaCl levels for 12 weeks, following 4 weeks of non-saline pre-treatment under greenhouse condition. The *C. uvifera* seedlings growth and nutrition were stimulated by *S. bermudense* regardless of salt stress. Although ECM colonization was reduced with increasing NaCl levels, ECM dependency on *C. uvifera* seedlings was increased. ECM plant tissue had significantly increased concentrations of P and K but lower Na and Cl concentrations than non-ECM plants. Higher K concentrations in ECM plants suggest a higher osmoregulating capacity of these plants. In this respect, the water status of ECM plants was improved despite their higher evaporative leaf surface. The results suggest that the reduction in Na and Cl uptake together with a concomitant increase in P and K absorption and a high water status in ECM plants may be important salt-alleviating mechanisms for *C. uvifera* seedlings growing in saline soils.

7.4 Arbuscular mycorrhizal symbiosis decreases proline accumulation in plants affected by nematodes

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Content of amino acid proline has been demonstrated to increase in plants under abiotic stress, which is also alleviated by the establishment of arbuscular mycorrhizal fungi (AMF) in roots. This study evaluated the effect of the nematode *Meloidogyne sp.* (N) on proline accumulation in leaves of *Impatients balsamina* , inoculated or not with the AMF-consortium MTZ-UV (10 g plant $^{-1}$), and with the application of 150 µg kg^{-1} of phosphorus (P). A 2x2x2 factorial experiment was established, with 8 treatments and 4 replications. After six weeks of AMF-inoculation, plants were inoculated with *Meloidogyne sp.* , and four weeks later plants were harvested. AMF-inoculation significantly ($P \leq 0.01$) reduced proline content, which was higher at non-AMF plants. Neither nematode inoculation nor P-application significantly affected proline content. Plant height, stem diameter, leaf area, number of leaves, and fresh weight were significantly ($P \leq 0.01$) affected by AMF. In addition, significant ($P \leq 0.01$) effects on leaf area and height were observed for P-application, while AMFxP interaction resulted in significant effects on height, and number of leaves. AMF-colonization was not reduced by either nematode or P-application. These results may contribute on understanding physiological mechanisms of AMF-plants involved in enhanced biotic stress tolerance. Further research should be addressed to elucidate the role of proline amino acid in plants affected by the nematode *Meloidogyne sp.*

7.5 *In vitro* evidences of mycoparasitism of the ectomycorrhizal fungus *Pisolithus tinctorius* by *Hypholoma fasciculare*

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In the northeast of Portugal macrofungi community associated to chestnut tree (*Castanea sativa* Mill.) is rich and diversified. Among fungal species, *Pisolithus tinctorius* and *Hypholoma fasciculare* are common in this habitat. *In vitro* interaction between *P. tinctorius* and *H. fasciculare* was investigated, to ascertain the potential mycoparasitic capabilities of *H. fasciculare* in the growth suppression of ectomycorrhizal *P. tinctorius*. The results show that in co-culture, the growth of *P. tinctorius* was drastically inhibited by *H. fasciculare*. Preliminary studies seem to evidence *P. tinctorius* hyphal injury and stress response in the contact zone between both mycelia. As the chestnut inoculation with these fungi showed higher hyphal adhesion of *H. fasciculare* to the roots in comparison with *P. tinctorius* adhesion, mycoparasitism of *P. tinctorius* by *H. fasciculare* could bring serious consequences to chestnut orchards, even more because the continuous contact of *H. fasciculare* with chestnut roots seems to promote the destruction of vascular root system. In addition, as both fungi exhibit high adhesion capacity to chestnut roots, the involvement of hydrophobins, proteins reported to be involved in the fungus adhesion to other organisms, is being studied by expression analysis in *P. tinctorius* or *H. fasciculare* after being in contact with *C. sativa* roots.

7.6 Impact of the sterol biosynthesis inhibitor (SBI) fungicides on the sterol metabolism of *Glomus intraradices*

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SBI fungicides were used in modern agriculture for control of pests and fungal disease agents. Very little information is available on the effects of SBI fungicides on sterol composition of the non-target arbuscular mycorrhizal (AM) fungi. Up to date, the sterol pathway of AM fungi is not clear. AM fungi are characterized by an absence of ergosterol and by two unusual fungal sterols : the 24-methylcholesterol and the 24-ethylcholesterol. Based on the root-organ cultivation technology, the present study aims to better understand the sterol metabolism of Glomus intraradices. Due to the knowledge on different fungicides, on their action sites, and the biosynthetic pathway of other organisms, the objectives of this study are (1) identification of enzymatic target of SBI fungicides, (2) establishment of sterol composition during SBI fungicides application and (3) establishment of the sterol biosynthetic pathway of AM fungi particularly between lanosterol and 24-ethyl or 24-methylsterol. The in vitro system using Ri T-DNA transformed carrot roots as the host and were used. Active matter of three SBI fungicides (fenpropimorph, propiconazole, fenhexamid) were tested at various concentrations (0 mg.l^{-1} (control treatment); 0.02 mg.l^{-1}; 0.2 mg.l^{-1}; 2 mg.l^{-1}; 20 mg.l^{-1}). Sterol analyses were performed on colonized roots and extraradical mycelium. Sterols were analysed by GC and identified by GC-MS.

7.7 *Glomus intraradices* increases photosynthesis and transpiration in strawberry plants, independently of nitrogen dose and water availability

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Strawberry is one of the most profitable horticultural crops in Michoacán (Mexico). To achieve the maximum profit per hectare, excessive nitrogen and water are used. In this context, inoculation of the strawberry plants with arbuscular mycorrhizal fungi would allow reducing these inputs. The plants associated with these fungi modify their structure and their metabolism and may achieve their growth and development with less contribution of environment resources. Photosynthesis and transpiration are the most important physiological processes involved in plant growth and their intensity depends on nitrogen and water availability for the roots. The hypothesis is that strawberry plants inoculated with arbuscular mycorrhizal fungi maintain their photosynthesis and transpiration rates under growth-limited water and nitrogen availability. In order to test this idea, during the 12 days following a stop in the irrigation, the rates of these processes were measured (IRGA LI-6400P) in inoculated and non inoculated strawberry plants, grown with 0.3 and 6.0 mM nitrogen in the nutrient solution. The statistical analysis showed a significant effect ($p < 0.05$) of nitrogen dose and water depletion on photosynthesis and transpiration. The rates of both processes were always major for inoculated than for not inoculated plants.

7.8 Aquaporin gene expression in *Medicago truncatula* roots colonized by *Glomus mosseae*

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The mechanisms active in the enhancement of plant resistance to water stress by arbuscular mycorrhizal (AM) are poorly understood. We speculate that the AM influence on plant response may be partially linked to modifications in aquaporin gene expression. Aquaporins (AQP) are a family of small pore-forming integral membrane proteins, widely present in nature. They are known to accelerate movement of water and other solutes across biological membranes where they are localized, but the molecular mechanisms of each AQP transport function and the diverse physiological roles played by AQP family members are still being defined. Little is known of the role of aquaporins in AM although there are previous studies which have reported up-regulation of a corresponding gene. Our research is aimed at characterizing AQPs involved in plant-fungal interactions in mycorrhiza of *Medicago truncatula* in order to understand mechanisms of mycorrhiza-facilitated water transport. In database searches we have identified 15 putative AQPs in the *Medicago* genome (MtAQPs). All 4 subfamilies of AQPs are represented: PIP (5), TIP (5), NIP (3) and SIP (2). Expression profiling of the different AQPs in an established mycorrhizal symbiosis between *M. truncatula* and *Glomus mosseae* showed differential regulation of the AQP genes. These data suggest that AM fungal colonization changes the properties of the plasma membrane and the tonoplast which may affect water transport relations in roots.

7.9 Effect of soil acidification on growth and physiological activities of four pine species seedlings inoculated with ectomycorrhiza

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The forest decline was observed in recent years near industrial areas and big cities of whole the world. Forest decline by acidic deposition is believed to be degradation of soil environmental condition. To study its effects, we grew seedlings of *Pinus densiflora*, *P. koraiensis*, *P. rigida* and *Larix kaempferi* with three ectomycorrhiza species in brown forest soil derived from granite, which had been treated with the acid solution, for 180 days. The five H^+ ion concentrations in the soil were studied (0 (control), 10, 30, 60 and 90 $\text{mmolH}^+\text{kg}^{-1}$). Ectomycorrhizal development significantly increased 10 and 30 $\text{mmolH}^+\text{kg}^{-1}$ treatments, however that of 60 and 90 $\text{mmolH}^+\text{kg}^{-1}$ treatments apparently decreased compared with other treatments. The concentrations of Al in roots and Mn in needles of each ectomycorrhizal seedlings of each species significantly decreased compared with non-ectomycorrhizal (NE) each seedling. Moreover, the maximum net photosynthetic rate at saturated CO_2 concentration (Pmax) of each ectomycorrhizal seedling at each species significantly increased than that of NE each seedling.The relative total dry mass (TDM) of *P. koraiensis* and *L. kaempferi* seedlings reached approximately 40% at a BC/Al ratio of 1.0, but that of *P. rigida* and *P. densiflora* showed approximately 50% at a BC/Al ratio of 1.0. However, ectomycorrhizal seedlings of each species increased relative TDM at a BC/Al ratio of 1.0 than NE seedlings of each species, 20 - 100%, even if those also reduced.

7.10 Factors determining mycorrhizal benefit under N limited conditions

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In this work we evaluate the response of mycorrhizal and non-mycorrhizal plants under N limited conditions, the role of mycorrhizae in the plant capacity to cope with nutritional stress, and how this role depends on the N availability. Groups of Pinus pinaster L. seedlings with different ages, and hence different photosynthetic capacities, and different nutritional status (starved or not starved prior to the experiment) were inoculated with alive or dead Pisolithus tinctorius. The seedlings were fed with ammonium at a constant supply of 1.33 or 2.66 mg N week^{-1}plant^{-1}, and followed for 4 months. The degree of mycorrhization was monitored through ergosterol quantification. Biomass increment, photosynthesis, N and C concentrations and daily chlorophyll a fluorescence, with the analysis of fast fluorescence kinetics O-J-I-P, were measured. Several plant growth parameters were calculated. Based on the results we conclude that the plants' age is determinant on the first responses towards mycorrhizal colonization. The presence of mycorrhizae can be an advantage or not in N limited conditions, depending on N availability and the internal N status of the plant. This effect is not a simple, direct one, but one who is deeply intertwined with the degree of mycorrhization.

7.11 Plant polyamine metabolism and arbuscular mycorrhizal colonization

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Polyamines are small organic cations that are absolutely required for eukaryotic cell growth. These compounds are thought to play an important role in plant responses to a wide array of environmental and biotic stresses, however little is known about their rol on the regulation of arbuscular mycorrhizal (AM) colonization. Our work was aimed at evaluating: 1-the influence of colonization by *Glomus intraradices* on polyamine metabolism of *Lotus*spp. under salt stress, and 2- the effect of altered polyamine levels of *Nicotiana tabacum* on root colonization by *Glomus intraradices*. In 1-, the experiments consisted of a randomized block design with two factors: (1) mycorrhizal treatments (with or without AM fungus) and (2) two salinity levels of 0 and 200 mM NaCl. Total free polyamines contents were higher in mycorrhized plants compared to non-AM ones. These results are discussed with regard to improvements in plant growth. In 2- we used transgenic tobacco plants containing the *Avena sativa* L. (oat) arginine decarboxylase (ADC) gene under the control of a tetracycline-inducible promoter. Transformed tobacco plants without the transgene and non-induced plants were used as controls. Our results showed higher percentage of AM colonization and number of infection points and vesicles in induced plants carrying the transgene compared to controls. We discuss these results in relation to tobacco polyamine levels.

7.12 Arbuscular mycorrhizal fungi protected *Capsicum annuum* L. cv. Piquillo against *Verticillium dahliae* Kleb. by inducing defence-related enzymes in roots

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Arbuscular mycorrhizal fungi (AMF) can reduce the deleterious effect of *Verticillium dahliae* on pepper growth and yield (1,2). Such bioprotection can result from the preactivation of defence responses that include structural modifications and the accumulation of Pathogenesis-Related proteins. Our first objective was to study if *V. dahliae* induced defence mechanisms in roots before infected-pepper developed visible symptoms of disease. The second aim was to determine if AMF induced defence-related enzymatic activities in pepper roots before or after pathogen's attack. The colonization of pepper roots by *Glomus deserticola* (Trappe, Bloss and Menge) induced the appearance of new isoforms of acidic chitinases, superoxide dismutase (SOD) and, at early stages, peroxidases. In contrast, *V. dahliae* neither stimulated the phenylpropanoid pathway nor elicited hydrolytic activities in infected pepper roots. Only in mycorrhizal plants, the inoculation with *V. dahliae* slightly increased both phenylalanine ammonia-lyase (PAL) and peroxidase activities 2 weeks later. Mycorrhizal-specific induction of new isoforms of acidic chitinases and SOD together with enhanced peroxidase and PAL activities 2 weeks after pathogen inoculation could be involved in the control of *Verticillium*-induced wilt in pepper by AMF. (1) Garmendia et al. (2004) Biol Control 31: 296-305 (2) Garmendia et al. (2004) Eur J Plant Pathol 110: 227-238 Fundación Universitaria de Navarra and Gobierno Vasco supported this work.

7.13 Oxidative reactions of axenically cultured olive seedling roots elicited by the defence phytohormones methyl-jasmonate and methyl-salicylate

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The aim of this work was to measure some oxidative reactions involved in the defence oxidative burst elicited by MeJA and MeSA in olive seedling roots, in order to compare these responses with those evoked by the presence of compatible mycorrhizating fungus and the possible significance of these reactions in the first steps of plant-fungus recognition. Axenically cultured isolated embryos from olive seed were used to obtain axenic and intact seedling roots. The roots oxidized NADH what was inhibited by KCN; this proved the existence of a CN-sensitive redox chain in the root cells PM. The roots generated O^2.- measured by the epinephrine/adrenochrome method, and this was enhanced by MeJA and MeSA. The roots also showed different POX activities: a guayacol-POX and an inespecific DMAB-MBTH POX, but a coniferyl alcohol-POX was not detected. Both inespecific and guayacol POXs were enhanced by MeJA and MeSA. These results proved that roots carried out oxidative reactions in the apoplast, with Reactive Oxygen Species (O_2.- and H_2O_2) generation, and these were enhanced by MeJA and MeSA that mimicked pathogen attack, it also proved that both phytohormones would be involved in the oxidative burst response. Both O_2.- generation and POX activities were strongly dependant on the root age: young roots (up to 1 cm long) showed the highest sensitivity to MeJA and MeSA. Supported by the Spanish Ministerio Educación y Ciencia (CGL2004-05987/BOS) and the Junta de Extremadura (2PR03A050).

7.14 Molecular mechanisms of nickel tolerance in a serpentine isolate of the ectomycorrhizal fungus *Cenococcum geophilum*: a proteomics insight

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Understanding the physiology of tolerance to metals in mycorrhizal fungi, namely ectomycorrhizal fungi, is important in both applied and fundamental biological settings. However, the molecular mechanisms associated with the response of ectomycorrhizal fungi to toxic levels of trace metals in general, and to Ni in particular, remain largely unknown. An isolate of the ectomycorrhizal fungus *Cenococcum geophilum*, originated from a serpentine soil in northeast Portugal was shown to tolerate high concentrations of Ni in the growth medium. We have investigated the molecular basis of this fungal isolate tolerance to Ni by using a proteomic approach. Proteomics provide an extraordinary means of evaluating the expression of many proteins simultaneously. It therefore allows us to gain a global view of the molecular processes underlying the physiological response of this fungal isolate to Ni exposure. The analysis of the two-dimensional electrophoresis maps when comparing treatment (100 ppm Ni) and control (0 ppm Ni) revealed that the presence of Ni induced significant changes in the proteome of this isolate. Selected spots were excised, trypsin digested and will be analysed by tandem mass spectroscopy for putative protein identification.

7.15 Arbuscular mycorrhizal fungi enhance *Sapindus saponaria* L. seedling nitrogen nutrition and growth in non-rotated ingrowth cores

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Arbuscular mycorrhizal fungi (AMF) in moist soil generally do not improve host nitrogen nutrition because inorganic nitrogen is mobile. Where low moisture prevents mass flow, however, AMF external hyphae might be beneficial for nitrogen uptake. In a well-drained, sandy South Florida soil, we used rotated ingrowth cores to determine if AMF could improve host nitrogen nutrition and growth. Along five 22 m long transects we buried 60 tubes (9 cm diam. x 20 cm long, with four 55 mm diam. side openings covered by 30 µm pore nylon cloth), each protruding 1 cm above the soil surface. These transects were evenly spaced 2 m apart and located between rows of *Litchi chinensis* L. trees. We filled the tubes with twice-autoclaved soil and closed their tops with nylon cloth. After one year, we planted one 8-mo-old AMF-free *Sapindus saponaria* L. seedling in each tube. Beginning 7 months after planting, we weekly twisted 30 tubes through one full rotation to sever AMF hyphae extending beyond the tubes. We watered the plants twice weekly to supplement rainfall for most of the experiment. Thirteen months after planting, there was no significant difference in plant survival between treatments, but non-rotated plants averaged almost 5 cm taller and had 1.37 times higher mean foliar nitrogen concentration than rotated plants. This suggests that hyphae of AMF acquired nitrogen beyond the cores and supplied it to hosts in amounts sufficient to enhance growth.

7.16 Cd-tolerant *Suillus luteus* protects its host against Cd-toxicity

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It has become quite clear that the ecological success of many plant species is – in large extent – established by microbial symbionts that protect them against all kinds of negative influences. The basidiomycete fungus *Suillus luteus* is a common mycobiont in pioneer pine forests. On severely polluted soils in the vicinity of former non-ferro smelters, metal tolerant populations of this fungal species have evolved. It has been established that Cu- and Zn-tolerant *Suillus* species can reduce heavy-metal stress in plants by limiting transfer of the respective metals to above-ground parts of the plant. In a dose-response experiment with mycorrhizal plants we evaluate the importance of Cd-adaptation in *Suillus*. Can the Cd-tolerance of *S. luteus* improve the health of a host plant exposed to toxic Cd-concentrations? Results indicate that isolates sensitive to cadmium do not perform well when the association is exposed to high Cd-levels. The uptake of essential minerals (ammonium, phosphate) from the test solution by Cd-tolerant plant-fungus associations is maintained in the presence of high Cd-concentrations.

7.17 Occurrence of mycorrhizal associations in mining tailings polluted with heavy metals

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Thousands of years of intense mining activity has caused the accumulation of tailings in the hills surrounding the mines in the Southeastern Spain. These tailings are polluted with high concentrations of heavy metals and this determines a scarce and dispersed presence of vegetation on soils. Mycorrhizal fungi are thought to be involved in improving plant tolerance to several stress conditions, including heavy metal stress. So, the establishment of mycorrhizal associations could contribute to achieve the successful restoration of plant-deprived soils. In this work we anlyze the presence of mycorrhizae in plants spontaneously growing in an ancient tailing pond located in Murcia. Microscopical observations of roots from *Zygophyllum fabago*, *Phragmites australis* and *Paronychia suffruticosa* revealed the generalized occurrence of these symbiotic associations. Furthermore, analysis of phosphatase, esterase and dehydrogenase enzymatic activities extracted from rhizospheric soils showed a good correlation with mycorrhizae abundance. Since these enzymatic activities are considered to be sensitive markers of soil quality, results obtained point to an improvement of soil attributes due to the presence of mycorrhizal associations. Acknowledgements: This work was partially supported by MEC and Fundación Séneca (REN2002-02952, PB/23/FS/02).

7.18 Genetic and functional diversity of ericoid mycorrhizal fungi tolerant to heavy metals

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The introduction of heavy metals in terrestrial ecosystems through human activities is one of the main causes of pollution, but soils derived from ultramafic rocks (serpentine) are also naturally enriched in heavy metals. Ericoid mycorrhizal fungi are known to be highly tolerant to heavy metals and to protect their host plants in contaminated sites. Aim of this study was to investigate the functional and genetic diversity of *Oidiodendron maius*, a widespread ericoid fungal species, in *Vaccinium myrtillus* roots collected (i) in a serpentinitic area of the Mont Avic Parc in Italy, (ii) in a heavily polluted industrial site in Poland, (iii) in unpolluted sites in Italy, Poland and Canada. Functional diversity of *O. maius* strains was evaluated by testing their growth in the presence of heavy metals (Cr, Ni, Zn). Some specificity in metal tolerance was found towards the contaminants abundant in the site of origin. Genetic diversity was analysed through AFLP analysis and sequencing of ITS and functional genes (e.g. SOD). For strains isolated in contaminated soils, genetic diversity was found to be higher for the SOD locus than for the ITS locus. Phylogenetic analyses showed different tree topologies for the two loci, indicating their different evolution. Interestingly, strains more tolerant to metals showed higher genetic diversity. One of the most tolerant fungal isolates, when tested in mycorrhizal synthesis experiments, was found to confer to the plant a higher tolerance to Cr.

7.19 Effect of AM symbiosis on antioxidant activities induced by water stress in *Lavandula officinalis* plants

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The effectiveness of allochthonous vs autochthonous AM fungi on growth and water stress tolerance of lavender plants was compared in an experiment carried out under drought conditions. Autochthonous fungal strains induced a higher plant biomass, mainly root biomass, higher water content and a more efficient N and K plant acquisition than that induced by the allochthonous strains. Autochthonous AM fungi developed more arbuscules and extraradical mycelium, and showed higher succinate dehidrogenase and alkaline phosphatase activities. The higher effectiveness of autochthonous fungal strains was related to the low accumulation of glutathione, ascorbate and hydrogen peroxide, which suggests an increased plant drought tolerance. Antioxidant activities such as catalase and superoxide dismutase, involved in cell metabolic functioning under water limitation were not affected, but glutathione reductase activity increased by 85% in plants colonized by the native strain. In conclusion, the water deficit had drastic consequences on plant growth, nevertheless AM symbiosis involving autochthonous adapted fungal strains, that enhanced cell antioxidant compounds, assure the greatest plant drought tolerance and defence against water stress. However, antioxidant enzymatic activities determined seem to be less correlated with the symbiotic efficiency.

7.20 Effects of sodium chloride on growths of *Eucalyptus globulus* seedlings inoculated with *Pisolithus* spp.

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To clarify the salt tolerance of ectomycorrhizal associations, we investigated the effects of sodium chloride (NaCl) on the growth of *Pisolithus* spp. and *Eucalyptus globulus* seedlings inoculated with the fungi *in vitro*. Two isolates of *Pisolithus* spp., 1307 and 1308, which were obtained at natural *Eucalyptus* stands in Western Australia and isolated into pure culture, were grown in liquid MMN media added with 4 different concentrations of NaCl (0, 10, 100, 500mM) at 25°C for 30days. Mycelial dry weights and the ion concentration of Na^+ and Cl^- within the mycelia were determined. Three-week-old seedlings of *Eucalyptus globulus* were inoculated with *Pisolithus* sp. (1307) and established ectomycorrhizal associations. After 4 weeks, the seedlings received 3 different concentrations of NaCl, 0, 50 and 100mM and were kept in a growth chamber for 5 weeks. Then ectomycorrhizal formation rate, dry weight of shoot and root parts and Na^+ and Cl^- concentrations were determined. Only little growth of the fungi was observed at 500mM NaCl. Up to 100mM, the addition of NaCl did not significantly reduce mycelial weight of the fungi compared to 0mM. Although Na^+ concentrations within mycelia showed a significant increase from 0 to 100mM, no difference was found for Cl^- concentrations among treatments. Analyses of ectomycorrhizal associations were under way. Based on this experiment, we will discuss the distribution pattern of Na^+ and Cl^- and the NaCl tolerance in the ectomycorrhizal association.

7.21 Effect of mycorrhization on the antioxidant systems in Pb-treated *Dittrichia viscosa* plants

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Stress conditions provoke an increased production of reactive oxygen species (ROS) in plants. ROS may lead to the oxidation of essential molecules for plant cell, impairing its performance. So, it is critical for plants to control the levels of these species. In heavy metal stress roots are the organs first facing the pollutant challenge. Almost all plants growing in field conditions form mycorrhizal associations and it is generally accepted that this fact modifies the response of plants to metals significantly. In the present study the evolution of antioxidant systems in response to a high Pb concentration is analyzed in both *Glomus intraradices*-mycorrhized and non-mycorrhized *Dittrichia viscosa* plants. *Glomus*-colonized plants showed a higher biomass production in response to Pb treatment than that displayed by non-mycorrhized plants. Levels of H_2O_2-consuming enzymes in shoots of both mycorhizal- and non-mycorrhizal plants tended to decrease with long exposure time to Pb. With respect to roots *Glomus*-colonized plant always showed an increase in antioxidant enzyme levels. This behaviour could only be observed for short incubation times in non-mycorrhizal plants, supporting the view that it is necessary for plants to maintain high levels of antioxidant enzymes in order to cope with heavy metal stress. Acknowledgements: This work was partially supported by MEC and Fundación Séneca (REN2002-02952, PB/23/FS/02). Thanks are given to BEG for providing the *G. intraradices* propagules.

7.22 Polyamines in vines infected by *Glomus intraradices* Schenk & Smith (BEG 72) and the white root-rot fungus *Armillaria mellea* (Vahl:Fr.) P. Kumm

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Polyamines, among plant hormones, may play essential roles both in pathogenic infections and in the process of mycorrhizal root colonization, and could be involved in the complex mechanisms of disease resistance in plants. Changes in polyamines levels in vine plants colonized by *Glomus intraradices* and infected with *Armillaria mellea* were studied. Experiments were conducted with the vine rootstock Richter 110 (*Vitis berlandieri* Planch x *V. rupestris*) under greenhouse and microplot conditions. Four treatments were considered: mycorrhizal and non mycorrhizal plants inoculated or not with *A. mellea*. HPLC in combination with fluorescence spectrophotometry were used to separate and quantify free root and leaf polyamines. Plant growth, mycorrhizal colonization and pathogen infection were monitored along the experiment. Polyamine levels decreased with plant development during the first growing season, but the decrease varied with the treatment. Mycorrhizal infection significantly increased free polyamine levels in plants not infected by *A. mellea*, whereas the presence of the pathogen reduced those levels. However, plants infected by both the pathogenic fungus and *G. intraradices* showed a higher level of free polyamines after one month growth when compared with non mycorrhizal plants infected by *A. mellea*. The potential role of free polyamines in the control of *A. mellea* in mycorrhizal vines is discussed.

7.23 Carbon cost of mycorrhization in addition to organic acid secretion is negligible for acid tolerant wheat cultivar expressing *ALMT1* gene

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Detoxification of Al by organic acids secretion from roots is one of the plant strategies to adapt to acidic soil. In addition, availability of P in acidic soil is generally low, thus plants depend on arbuscular mycorrhizal (AM) fungi for P acquisition. The expense of organic acids secretion and mycorrhizal formation of an Al-tolerant wheat cultivar, ET8, which expresses the *ALMT1* (Al-activated malate transporter) gene and secretes malate in response to Al^{3+} was assessed. The ET8 and an Al-sensitive near-isogenic line, ES8, were grown in acidic soil (pH 4.6) in a greenhouse. In the absence of P-fertilizer and AM fungi, dry mass of the ET8 was at the same level of that of the ES8. P-fertilizer significantly increased the growth and P concentration of the ET8 but not those of the ES8. The inoculation of AM fungi also increased the growth and P concentration of the ET8 to the same extent as those by the P fertilizer. Dry mass of the ES8 was not increased by AM formation although the P concentration was increased. The expression levels of the *ALMT1* were constant irrespective of P-fertilization and mycorrhizal status in the two cultivars and were about 20-fold higher in the ET8 than in the ES8. These results indicated that the secretion of malate functioned efficiently in the presence of AM fungi and that carbon cost for mycorrhization in addition to the secretion was not the extent as to reduce the growth of the plant under the P-deficient and low-pH conditions.

7.24 Abiotic and biotic stresses management on maize using arbuscular mycorrhiza fungus

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Maize (*Zea mays* L) growth and yield are adversely affected by abiotic stresses such as drought, poor nutrient availability and biotic stresses such as nematodes *Pratylenchus* spp. Among other factors that affect maize productivity is presence or absence of beneficial soil microsymbionts. The effects of root colonization by the mutualistic arbuscular mycorrhiza fungus *Glomus mosseae* application, on yield, growth and *Pratylenchus* spp., density on a maize cultivar were evaluated under low and high water regimes. Arbuscular mycorrhiza fungus treatments used for the experiment were compared against a nematicide (carbofuran), nematode only treatment, each plant inoculated with 5000 *Pratylenchus* spp. (biotic stress) and a no nematode control. Water stress significantly reduced *Pratylenchus* spp. density, maize growth and grain yield when compared across the water regimes. *G. mosseae* increased maize growth and grain yield significantly ($P<=0.05$) under low and high water regimes, when compared with treatments without *G. mosseae*. The arbuscular mycorrhiza fungus used did not significantly reduce *Pratylenchus* spp. density, but enhanced maize growth and grain yield significantly when compared with nematode only treatment. Also, abiotic stress did not reduce mean percentage mycorrhiza colonization significantly. Therefore, *G. mosseae* could be used to improve maize grain yield, manage abiotic and biotic stresses in agroecological zones where maize are grown.

7.25 Effects of arbuscular mycorrhizal fungi and endophytic fungi on growth of red pepper (*Capsicum annuum* L.)

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This study was conducted to investigate effects of foliar endophytic fungi and arbsculuar mycorrhizal (AM) fungi on growth of red pepper (*Capsicum annuum* L.). Leaves were collected from *C. annuum* growing in arable fields, Chungbuk and Gyeonggi. Endophytic fungi were isolated from the surface sterilized leaves and morphological and molecular techniques were used to identify these fungi to species. Three isolates of the endophytic fungi were inoculated to the leaves of *C. annuum*. Also, three species of AM fungal spores were extracted from pure cultures and the mixture of the three species were inoculated to the roots of the plants. After two month of growth in a growth chamber, a pathogen, *Phytophthora capsici* was inoculated to the plants to test the effects of both symbiotic fungi to the pathogen on the growth of the host plants. The biomass of host plants was significantly increased when AM fungi were inoculated, but the plant inoculated with endophytic fungi showed no significant effects on the growth of the host plants. However, the host plants with AM and endophytic fungi showed better growth than controls when the pathogen was inoculated.

7.26 Arbuscular mycorrhizal fungi increased olive trees growth, nutrition and survival after transplanting and tolerance to saline soil conditions

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Arbuscular mycorrhizal (AM) fungi (*Glomus mosseae*, *Glomus intraradices* or *Glomus claroideum*) increased survival, growth and nutrition of olive trees after transplanting to non-saline and saline soil. Salt reduced number of olive shoots, trunk diameter and shoot length and these detrimental growth effects were correlated with a lower root growth and nutrients acquisition. The mycorrhizal growth response was larger under salt conditions. The effectiveness of *Glomus mosseae* in increasing olive tree growth ranged from 163% (shoot) to 295% (root) in non-saline soil and from 239% (shoot) to 468% (root) in saline soil. These mycorrhizal effects were different from the rest of AM inocula applied. The enhanced olive tree salt tolerance seemed to be a consequence of increased nutrients acquisition, particularly K. As K plays a key role in the osmoregulation processes it is important that AM colonization increased K content by 4 fold (*G. mosseae*), by 2.2 fold (*G. intraradices*) and by 2.3 fold (*G. claroideum*) compared to non-mycorrhizal olive trees. Concomitantly with an increase of salt in soil, the AM effect on K acquisition by olive plants was increased by 6.4 fold (*G. mosseae*), by 3.4 fold (*G. intraradices*) and by 3.7 fold (*G. claroideum*). This effect may be the main cause involved in compensating the detrimental effect of salinity. In conclusion, AM association plays an important role in enhancing olive tree growth and nutrition in saline soils.

7.27 Influence of arbuscular and saprobe fungi on the phytotoxic effects of dry olive residue in tomato plants

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The dry olive residue (DOR) obtained from the olive oil two-phase extraction system contains phytotoxic components, mainly phenols, capable of inhibiting microorganisms and plant growth. Although contamination of soils with DOR can be a serious problem, their remediation may be possible using saprobe fungi. On the other hand, there are evidences that AM fungi help the plant to thrive under polluted conditions. The aim of the work was to investigate the influence of saprobe fungi on the effect that has the DOR on AM symbiosis and plant growth. The application of DOR decreased the shoot and root dry weight of tomato inoculated with *G. deserticola*. The inoculation of saprobe fungi to DOR during 20 weeks led to a significant decrease of DOR phytotoxicity when was applied to plants cultivated in sterilized soil. The AM fungi *G. deserticola* improved plant growth in presence of DOR when its was previously incubated 20 weeks with saprobe fungi, therefore, both fungi can be used to transform the dry olive residue in organic fertilizer.

7.28 Apoplastic invertases in mycorrhizal Solanaceae

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In arbuscular mycorrhiza (AM) the obligate biotrophic AM-fungus is supplied by the plant symbiont with carbohydrates, most likely in form of hexoses. To fulfil the demand of apoplastic hexoses, extracellular invertases are suggested to play a crucial role. Therefore, apoplastic invertases were analyzed in *Glomus intraradices*-colonized wildtype plants. Additionally, effects of modulated invertase activity on AM were investigated using transgenic tobacco (*N. tabacum*) plants expressing an apoplast-located yeast invertase either under control of the alcohol inducible promoter system or constitutively using the 35S promoter. Invertase activity staining of wildtype tomato (*L. esculentum*) and tobacco roots revealed increased invertase activity near fungal structures. Analysis of the apoplastic tomato invertase *LIN6* showed promoter activation and transcript accumulation in arbusculated cells and near fungal hyphae as well as in the root central core upon AM. Root-specific induction of the apoplastic yeast-derived invertase led to enhanced hexose contents in the roots, but did not affect mycorrhization. In contrast, plants expressing the yeast invertase constitutively showed either a repression or a stimulation of mycorrhization, depending on the degree of invertase induction in the leaves. A strong invertase induction led to accumulation of leaf hexoses and reduced mycorrhization with less formation of fungal storage organs, whereas a moderate induction stimulated the mycorrhization.

7.29 Mycorrhizal inoculation enhances chromium accumulation in three plant species grown in chromium sludge

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Leather tanning, refineries, and paper-pulp industries generates large quantities of chromium which causes soil and water pollution and creates large number of wastelands through sludge disposal. Remediation of such environmentally vulnerable lands is a matter of concern. Phyto-bioremediation is a promising low cost biotechnological solution for remediation of such sites and extracting chromium as value addition. A study was undertaken to find out the response of AMF towards higher accumulation of chromium in three plants namely *Vetiver zizanoides*, *phragmites cummunis* and *Aloe vera* grown in chromium sludge (23,000 ppm Cr). The experiment was conducted in pots (size 1.7L) using completely randomized design. There were four types of AMF (AM1: *Glomus lamellosum*; AM2: *Glomus intraradices*; AM3: *Glomus proliferum*; AM4: mixture of 3 AMF) and one control and three plants giving 15 treatments and each treatment was replicated by five times. AMF inoculation was done @ 250 infectious propagules per plant. Experiment was harvested after three months of plantation and roots and shoots were analyzed for chromium accumulation. The analysis of results revealed that Mycorrhizal inoculation enhances chromium accumulation in shoots and roots of all three species used. Highest accumulation of chromium was found in the plants inoculated with *G. lamellosum* and *G. intraradices*. Whereas *Aloe vera* inoculated with *G. lamellosum* showed higher accumulation of Cr (1548 ppm) than other plant species.

7.30 *Agrostis capillaris* clones from contaminated and uncontaminated site vary in their response to inoculation with different *Glomus intraradices* strains

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Arbuscular mycorrhizal (AM) fungi have been repeatedly shown to affect plant growth and fitness under heavy metal (HM) stress. However, considerable variability among different plant-fungus combinations has been observed and the question remains about interaction of plant and fungus tolerance to HMs. The aim of the present study was to compare HM tolerance of six *Agrostis capillaris* clones associated with three different *Glomus intraradices* strains. Both plants and AM fungi originated either from contaminated or uncontaminated site. Three plant clones from contaminated and three from uncontaminated substrate were grown for 5 months in HM-contaminated substrate either uninoculated or inoculated with one of the three AM strains. There was a considerable difference in the development of *G. intraradices* isolates: The roots of plants inoculated with the isolate from contaminated substrate were highly colonised, while the isolate from uncontaminated substrate with high pH showed only negligible development. Also the plants from the uncontaminated substrate had generally lower shoot biomass than their counterparts from the contaminated site. A significant interaction between plant clone and AM treatment was observed, both in terms of plant growth and root colonisation. The study was financially supported by the Grant Agency of the Academy of Sciences of the Czech Republic, project No. B600050636.

7.31 Evaluation of the impact of mycorrhization with *Piriformospora indica* via the biophysical phenomics of the host plant accessed by the JIP-test

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Mycorrhizal activity has multiple effects on the physiology and vitality – activity and adaptability – of the host plant. For the detection and evaluation of mycorrhization effectiveness we apply an *in vivo* vitality analysis of photosystem II, a highly sensitive component of the plant to any stress. This analysis uses the JIP-test, which translates the fluorescence kinetics OJIP exhibited by all photosynthetic organisms upon illumination to behaviour patterns, thus leading to the description of a biophysical phenotype and, hence, termed as biophysical phenomics. We present a study of the impact of *Piriformospora indica*, an emerging growth booster with arbuscular mycorrhizal fungi (AMF)-like characteristics and the added advantage of being able to grow axenically, on Chick Peas (*Cicer arietinum* L. Chafa variety) exposed to cadmium stress, applying (a) the JIP-test equations – to derive and compare biophysical parameters, (b) normalisations and subtractions of transients of inoculated and non-inoculated plants without or under Cd stress – to deduce bands providing specific information for the impact on different microstates of the phenotype-macrostate. We demonstrate that the multi-level beneficial role of *P. indica* concerning the tolerance of the host plant to Cd is clearly revealed through pattern recognition and that biophysical and physiological parameters are strongly correlated. We also show that *P. indica* is as effective as typical AMF (*G. mosseae* and *G. caledonium*).

7.32 Effect of Cd on growth of three ectomycorrhizal fungi and *Pinus thunbergii* seedlings

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To clarify Cd tolerance of ectomycorrhizal fungi associated with *Pinus thunbergii*, we determined the growth of ectomycorrhizal fungi and of *P. thunbergii* -seedlings inoculated with the fungi in vitro. *Amanita rubescens*, *Pisolithus tinctorius* and *Rhizopogon rubescens*, were grown in MMN liquid media added with different concentrations of Cd: 0, 0.8, 8, 80 mg/l. After 40 days, dry weights were measured and the concentration of organic acids in the media was determined. Each fungal species was inoculated into *P. thunbergii* seedlings and then Cd was added into the seedlings at 0, 10 or 100 mg/kg. After Cd treatments, we determined the mycorrhizal formation rate. The dry weight of *A. rubescens* in the 8 mg/l was not significantly different from that in the 0 mg/l. The concentrations of organic acids in the 8 mg/l media of *A. rubescens* tended to be higher than those in the 0 mg/l. Mycelial weights of *P. tinctorius* and *R. rubescens* at 0.8 mg/l were significantly lower than those at 0 mg/l. In the *A. rubescens* -*P. thunbergii* mycorrhizal system, the mycorrhizal formation rate was significantly decreased at 10 mg/kg than that at 0 mg/kg. In *P. tinctorius* -inoculated seedlings, the rates were not significantly different among Cd treatments. These results suggested that *A.rubescens* may have a Cd tolerance, but the tolerance of fungi under mono cultural condition may not always reflect the tolerance of ectomycorrhizal associations.

7.33 Expression libraries-based investigation on cadmium molecular response in a metal tolerant fungal strain

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Fungi are ubiquitous in the natural environment, where they affect the form and the distribution of toxic metals. Physiological responses to metal contamination in fungi involve several defence mechanisms that can help them to resist or to reduce metal toxicity. Understanding these mechanisms, governing heavy metal resistance in fungi, is of great interest because of their potential applicability in bioremediation. Our investigations are focused on a strain of the ericoid mycorrhizal fungus *Oidiodendron maius*, sampled in a heavy metal contaminated soil. This strain can tolerate higher concentration of zinc and cadmium compared to isolates from unpolluted areas. Cadmium is a widespread pollutant, very toxic to all organisms. To identify new fungal genes involved in cadmium resistance, we screened an *O. maius* cDNA library using a yeast expression system. We employed the Cd-sensitive *S. cerevisiae* yap1 mutant as a recipient for the cDNA library, and the screening on Cd-containing medium yielded a sequence coding for a 176-aa protein. Database searches revealed homologues among fungal and plant sequences, but all sequences corresponded to "protein of unknown function DUF614". DUF614 indicates a Cys-rich protein family whose only member characterized so far is AtPcr1 (Plant cadmium response) a gene that confers Cd resistance to Arabidopsis. Likewise, we named our sequence OmFcr1 (Fungal cadmium response). The putative identity of this gene product will be discussed.

7.34 Effects of polycyclic aromatic hydrocarbons (PAH) on mycorrhization: contribution of the symbiotic association to pollutant dissipation

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PAH represent a group of persistent and toxic soil pollutants that are of major public concern due to their mutagenic and carcinogenic properties. The use of vegetation to improve bioremediation of PAH-polluted soils is an economic and low maintenance approach. Mycorrhizal plants are also known to be involved in the biodegradation of PAH and in the increase of stress tolerance. However, mechanisms involved in PAH degradation by mycorrhizal plants are still not clearly understood. An indirect degradation associated with the mycorrhiza-associated microflora has been demonstrated but no data about the direct implication of arbuscular mycorrhizal fungi and/or the colonized roots without other associated microorganisms are available. Ri T-DNA transformed chicory roots colonized by *Glomus intraradices* were cultivated in presence of two PAH: anthracene and benzo[a]pyrene. In spite of a reduced development of extraradical mycelium and low root colonization the fungus is able to fulfill its life cycle on polluted medium. Our results suggest that chicory roots, free of endomycorrhizal fungi, growing *in vitro* were able to contribute to PAH dissipation, but the presence of mycorrhizal fungi significantly enhanced the PAH dissipation in roots. These monoxenic experiments demonstrated a positive contribution of the symbiotic association in absence of any telluric microbial communities in PAH dissipation.

7.35 Root colonization by *Glomus mosseae* primes tomato plants for jasmonate-dependent defense responses

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Root colonization by arbuscular mycorrhizal (AM) fungi can improve plant resistance/tolerance to biotic stresses. Although this bioprotection has been observed in different plant systems, the underlying mechanisms remain largely unknown. In contrast to the Systemic Acquired Resistance triggered after infection with necrotizing pathogens, a direct activation of defense related mechanisms has not been observed. To investigate the mechanisms and signalling pathways involved in bioprotection by AM fungi, we compared the response of non mycorrhizal plants or plants colonized by either *Glomus mosseae* or *G. intraradices* to different defense related signals. Defense related gene expression and protein activities were monitored in a time course experiment after shoot treatment with jasmonic acid (JA), absisic acid (ABA), ethylene (ET) and salicylic acid (SA). Differential regulation of the defense responses triggered was observed in mycorrhizal plants. Remarkably, upon JA treatment, shoots of *G. mosseae* colonized plants showed 2 to 5 fold higher protease and glucanase activities and expression levels of typical defense-related JA responsive genes. Our results evidence different regulation patterns in plants colonized by *G. mosseae* or *G. intraradices* and point to a prominent role of priming for JA dependent responses in AM-induced resistance.

7. 36 Mycorrhizal responses to drought

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Climate models predict for Spain an increase in temperature along with a decline in rainfall and modifications of its seasonal patterns, leading to larger events separated by longer drought spells. These changes may affect mycorrhiza development in several ways, therefore having implications for terrestrial ecosystems. In semi arid SE Spain, where water is the most limiting factor, we expected changes in root colonization and mycorrhizal fungi species composition along a gradient in ecologically representative areas where there are significant changes in precipitation and temperature with elevation. To assess these effects we monitored *Stipa tenacissima* (a tussock grass species) and *Anthyllis cytisoides* (a leguminous shrub) for one year in the Sierra Alhamilla range. Preliminary results show different level of mycorrhizal colonization in the two species. While colonization of *Anthyllis cytisoides* increased with elevation, it remained steady of *Stipa tenacissima*. It seems that mycorrhizal associations respond to environmental conditions depending on the host species. It could be then expected that dryer conditions in arid ecosystems caused by climate change will alter mycorrhizal interactions, having large effects on the structure of plant communities.

SESSION 8

Functional diversity in mycorrhiza

Structure and function of an ectomycorrhizal fungal community

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We have investigated factors that influence the structure of an ectomycorrhizal fungal community and, to a limited extend, functional variation among species within that community. We monitored species frequencies over time, at different soil depths, and in different forest floor substrates. Soil depth, forest floor substrate and time significantly influenced species frequencies. Competition among species may have also occurred. Our results illustrate the roles of space, time and competition in structuring mycorrhizal fungal communities. We also found that different species possess different activities of enzymes important for nutrient uptake from the soil. Because of this functional variation among species, the structure of ectomycorrhizal fungal communities may influence ecosystem properties such as nutrient cycling, carbon cycling and productivity.

Linking functional and genetic diversity in the arbuscular mycorrhizal fungal symbiosis

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Identification of AMF communities in the field has revealed valuable information about the distribution of AMF and the factors that influence AMF community composition. However, it has proven difficult to link these observations with functional traits of AMF isolates. Several recent studies showed that AMF species or taxa, identified either by spore morphology or by ribosomal DNA are functionally diverse, even when these fungi were isolated from the same location. In this study we overcome this problem by looking at overall genetic and functional diversity of the AMF symbiosis. Some plant species associate with many fungal partners and host surprisingly diverse AMF communities while others enter into specialized relationships with only a few AMF. Until now there has been no functional explanation for the variation in AMF diversity among host plants. Here we propose that AMF diversity of a specific plant species in the field is inversely related to functional diversity in plant growth responses to AMF. It is expected that relatively low AMF diversity in a plant species in the field results from a preference for certain AMF types, and that this preference is found in plant species with highly variable responses to different AMF. In contrast, plant species that are unresponsive to AMF are not expected to select AMF, because this does not result in additional growth or fitness.

Does carbon flux from ectomycorrhizal fungi drive soil biodiversity?

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Several studies have identified that plants allocate substantial amounts of carbon to their ectomycorrhizal fungal partners, and that this carbon is allocated very rapidly. Given the extent and biomass of ectomycorrhizal fungi in forest soils, this carbon is likely to be an important energy source for free-living heterotrophic soil microbes. In this paper I will describe some recent experiments using radio- and stable isotopes of carbon applied in combination with molecular microbiological methods and community level physiological profiling to link the function of soil microbes with their diversity. The results demonstrate a close coupling between the flux of recently assimilated carbon from ectomycorrhizal fungi with the activity and community structure of free-living soil microbes.

A novel assembly of ericoid mycorrhizal fungi from arctic tundra varies in function and fungicide tolerance

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We synthesize three years of studies on the species and functional diversity of ericoid mycorrhizas (ErM) in an arctic long-term ecological research site in Alaska. The ErM fungi were isolated and molecularly identified as a novel assembly of *Phialocephala* sp. (195 isolates), *Cryptosporiopsis* sp. (72), cf. *Leptodontidium* sp. (12), *Irpex* sp. (12), two *Hymenoscyphus* spp. (10), *Lachnum* sp. (6), and *Mollisia* sp. (3). When tested in liquid medium with single organic and inorganic N sources or in a resynthesis system (with *Vaccinium uliginosum* or *V. vitis-idaea*) with the same N sources, the ErM fungi varied substantially in their ability to utilize different N sources. The ErM species also varied vastly in their growth and morphological responses when exposed to two broad-spectrum fungicides (azoxystrobin and propiconazole). The functional assessment of the ErM in a microcosm with intact tundra supports results from *in vitro* and *in planta* studies. The uptake of ^{15}N by *V. vitis-idea* from organic N was 40% lower when fungi were inhibited by the fungicides; inorganic N uptake was unaffected. We conclude that 1) the ErM communities in the high latitudes are novel; 2) the ErM species plus individuals within species vary vastly in their functional and fungicide tolerance attributes; and, 3) the ErM fungi are largely responsible for organic N uptake while the plants may access the inorganic N without mycorrhizal facilitation.

Nitrogen stable isotope measurements link carbon and nitrogen fluxes in ectomycorrhizal symbioses

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In field studies, it is difficult to partition carbon use and nitrogen uptake between fine roots and mycorrhizal fungi, and equally difficult to assess nitrogen availability and forms of available nitrogen in soil. Here, I use natural abundance stable isotopes (^{15}N:^{14}N ratios) to reveal carbon and nitrogen cycling patterns. ECM fungi with proteolytic capabilities have higher ^{15}N content and presumably greater carbon demand than fungi without such capabilities. Comparing host specificity of ECM fungi against ^{15}N patterns suggests that high host specificity may also be associated with proteolytic capabilities. Plant carbon allocation to ECM fungi in culture studies ranges from 1% to 20% of plant net primary production (NPP) and scales linearly with total belowground allocation. Such relationships could help to incorporate mycorrhizal fungi into ecosystem models used to predict forest responses to shifts in management practices, climate, or nitrogen deposition. Using several realistic assumptions, I derive relationships among carbon allocation to ECM fungi, nitrogen uptake by those fungi, nitrogen transport to host plants, and ^{15}N measurements in plants, available N, and ECM fungi. Decreased plant ^{15}N content in culture and field studies with declining nitrogen availability suggest that as nitrogen availability declines, both the importance of ECM fungi for plant nitrogen supply and the percent of plant NPP allocated to ECM fungi increases.

Plant phosphorus status represses the symbiotic phosphate uptake pathway in the tomato mycorrhiza

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In a mycorrhizal plant, orthophosphate (Pi) is delivered via two pathways, *i.e.* the direct uptake pathway at the plant-soil interface, and the mycorrhizal uptake pathway via the fungal mycelium. The mycorrhizal uptake pathway can be dominating in mycorrhizal plants, even in the absence of detectable growth responses. The molecular mechanisms involved in the regulation of the mycorrhizal Pi uptake pathway are not well understood and represented the major focus of the present work. We investigated the molecular physiology of AM-specific Pi uptake in the tomato variety Micro-Tom and the Pi transporter *LePT4* loss-of-function mutant *lept4-1* in association with *G. intraradices*. The AM symbiosis in *lept4-1* was functional, indicating that LePT4 activity is dispensable independent of the soil P availability most likely due to considerable redundancy between LePT4 and its mycorrhiza-inducible paralog(s). At high available P levels, high plant P status of both genotypes coincided with repression of the expression of the mycorrhiza-inducible Pi transporter genes. Moreover, repression of Pi transporters, reduced development of the extraradical hyphal network, and reduced root colonization resulted in an almost complete repression of symbiotic Pi transport. These and previously published data are consistent with a model in which both fungal and plant P status, and intracellular colonization of root cortex cells are the major regulators of the activity of the mycorrhizal Pi uptake pathway.

8.1 Functional anatomics of ectomycorrhizae

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Many features of ectomycorrhizae (ECM) appear to be functionally important. Mantles may provide protection against microbial attack of roots and buffer against rapid loss of water. They themselves can be protected by an array of mechanisms. Hydrophilic mantles are very likely able to acquire water and nutrients directly, whilst hydrophobic ECM with well developed rhizomorphs can transport nutrients over distances of several decimetres. Internal structures of mantles and rhizomorphs and modes of hyphal development may contribute to the efficiency of exploration, exploitation, and transport. A great diversity of differentiation patterns can be discerned and many of the characters are fungal species- or genus-specific. Others can characterize relationships of even higher systematic levels. However, in most of the cases, anatomical features can only provide hints at their physiological and ecological significance. Therefore detailed biological studies are urgently necessary to prove hypotheses regarding function. Analyses of putatively functionally and ecologically crucial features are the first aim of Functional Anatomics of ECM. A concert action of the ectomycorrhizal scientific community is necessary to study functions that are possibly mirrored by anatomical structures. This will provide important insights in the functional diversity of ECM in ecosystems, the ultimate aim of diversity studies. A selection of features will be discussed regarding possible functional benefits.

8.2 Too much of a good thing? The extraradical mycelium of 7 ectomycorrhizal fungi associated with *Pinus sylvestris* grown under ambient and elevated CO_2

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Responses to elevated CO_2 have often been studied from the plant's perspective (phytocentric approach) and less often from the fungal perspective (mycocentric approach) in which carbon and nutrient sink strength of the fungus plays an important role. This study was performed to investigate fungal species-specific responses of ectomycorrhizal (ECM) Scots pine (*Pinus sylvestris*) seedlings under ambient and elevated CO_2. Each seedling was associated with one of the ECM species: *Piloderma croceum*, *Hebeloma cylindrosporum*, *Paxillus involutus*, *Laccaria bicolor*, *Suillus bovinus*, *S. luteus*, *Boletus badius* or uninoculated under ambient and elevated CO_2 (350 or 700 $\mu l\ l^{-1}$ CO_2); each treatment contained 6 replicates. The trial lasted 156 d. During the final 28 days we labeled seedlings with $^{14}CO_2$. We measured plant biomass, nutrient content, ^{14}C allocation and length of the extraradical mycelium. Almost all parameters were significantly affected by fungal species and/or CO_2. There were very few significant interactions. Elevated CO_2 decreased shoot : root ratios. There were also large fungal effects on that ratio. Extraradical hyphal length was significantly negatively correlated with shoot weight and shoot N content, independently of CO_2 level. Apparently, extraradical hyphal length may be much more than is optimal from the plant' perspective. We'll discuss the role of fungal sink strength for carbon and nitrogen in constraining plant responses to elevated CO_2.

8.3 Ericoid mycorrhizal fungi of arctic tundra differ in their ability to use different inorganic and organic sources of nitrogen

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Ericaceous plants are a conspicuous component of tundra communities, in which most nitrogen occurs in organic form and nitrogen mineralization is low. Understanding functional diversity of ericoid mycorrhizal (ErM) fungi is essential to elucidating plant nitrogen acquisition in arctic ecosystems. Previous work in our lab found that the ErM fungal community in tundra near Toolik Lake, Alaska differs from those described for temperate and boreal ecosystems. Fungi forming ErM structures in roots of common ericaceous plant species at the site fell in lineages that had not been previously described as ErM. To test for functional diversity among ErM fungi in nitrogen acquisition, we grew isolates of the most frequently detected species in liquid culture with six different nitrogen sources. We observed both inter- and intra-specific variation in growth. To further explore intraspecific variation in nitrogen source use, we compared biomass between five nitrogen sources (ammonium, aspartic acid, glycine, serine, and threonine) for 23 isolates of the most frequently encountered species, *Phialocephala fortinii*. All isolates of *P. fortinii* grew most poorly on ammonium, but many differed from each other in the nitrogen source on which they grew best. Our work suggests that variation both among and within species of ErM fungi in use of different nitrogen sources may play a key role in maintaining fungal diversity and maximizing efficiency of nitrogen cycling in arctic tundra.

8.4 Arbuscular mycorrhizal fungal species determine their host's physiological identity

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We inoculated seedlings of a single host plant species, *Trichilia tuberculata*, with isolates of two arbuscular mycorrhizal fungal (AMF) species, *Acaulospora mellea* or *Scutellospora calospora* and compared differences in growth, allocation, photosynthetic capacity, and water uptake efficiency with nonmycorrhizal controls. We related growth and allocation patterns to underlying differences in host physiology. Mycorrhizal plants grew significantly faster than nonmycorrhizal controls. Moreover, host biomass gain varied quantitatively with their AMF symbiont (*S. calospora* > *A. mellea* > control). In *S. calospora*-inoculated seedlings, higher growth was associated with improvements in the efficiency of whole plant carbon gain (net assimilation rate per unit leaf area: ULR) without adjustments in allocation of their total biomass to leaf area (leaf area ratio: LAR) relative to controls. This increase in carbon gain efficiency for *S. calospora* plants was directly correlated with a significant increase in water uptake efficiency. In contrast, increased growth in *A. mellea* seedlings was associated with an increase in LAR. Thus, growth differences between AMF species were not only quantitavely different, but the identity of the fungal mutualist qualitatively affected a single plant species by contrasting physiological mechanisms, each of which could offer variable degrees of advantage under different environmental conditions.

8.5 Induction of phytochemicals in AM purple coneflower

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Purple coneflower, *Echinacea purpurea* L., is an important phytomedicinal plant species. Phenolics and alkamides produced in this species were shown to have anti-pathogenic properties. This factorial greenhouse study aimed to determine the time-lapse effects of arbuscular mycorrhizal (AM) colonization by *Glomus intraradices* Schenck & Smith, in combination with simulated herbivory (jasmonic acid), on plants of purple coneflower. Overall, our results showed three-fold greater biomass for AM than non-AM plants, as well as higher total content of alkamides and phenolic compounds (e.g., cynarin, and cichoric, caftaric and chlorogenic acids) in roots and/or shoots. We detected similar trends when taking into account the herbivory factor or the plant phenological stage. The AM plants also had enhanced protein concentrations, whereas our indicator of nitrogen assimilation (glutamine synthetase) did not vary significantly among the treatments. By optimizing the environmental conditions of purple coneflower, these studies, collectively, will contribute to improving phytomedicinal production.

8.6 Astronomical density of the *Rhizopogon* soil spore banks in California pine forests

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An important functional guild is defined by ectomycorrhizal species that form spore banks, as these species provide inoculum following severe disturbance. *Rhizopogon* species are a significant component of this guild in Western North American pine forests. Prior work has shown that roughly half the test seedlings planted into highly diluted pine soils will be colonized by *Rhizopogon* , and five to eight species are usually retrieved from each site. We used serial dilutions of known quantities of spores from three difference species to quantify the concentration needed for colonization of pine seedlings. We found that all three species exhibited a sigmoid relationship between spore concentration and percent of seedlings colonized. We used regressions of these relationships to predict spore concentration of soil from observed percentages of seedlings colonized from dilute soil samples taken from 41 diverse pine forests. For several technical reasons we are likely to have underestimated the true values, yet we find that an average hectare of pine forest has at least 1.6 X 10^{12} ± 2.3 X 10^{11} (SE) spores. These spore banks are patchy, with hot spots exceeding 4800 spores/ml, but even the least dense zones contain 36 spores/ml, which translates to 3.6 million spores per square meter in the top 10 cm of soil. These astronomical values demonstrate the abundance of the standing crop of spores for a portion of a single genus, but represents a fraction of the entire spore bank.

8.7 Impact of the plant-host (conifer/hardwood) on the specific and functional diversity of ectomycorrhizal fungi

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We studied the impact of trees substitutions on the ECM diversity in a temperate forest. This study also aimed at determining up to which point the plant host (conifer/hardwood) can affect the functional role of mycorrhizal fungi in the hydro-mineral nutrition. To study the fungal diversity of an experimental French forest site (Burgundy), the fruit bodies (saprotroph and mycorrhizal) were sampled and identified in different treatments (conifer and hardwood plantations) during 4 years; and morphological typing of ECM tips was realised during one autumn. Near to 300 fungal species were identified whose more fifty percent were ectomycorrhizal. The strong impact of the hardwood substitution by conifer plantations will be discussed. Moreover, to compare the influence of two plant hosts (Douglas fir and beech) on the ECM activities, hydrolytic and oxidative enzymes activities (involved in the decomposition of ligno-cellulose, chitin and P-containing organic compounds) of ECM fungus *Laccaria bicolor* were measured during two years in nursery. A microplate multiple enzymatic test was used to reveal a high diversity of activity profiles between *L. bicolor* associated with the conifer and the same fungus associated with the hardwood, in particular during the winter period. The potential saprotrophic functions of *L. bicolor* in specific conditions (winter period in temperate forests) and the role of ECM fungi during the bud break will be also discussed.

8.8 Effects of three AM fungi on growth, distribution of peltate hairs and essential oil production in *Ocimum basilicum* L. var. Genovese

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The essential oils of basil are widely used in the cosmetic, pharmaceutical, food and flavouring industries. Little is known about the potential of arbuscular mycorrhizal (AM) fungi to affect the production of essential oil in aromatic plants. The effects of colonization by three AM fungi *Glomus mosseae*, *Gigaspora margarita* and *Gi. rosea* on shoot and root biomass, abundance of glandular hairs and essential oil yield of *Ocimum basilicum* L. var. Genovese were studied. The AM fungi induced various modifications of the considered parameters, but only *Gi. rosea* significantly affected all of them in comparison with control plants or the other fungal treatments. It significantly increased biomass, leaf area, root branching and length, and the total amount of essential oil (especially ·-terpineol). Increased oil yield was associated to a significantly larger number of peltate glandular trichomes (main sites of essential oil synthesis) in the basal and central leaf zones. Futhermore, *Gi. margarita* and *Gi. rosea* percentually increased eugenol and reduced linalool yield. Near Infra Red Spectroscopy of intact leaf and Electronic Nose analysis both confirmed a high level of diversity ($R^2>0.5$), therefore NIR well distinguished inoculated and control plants while EN figures were closer to the chemical aromatic profiles. Results showed that different fungi can induce different effects on the same plant and that the essential oil yield can be modulated according to the colonizing AM fungus.

8.9 Seasonal changes of species and enzymatic activity profiles in the ectomycorrhizal community of an oak forest

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The structure of an ectomycorrhizal (ECM) community has been monitored in a mixed hardwood forest (*Quercus robur*, *Quercus petraea*, *Carpinus betulus*) in north-eastern France over more than one year. In addition to species identification by sequencing the ITS rDNA, populations of individual ECM tips of the different morphotypes were subjected to a range of enzyme assays relevant with the mobilization of soil nutrients. The abundance and the potential enzyme activity profiles of the ECM tips vary seasonaly and with vertical and horizontal distribution. The morphotypes formed by *Lactarius quietus*, *Tomentella sublilacina* and *Cenococcum geophilum* remained present the whole year long. In contrast, other morphotypes were very seasonal (*Clavulina cristata* in winter) or sporadically abundant (*Entoloma nitidum* in July). According to their abundance and their activity profiles, we modelized the annual global activity of the ECM community. The seasonal global activity of the community is due to a relay between species : several species contibute to the global activity at one time, but it is not necessarily the same ones all the year long. The specific composition of the community is changing whereas the specific functions seems to be constant, being the sign of a kind of homeostasis at the community scale. Here, some key concepts of Ecology such as functional diversity, redundancy or complementarity of functions between populations can be applied to ECM communities.

8.10 Plant functional group responses to arbuscular mycorrhizal fungi

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Plant species with similar traits or similar effects on major ecosystem processes are often clustered into plant functional groups. Until now it is unclear whether plant functional groups can be distinguished from each other based on their interaction with mycorrhizal fungal symbionts. In this study we tested whether members of two important plant functional groups (legumes and grasses) responded differently to arbuscular mycorrhizal fungi (AMF). Two legume species (*Lotus corniculatus* and *Trifolium repens*) and two grasses (*Anthoxantum odoratum* and *Poa pratensis*), which are characteristic for dune grasslands, were grown without AMF, inoculated with two distinctive AMF types or were inoculated with a mixture of both AMF types. The impact of these different AMF treatments on plant growth was investigated. The two legume species were highly dependent on AMF and the presence of AMF increased their biomass up to 20 fold. Moreover, both legumes responded differently to the different AMF treatments indicating that the composition of AMF communities influences their growth. In contrast, both grasses did not profit from AMF. Our results suggest that these two important plant functional groups can be distinguished from each other based on their response to AMF. Moreover, our results indicate that growth of legumes is not only dependent on the presence of nitrogen fixing rhizobium bacteria, but that legumes also require mycorrhizal fungal symbionts to be successful.

8.11 Prepackaged symbioses': propagules on roots of the myco-heterotrophic plant *Arachnitis uniflora*

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Arachnitis uniflora is a myco-heterotrophic, achlorophyllous plant species with a restricted distribution. Plants are visible above ground only at the time of flowering. Each plant consists of fleshy tuberous roots colonized by fungal hyphae, and a flower stalk that bears a single flower. The fungus present in the roots belongs to the genus *Glomus*, an arbuscular mycorrhiza genus in the phylum Glomeromycota. This is the only myco-heterotrophic plant species that is known to associate with this group of fungi. Hyphal connections are formed between *A. uniflora* roots and the roots of surrounding photosynthetic plants through which carbon compounds needed for growth and reproduction are acquired. The fleshy roots produce propagules from their apices and from lateral sites along the root axis. The former are initiated immediately beneath the root cap and, with development, a shoot apical meristem is initiated and the basal region of the propagule becomes colonized by fungal hyphae that originate from colonized cells in the parent root. Lateral propagules are initiated in cortical cells and these also develop a shoot meristem and become colonized by fungal hyphae. Preliminary molecular evidence (A. Schüßler) confirms that the same mycosymbiont is present in roots and propagules. When propagules detach from the parent root, new plants are established that are already colonized by an arbuscular mycorrhizal fungus. This method of vegetative propagation is unique to this species.

8.12 Changes in phosphatase activities and root system morphology induced by *Glomus mosseae* in palm roots

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In the present work we study the effect of different P and N doses supplied to the plants *Brahea armata, Chamaerops humilis, Phoenix canariensis* and *P. dactylifera* on the activity of acid (ACP) and alkaline (ALP) phosphatases in mycorrhizal and nonmycorrhizal roots. Further, the changes induced in AM colonization under the different fertilization levels on the root system morphology have been evaluated. Although the ACP and ALP activities were in general higher in mycorrhizal than in nonmycorrhizal palm roots, it was demonstrated that this depended on both the fertilization level and the palm species. *B. armata* and *C. humilis* responded with an increase in phosphatase activities only in the absence of exogenous phosphate or nitrate sources, while both *Phoenix* species showed increased phosphatase activities independently of the fertilization levels. The ultrastructural localization of ACP for the palm root-*Glomus mosseae* system, characterized by the formation of intermediate types of mycorrhizal morphology, is also described here. The AM colonization induced changes in the root system morphology of *P. canariensis* and *P. dactylifera* at all fertilization levels, while in *B. armata* and *C. humilis* the changes were limited to certain fertilization treatments. The results presented here are discussed in regard to mycorrhizal efficiency. This work was supported by Project REN2003-08241/GLO from MEC, Spain.

8.13 Symbiotic soil fungi drive carbon allocation below ground

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In recent years the potential for ectomycorrhizal (EM) fungi to influence forest carbon and nutrient cycles has become increasingly evident. Carbon availability to EM fungi is likely to increase at elevated atmospheric CO_2, and fungi are potentially an important component of how forests respond. To find out whether there are any broad patterns in species' responses which relate to their ecology, we compared the growth, respiration and C-exudation of 17 EM isolates (13 species) in liquid culture. We used three different C:N ratios (10:1, 20:1 and 40:1), in effect moving from conditions of C-limitation to conditions of N-limitation. Responses to increased C-availability were species-specific. Suilloid fungi were the most responsive in terms of growth and respiration (up to 4.2 times higher at high C-availability than that at low). In contrast, a group of 8 isolates showed no growth increase above C:N 20:1. This inability to respond was not due to N-limitation, although there were marked differences in N uptake between isolates. At higher C-availability isolates generally became more efficient in converting C into biomass. Seven isolates showed net release of C-based exudates into the medium. These results are discussed in relation to the role of EM fungi in nutrient cycling at elevated CO_2, to previous work on the effect of elevated CO_2 on EM communities and to current ideas about functional groups and ecological strategies of EM fungi.

8.14 Genetic and functional diversity within the *Hymenoscyphus ericae* aggregate

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The *H. ericae* aggregate has recently been re-classified to comprise 2 related groups of fungi, *Meliniomyces* species and *Rhizoscyphus ericae*. Representatives of these groups are found worldwide in diverse habitats such as forests or heathlands. Some strains may form both ericoid and ECM associations. Although the genetic diversity of this group is being unravelled, its functional significance is still under-explored. We have screened 3 closely related strains of *Rhizosyphus ericae* and assessed their growth in pure culture when fed with 3 different sources of N. The 3 strains showed significant variations in their patterns of N source utilisation. When seedlings of *Vaccinium* species were infected with these 3 strains, N transfer to plant shoot was well predicted by the ability of fungal endophytes to utilize N source in pure culture. Our result is the first report of a direct *quantitative* relationship between fungal N use in pure culture and plant N uptake in symbiosis. Our study clearly demonstrates that relatively small genetic variations in mycorrhizal fungi may translate into functional diversity at the symbiosis level. To continue this work, we are currently questioning whether different *Meliniomyces* genotypes colonize roots of co-occurring ericaceous and coniferous plant species, with the aim of assessing how such fungal-plant relationship function and / or differ when different partners are involved.

8.15 The potential of the in-growth mesh bag system to study functional diversity of ectomycorrhizal mycelia in the field

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The ectomycorrhizal (EM) mycelium has been shown to play an active role in nutrient acquisition from sources not available to the host roots. EM-species vary in their ability to colonise substrates with different nutrient contents, both when growing individually and in competition with other EM-species. Different species also vary in how their external mycelium explores the soil substrate. The external EM mycelia are thus a vital part of the expressed functional diversity in the field situation. The soil is a diverse environment and both potential resources and occurrence of single mycorrhizal species are patchy. Therefore we think a method where direct observation in the field of mycelia exploring a specific bait substrate would be an important contribution for studies of functional diversity. In the present experiment, sand filled in-growth mesh bags were used to selectively attract EM mycelia. Half of the bags were amended with apatite in order to select for EM species that weather apatite in P-deficient forest soil. The bags were buried between the humus layer and mineral soil for one growth season before harvesting. The mycelia colonising the bags were subsequently subjected to DNA extraction, PCR of the ITS, cloning, sequencing and identification against the UNITE database. The results are discussed in relation to the potential of the method to detect functional diversity in the field. See also presentation by Wallander & Thelin.

8.16 Do arbuscular mycorrhizal fungi differ in their foraging strategies?

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The arbuscular mycorrhizal fungus *Glomus hoi* has been shown to capture nutrients from decomposed organic material. To extend the study of this nutrient scavenging behaviour from organic material, we measured nutrient uptake and growth of *Plantago lanceolata* plants colonised by either *G. hoi* or *G. intraradices*. Growth containers consisted of plant and fungal compartments separated by meshes to exclude roots from the fungal compartment. Both roots and hyphae were excluded in control groups. A patch of $^{15}N/^{13}C$ dual-labelled grass shoot material was added to the fungal compartment. Plants colonised by *G. hoi* captured more P than those colonised with *G. intraradices* grown without an additional fungal compartment, but when access to the patch was allowed both fungi were equally effective at promoting host plant total P capture. Plants colonised by *G. intraradices* showed significant ^{15}N enrichment compared to those colonised by *G. hoi* when allowed access to the ^{15}N labelled organic patch, but colonisation by *G. intraradices* significantly reduced plant size probably because they supported a greater fungal biomass. *G. hoi* seems better than *G. intraradices* at taking up nutrients from a restricted soil volume under nutrient limiting conditions. *G. intraradices* however seems to have the greater capability for nutrient uptake from further distances and from patches of decomposing organic material, although at a greater carbon cost of producing additional fungal structures.

8.17 Comparative studies of arbutoid mycorrhizas in several genera of Pyrolaceae (Ericales) from Western Canada

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In spite of their widespread distribution in north temperate environments and their importance in ecosystems in terms of maintaining the diversity of mycorrhizal fungi, the colonization patterns of many genera in the Pyrolaceae that form arbutoid mycorrhizas are poorly studied. We sampled five species, including *Moneses uniflora* (Single delight), *Chimaphila umbellata* (Prince's pine), *Orthilia secunda* (One-sided wintergreen), *Pyrola asarifolia* (Common pink wintergreen) and *P. chlorantha* (Greenflowered wintergreen) in three locations near Prince George, British Columbia. Samples were processed for light, scanning electron, and laser scanning confocal microscopy to compare colonization patterns of roots by mycorrhizal fungi. All species had thick rhizomes (several cm to much longer) with clusters of first and second order mycorrhizal roots. Mycorrhizal morphotypes were numerous and included both ascomycetes and basidiomycetes. In some, well-developed fungal mantles were present whereas in others there was little apparent external colonization. In all species a paraepidermal Hartig net and intracellular hyphal complexes, restricted to epidermal cells, developed. Hartig net hyphae adjacent to the inner tangential wall of epidermal cells initiated hyphal complexes. Differences in degree of hyphal complex colonization, abundance, and location along the mycorrhizal root axis occurred. This study extends the number of species in the Pyrolaceae that show a typical arbutoid mycorrhiza.

8.18 Identification of arbuscular mycorrhizal isolated from Algerian *Olea* plantations and analysis of their growth effect on micropropagated olive trees

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A field survey of the arbuscular mycorrhizal (AM) status of *Olea* tree plantations, located in three distinct sites of Algeria, was performed throughout the various seasons. Six AM fungal morphotypes have been successfully isolated by trap cultures with leek and clover plants. All the AM fungal morphotypes isolated belonged to the genera *Glomus* and *Entrophospora*. The symbiotic efficiency of five of them was tested with micropropagated olive trees, and compared to the efficiency of the indigenous soil microflora in the three native soils. Three fungi were phylotyped by sequencing the 5' end of large ribosomal subunit DNA, and identified as *Glomus* isolates (A, B and C). All three colonized olive roots intensely and promoted plant growth by 400%. Information on the AM symbiosis is still scarce or absent for many North African countries. The effectiveness of AM fungi for olive tree production is discussed.

8.19 Detection of extracellular proteases produced by ectomycorrhizal fungi

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In northern forest ecosystems, most soil nitrogen (N) is in organic form and forest trees are dependent on symbiotic ectomycorrhizal (ECM) fungi and their degradative abilities for N uptake. The ability of ECM fungi to acquire N from organic substrates should, therefore, be a widespread trait given its ecological importance. However, little is known about the degradative abilities of the dominant ECM fungi as most taxa are considered intractable. Here, we present data on extracellular protease production by 26 species of ECM fungi, the majority belonging to this category. Protease activity was detected using either a fluorescently labelled protein (FITC-BSA) as a substrate or visualized using gel electrophoresis (zymograms) or milk powder plates by the breakdown of insoluble proteins. The majority (24/26) of species produced detectable protease activity. However, detection was method dependent. In general, activity was too low to be detected by the FITC-BSA assay. Zymograms detected proteases in *Amanita muscaria, Russula chloroides, Lactarius deterrimus* and *L. quieticolor*. Growth on milk powder plates was the most effective method for detecting protease production by intractable ECM species. The study supports the hypothesis that protease production is a widespread physiological trait in ECM fungi and that this ability is of considerable significance for nitrogen uptake in forest ecosystems.

8.20 *Hymenoscyphus ericae*, an ericoid mycorrhizal fungus, utilizes cleaved DNA rather than intact DNA as phosphorus sources at low concentrations

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Phosphodiesters such as DNA are important phosphorus (P) sources for plant growth due to the fact that their turnover is rapid in the soil. However there is little information on the ability of mycorrhizal fungi to utilize DNA-P. Growth of *Hymenoscyphus ericae*, an ericoid mycorrhizal fungus, was studied in liquid culture containing different forms and concentrations of DNA as P sources. These P sources were incorporated into the medium as salmon testes DNA or DNA from cells of *Arabidopsis thaliana* which were either intact or had undergone programmed-cell-death (PCD). The production of cells with PCD morphology was achieved in cultures of *A. thaliana* after exposure to heat stress. The DNA extracted from salmon testes and intact cells of *A. thaliana* was less degraded than DNA extracted from *A. thaliana* which had undergone PCD. Mycelial growth of *H. ericae* increased in the range 0-2 mM P of salmon testes DNA. At 0-250 µM P of salmon testes DNA, growth was similar in the ranges of 0-10 µM P and 50-250 µM P but mycelial dry mass was significantly less at 0-10 µM P compared with 50-250 µM P. At 2 µM P, mycelial dry mass of *H. ericae* on media containing DNA extracted from PCD cells of *A. thaliana* was significantly greater than that grown on media containing no P or DNA extracted from intact cells. The DNA in the soil is likely to come from cells having undergone PCD. This study demonstrates that PCD-induced DNA is a more accessible P source to *H. ericae* at low DNA concentrations.

8.21 Space occupation of ectomycorrhizal mycelia

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In most species of ectomycorrhizae (ECM), nutrient uptake is performed by the extramatrical mycelium consisting of single hyphae or rhizomorphs. Depending on the organisation of the mycelium, four different exploration types (ETYs) of ECM can be distinguished, which differ strongly in structure and extension as well as in mycelial biomass. In general, there is a broad range from ECM with almost no mycelium (contact ETY) up to the long distance ETY forming extensive and far reaching mycelia. Our investigations focussed on the calculation of carbon allocation to different ETYs and on their space occupation. To get data for each ETY, synthesized mycorrhizal systems were photographed with a high resolution digital camera. Image analysis allows measurement of mycelial densities according to defined distance areas from the mantle. The following data set was obtained for typical representatives of each ETY (e. g. *Tylospora, Piloderma, Cortinarius*, and *Rhizopogon*): biomass in mg per unit length of ECM, maximum reach and two dimensional space occupation per unit length of ECM. These data can now be used to estimate mycelial biomass and space occupation in the field. Depending on the ETY, space occupation of root systems increases significantly when the mycelia of associated fungal partners are taken into account. In combination with the results of enzymatic activity of ECM, conclusions on kind and amount of nutrient mobilization and transfer to the tree partners might be possible.

8.22 *Arabidopsis thaliana* colonization by ascomycetous root endophytes

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Arabidopsis thaliana is generally considered non-mycorrhizal. However, it can be colonized by a basidiomycetous endophyte that promotes its growth. Our preliminary data suggest that *A. thaliana* hosts a range of other endophytes that also stimulate its growth. We have acquired several root-colonizing ascomycetes from five Long Term Ecological Research sites representing tallgrass prairie, shortgrass prairie, arid grassland, oak savanna, and high montane meadow ecosystems in the western US. Resynthesis studies with *Allium porrum* confirm these fungi are endophytic. Many of these endophytes also colonize *A. thaliana* and may stimulate its growth. To screen for *A. thaliana* colonization, seeds were germinated on lawns of putative endophytes, grown in a closed resynthesis system and analyzed microscopically. Here, we also report studies that focus on the endohytes' effects on *A. thaliana* seed germination and growth as well as their intracellular colonization. Our studies thus far show that endophytes (*Periconia macrospinosa*, *Periconia sp.* and *Microdochium sp.*) from a tallgrass prairie readily colonize *A. thaliana*. We present these results and results from ongoing *A. thaliana* resynthesis studies that include a number of additional putative endophytes (*Microdochium sp.*, *Neophaeosphaeria barii*, and *Alternaria longissima*) from a shortgrass prairie. Based on the results thus far, we expect that these endophytes could colonize and form functional mutualisms with *A. thaliana*.

8.23 Characterization of tomato response to inoculation with two cuban isolates of arbuscular mycorrhizal fungi

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During several years our Institute has worked in the application of arbuscular mycorrhizal fungi (AMF) to different crops in many Latin American countries and in Spain, obtaining significant increments in agricultural yields. Among studied strains the better results were found with two cuban isolates from *Glomus* genus: *Glomus mosseae* and *Glomus* sp. The aim of this work was to study comparatively the biochemical-physiologic responses of tomato (cuban cv. Amalia) to inoculation with these two AMF strains. Some experiments under controlled conditions were carried out, using a sterile mixture of Red Ferralitic soil and filter cake. Fungal variables (percentages of mycorrhizal colonization and fungal density); growth variables (height, total fresh and dry weights, leaf number and foliar surface); as well as biochemical variables (activity and expression of enzymes related to symbiosis) were determined. Moreover, the protective effect of both strains against different pathogens was evaluated. This study revealed very interesting information about tomato responses to inoculation with two different AMF strains. Results constitute an excellent complement to crop behavior observed under field conditions. In general, the best results were obtained in tomato plants inoculated with *Glomus* sp, suggesting a higher compatibility of tomato with this strain and, fundamentally, a best functioning under studied conditions.

8.24 Mycorrhizal type affects carbon allocation and isotope patterns in alder

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Mycorrhizal root colonization affects a variety of parameters that influence plant carbon (C) isotope patterns. Comparisons between arbuscular (AM) and ectomycorrhizas (EM) indicate similar effects on host plant physiology. However, different host plants complicate interpretation. The present study was conducted to directly compare the effect of AM versus EM on C allocation and $\partial^{13}C$ of the same host plant species. Grey alders, either non-mycorrhizal, AM or EM were grown in a semihydroponic system under controlled environmental conditions. Plants were not inoculated with *Frankia* and supplied with either sufficient or limiting amounts of nitrogen (N). Harvested plant material was analysed for C content and isotopic composition by isotope ratio mass spectrometry. Leaf photosynthetic and transpiration rates were also measured. Mycorrhizal root colonisation had either no or a negative effect on plant growth. Biomass distribution between leaves and stems was not affected by mycorrhizal status. In contrast, C allocation was significantly affected, but only under N limitation. Nitrogen-limited mycorrhizal plants invested more C into leaves than non-mycorrhizal plants. Leaf C isotope patterns were significantly affected by N supply as well as mycorrhizal status. Especially under N limitation, mycorrhizal plants tended to be more depleted in ^{13}C than non-mycorrhizal plants indicating a lower water use efficiency.

8.25 The relationships between P dynamics in AM hyphae and mycorrhizal responses on plants

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Plant growth of leek was greatly improved by arbuscular mycorrhizal (AM) fungus, *Scutellospora calospora* in low-P soil-sand mix, while the response of cucumber by the fungus was less than that of leek. Light intensity, soil P availability and particular combinations of fungi and plants have been believed to cause the different mycorrhizal responses. It also has been pointed out that P dynamics (P metabolism, P transport rate and amount of P transported) in AM hyphae are different in each combination, and that soil P availability affects P dynamics. We hypothesize that P dynamics in AM hyphae is closely related to different plant responses. Cucumber and leek were grown in a pot as host plants with *Scutellospora calospora* in –P or +P soil, plus a small container which only hyphae could access inside was buried in the pot. Twenty-four and 72 hours before harvesting, radioactive phosphorus (^{32}P) was applied to the container to measure the speed of P transported. The hyphae were collected from the container, and then Poly-P, a main compound in AM hyphae, was quantified by Polyphosphate Kinase (PPK)/Luciferase method, the localization of poly-P was determined by Toluidine Blue O. The influence on Poly-P accumulation in the hyphae by soil P availability will be discussed.

8.26 *Cheating* revisited: new insights into functional diversity in arbuscular mycorrhizal symbioses and its ecological significance

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The responsiveness of arbuscular mycorrhizal (AM) plants in terms of increased uptake of nutrients such as phosphate and increased growth over non-AM controls depends on genomic properties of both the plants alone and those of the AM fungi with which they associate. It is conventionally assumed that unresponsive AM plants gain no nutritional benefits (i.e. the fungus 'cheats' or 'exploits' its host). Sometimes it is even assumed that the symbioses have no nutritional function in plant ecosystems. Here we use results from compartmented pot systems to show that unresponsive AM plants can, depending on the individual fungi, gain large amounts of P via their fungal partners. This physiological evidence is increasingly supported by molecular biology, in that genes for AM-inducible plant P transporters are expressed in unresponsive AM plants, just as they are in responsive ones. We also show that hidden P transport into AM plants that are unresponsive in simple pot experiments confers benefits to their hosts when they are in competition with other plants, whether AM or otherwise. These findings will help to put a functional basis on ecological models for roles of AM fungal symbioses in plant competition. However, to disentangle AM functional diversity at the plant population or community level it will be necessary to do 'reductionist' experiments. We will argue that to consider such an approach as ecologically irrelevant, as is sometimes done, will be very unwise.

8.27 Dispersed and aggregated forms of polyphosphate are observed in freeze-substituted intraradical hyphae and germ tubes of *Gigaspora margarita*

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Arbuscular mycorrhizas are well known to enhance phosphorus uptake of host plants. Phosphate is collected by extraradical hyphae extending into soil, translocated to intraradical hyphae and finally transferred from the fungus to the plant. An extensive tubular vacuole system has been documented in germ tubes, intraradical and extraradical hyphae in *Gigaspora margarita*. This system is thought to be responsible for translocating and storing phosphate in the form of polyphosphate (polyP). To visualize polyP in the vacuoles, quick-freeze and freeze-substitution techniques were employed to germ tubes, and to onion roots colonized by the fungus with a modification. Semi-thin sections of resin-embedded material were stained with DAPI which binds to polyP, a complex that emits yellow fluorescence under UV excitation. Ultra-thin sections were observed by TEM to exclude dead cells or artifacts caused by sample preparation. In strongly-stained germ tubes, polyP was found mostly in a dispersed form in spherical and tubular vacuoles. Intense yellow spots were also occasionally found associated with these vacuoles. Using fluorescence microscopy, endosymbiotic bacteria of this fungus were recognized as blue and present as a single or diplococcal dot. Fluorescence microscopy and TEM showed that vacuoles of intraradical hyphae simultaneously contain dispersed and aggregated forms of polyP. The aggregate form was confirmed to be present within vacuoles, associated with the membrane.

SESSION 9

Biodiversity and ecological impacts

Fine scale distribution of pine ectomycorrhizal fungi

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The extramatrical mycelium (EMM) of ectomycorrhizal (ECM) fungi is an important sink for host carbon and is functionally important in nutrient and water capture. Further understanding of how ECM mycelial communities are structured, particularly in relation to colonised root tips, is required to advance our understanding of the functional importance of individual species in mixed communities. To investigate the fine scale distribution of pine ECM fungi, we sampled four 20 cm x 20 cm x 2 cm forest soil slices. Each slice was divided into one hundred 2 cm cubes. For each cube, ECM root tips were identified and the presence of EMM of those fungi determined by ITS TRFLP analysis. In addition, other ECM species present in the cubes but not detected as tips were identified using a TRFLP database containing ECM fungi collected from the field site. For the seven ECM species detected as tips, there was a clear spatial segregation between mycorrhizas and EMM with some species producing EMM in different soil-layers to their mycorrhizas. The spatial relationship between mycorrhizas and their EMM generally conformed to their reported exploration types, but EMM of smooth types (eg *Lactarius rufus*) was more frequent than expected. In addition to the EMM of the 7 species detected as mycorrhizas, DNA of a further *ca.* 40 ECM species not present as tips were detected. Their spatial distribution patterns and potential biological origin is discussed.

Fragmented landscapes – The role of dispersal, abiotic factors and competition for community assemblage of AMF

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A mounting body of literature indicates that while AMF species might be distributed world-wide, local AMF communities might differ between host plants, soil types, and seasons. Therefore, the environment for AMF species might be a fragmented mosaic of differing habitat qualities. We combined controlled greenhouse experiments and field samplings in two areas to address the relative importance of dispersal, abiotic factors and competition for the composition of AMF communities. In an agroecosystem in Zimbabwe, consisting of a patchwork of sand and clay soils, abiotic factors and competition affected the family-level distribution of species. Within each soil type, sites that were closer together were more similar than sites that were farther apart, suggesting that dispersal influenced the community composition on a sub-family level. In Yellowstone National Park, USA, geothermal areas are interspersed between non-thermal areas that possess very different soil temperature, pH regimes, and plant communities. Here we found little or no difference in AMF species between thermal areas, suggesting that dispersal occurs across the mosaic. The influence of abiotic factors and competition on AMF community composition in thermal and non-thermal areas is currently researched and will be discussed. Our work indicates that dispersal, abiotic factors and competitive interactions could influence community composition of AMF, but their relative importance might differ between environments.

Some trends in the global distribution of the genus *Scutellospora*: a preliminary study

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Studies about geographical distribution of arbuscular mycorrhizal fungi (AMF) at a regional or global scale are scarce, and knowledge of the ecological conditions associated with their distribution is poor. However, some hypotheses have been proposed for the genus *Scutellospora*. Local studies suggest that this genus is susceptible to disturbance and generally disappears with anthropogenic pressure; it seems to be associated with sandy soils and it has been suggested that may be present in greater diversity in tropical regions. In this work we performed a bibliographical review to compile records of the presence of *Scutellospora* species in different ecological conditions and at different latitudes, in order to evaluate the existence of these trends in *Scutellospora* distribution. In spite of preliminary character of this review, we find that: a) the presence of *Scutellospora* was not restricted to environments with low disturbance, b) a clear association exists between *Scutellospora* and sandy soils and c) there is a strong bias in sampling efforts toward temperate regions over tropical areas. This bias makes conclusions about the existence of an increase of diversity toward the tropics difficult at the present time. Nevertheless, it would be valuable to further describe AMF diversity in tropical regions, as well as to do greater efforts to integrate available information to understand the distribution patterns of AMF.

Communities of AM fungi in agricultural habitats and landscapes of Central Europe

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We studied the occurrence of arbuscular mycorrhizal fungi (AMF) in selected grasslands and arable lands in Central European lowland, mountainous and alpine areas. We asked which impacts type of land use, farming practices, geology, chemical and physical soil parameters and elevation have on different AMF species and on the AMF community structure. Morphological methods were used to analyze AMF spore populations from field soils and trap cultures. Uni- and multivariate analyses were applied to model spore, land use and environmental data. About 70 species were detected. Parameters of all mentioned categories influenced the spore populations. Intensified land use and farming practices, but also soil pH > 7.0 affected in particular spore and species numbers of *Acaulospora* and *Scutellospora* spp. negatively. Spore and species numbers of *Gigaspora* and *Scutellospora* declined with increasing altitude, while *Pacispora* numbers were highest at the highest alpine elevations investigated (2700-3000 m asl). A few species occurred in all habitats (e.g. *Glomus intraradices* and *G. versiforme*). We called those 'ecological generalists'. We identified also 'ecological specialists', found only under particular conditions (e.g. *A. alpina* and *P. robigina*). The studies contribute to the understanding of the ecological conditions which control the occurrence of AMF species. They improve the scientific base for species selection for inoculation technology, or for revegetation/rehabilitation practices.

Role of mycorrhizal response diversity in forest succession following disturbance

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A diversity of responses to disturbance among ectomycorrhizal fungal (EMF) species contributing to similar ecosystem functions is important to forest succession. Response diversity is particularly important in ecosystems subject to frequent or catastrophic disturbance, providing insurance that succession proceeds within an historical stability domain. Here, we examine patterns of EMF species composition, diversity, and function in relation to succession following wildfire and clearcutting in Douglas-fir dominated ecosystems. Both disturbance types alter EMF communities, but succession proceeds predictably and similarly. However, severe disturbances, such severe burns or destumping that remove living trees, root systems or forest floor, can reduce ECM colonization and diversity on regenerating seedlings, often corresponding with reduced growth rates and foliar nutrients. Concurrent increases in invasive plants augment these changes. These results suggest a reduction in function with reduced response diversity. By contrast, retaining key functional groups of plants, such as broadleaf trees, helps retain diverse EMF that persist through succession. Many of these fungi are host-generalists, linking broadleaves with conifers, and facilitating inter-tree carbon transfer that fluctuates with EMF species, phenology and shading. This dynamic interplay with broadleaves and EMF may allow understory conifers to eventually dominate, in congruence with historic forest succession patterns.

Evidence for ectomycorrhizas in *Pakaraimaea dipterocarpacea* from South America and an ancient origin of ectomycorrhizas in Dipterocarpaceae

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The Dipterocarpaceae are one of the most important ectomycorrhizal (EcM) tree families in the tropics, especially in SE Asia. The consistent association of Paleotropical Dipterocarpaceae with EcM fungi suggests that EcM status is a ancestral character in the family. Despite its distinctive morphology, *Pakaraimaea dipterocarpacea*, a Neotropical Dipterocarpaceae endemic to the Guayana Region, is phylogenetically related to the Paleotropical Dipterocarpaceae. The confirmation of *P. dipterocarpacea* EcM status would indicate that Paleotropical Dipterocarpaceae and *P. dipterocarpacea* probably had a common EcM ancestor. The capacity of *Pakaraimaea* to associate with ectomycorrhizal fungi was investigated using a combination of molecular and anatomical analyses. This paper provides the first documented evidence of EcM in *Pakaraimaea* and indicates that EcM possibly evolved in Gondwana in ancestors of Dipterocarpaceae before the separation of South America from Africa by the Atlantic, ca. 135Ma. The observation of *Sebacinaceae* and *Clavulinaceae* suggests that broad host range fungi are important components of *P. dipterocarpacea* EcM communities. The implications of *Pakaraimaea* natural history on the diversity of associated ectomycorrhizal fungi are discussed.

9.1 Necessity to manage mycorrhizae in *Tetraclinis articulata* woodland ecosystems

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In Mediterranean regions, plant growth is limited by water stress and soil-related constraints. The ecosystems by their fragility change their physicochemical and biological soil properties. All operations of ecosystem restoration become complex and require suitable technologies. In Morocco, this situation is observed in the natural surfaces of *Tetraclinis articulata* woodlands. Our first research on the relationship between mycorrhizae and *T. articulata* revealed that all root samples of this plant formed only AM. The level of colonisation was generally high (80%). The mycorrhizal structure was found to be the "Paris" type in all studied samples. The number of AM fungal spores occurring in field-collected soil samples was relatively high and all recovered spores belong to the Glomineae order. Actually, it is established that this type of micro-organisms are of fundamental importance for growing forest trees. This \"mycorhizal\" effect on the growth of *T. articulata*can be obtained either by the introduction of selected fungi or by the management of the Mycorrhizal Inoculum Potential (MIP) in situ. That's why in our research work we are giving importance to an integrated management of the mycorrhizal symbiosis properties within the natural area of *T. articulata* in order to define management practices able to optimize survival and development of *T. articulata* and to prevent the phenomena of soil degradation.

9.2 Community of arbuscular mycorrhizal fungi and their host plants in an ecosystem in Algeria

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Terrestrial ecosystems contain many arbuscular mycorrhizal fungi (AMF) and plants that coexist in communities. AMF form symbiosis with the roots of most plant species. They are known to be non host specific although experimental studies have revealed that plant species differ in their responses to individual species or isolates of AMF. This study has been carried out on roots collected from a field located in the north of Algeria where plant community is made of *Orysopsis caerulescens*, *Fraxinus sp.* and *Eucalyptus camaldulensis*. The survey of the arbuscular mycorrhizal status of these different plant species revealed the presence of a lot of AMF. Total root length colonized was assessed for each plant.Two arbuscular mycorrhizal fungi colonized the roots of the three plants. One fungal species was observed within both the roots of *Eucalyptus* and *Orysopsis caerulescens*. The others are observed exclusively within the roots of *Eucalyptus* or exclusively within *Fraxinus* roots or roots of *Orysopsis caerulescens* These results would bring a contribution to the controversial debate of the existence of any specificity in the arbuscular mycorrhizal symbiosis.

9.3 The Impact of tillage practices on AM fungal diversity in subtropical crop fields

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Arbuscular mycorrhizal fungi (AMF) are a main component of the soil microbiota in most agrosystems. As obligate mutualistic symbionts, they colonize the roots of the vast majority of plants, including most crop plants. We used molecular techniques to investigate how different tillage systems (mouldboard, shred-bedding, subsoil-bedding and no tillage) may influence the AM fungal community colonizing maize, bean and sorghum roots in an experimental site located in Northern Tamaulipas (Mexico). Roots from 36 plants were analysed using AM fungal-specific primers to amplify sequences from the partial small subunit (SSU) of ribosomal RNA genes. More than 690 clones were screened for random fragment length polymorphism (RFLP) variation and 149 out of them were sequenced. Nine AM fungal types were identified, clustering into three families of AM fungi: Gigasporaceae, Glomaceae and Paraglomaceae. Glomus species was the dominating taxon in all samples. Six out of the nine identified types are distinct from any previously published sequences and might correspond to new isolates. Taken in the whole, fungal diversity was low, even if the crops under no tillage system showed the highest number of AM fungal clones, and among them sorghum showed the highest values. Gigasporaceae seemed to be specific for maize and sorghum under subsoil-bedding system. In conclusion, agricultural practices seemed to influence AM biodiversity, since some fungal types were treatment specific.

9.4 Vegetation cover diversity and mycorrhizal fungi patterns in a biogenetic reserve: after fire communities in Serra da Estrela grassland, Portugal

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The Natural Park of Serra da Estrela (PNSE) includes interesting high altitude ecosystems (up to 1991 m) and 10.000 ha of biogenetic reserves. Severe fires over the last decade have damaged vast grasslands with endemic species resulting in the loss of habitat balance above and below-ground. These changes had also relevant socio-economical impact on the production of the unique Serra da Estrela sheep cheese. We hypothesized that those fires did affect the vegetation cover and soil microflora properties, in particular the mycorrhizal fungi diversity patterns and their functioning. We analyzed the effects of fire on vegetation cover diversity and on predominant mycorrhizal types. Plants were collected during the peak of their growing season; the mycorrhizal types and the diversity patterns of mycorrhizal associations were assessed for 38 species (19 families) present in two adjacent grassland areas, one unburned and the other affected by a severe wildfire 2-years ago. Our study was specially addressed to the arbuscular mycorrhizal fungi (AMF) associations, since they are the main type represented in the area (AMF, 92%; ERM, 5%; ECM, 3%). Preliminary conclusions on the 1) impact of fire on AMF abundance and diversity patterns, 2) re-establishment of endemic species and their mycorrhizal dependency, and 3) relevance above and belowground of AMF communities in the PNSE grassland resistance and resilience are discussed.

9.5 Molecular diversity of mycobionts of *Polygonum viviparum* growing in primary successional sites in the Austrian alps

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The herbaceous plant *Polygonum viviparum* (Polygonaceae) is known to be ectomycorrhizal since 1977 (Fontana). However, knowledge about the mycobionts of this plant is still poor. The aim of this work was to identify the ectomycorrhizal fungal partners of *P. viviparum* in a primary successional habitat. Whole plants were sampled three times (Jun., Aug., Sept.) during the vegetation period 2005 on the glacier forefront of the Rotmoosferner (Austria). Fife sampling plots were selected behind the moraine ridge of 1920 (2200 m a. s. l.) and fife samples, each including several plants, were taken from each plot. 100 root tips per plot were randomly selected. The mycorrhizal root types were sorted into 23 morphotypes using the following characters: colour, mantle surface structure, and presence of rhizomorphs. Several representatives of each mycorrhizal type were identified by molecular methods (rDNA ITS sequencing). *Cenococcum geophilum* (Fr.) was the dominant morphotype with an abundance of 14 %. Our molecular approaches confirmed this result. But molecular methods also demonstrated that different fungal species can develop the same morphotype. Thus, all together 35 fungal species were detected as mycobionts of *P. viviparum*: 82 % belong to the Basidiomycetes, and 18 % to the Ascomycetes. Fungi not forming conspicuous fruitbodies such as *Cenococcum* or representative genera of Thelephoraceae are the dominating symbiosis partner of *P. viviparum* in this primary successional habitat.

9.6 Hypogeal mushrooms reported in Armenia

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Hypogeal mushrooms (HMs) belong to phyla Ascomycota (Pezizales, Elaphomycetales), Basidiomycota (Agaricales, Boletales, Phallales, Russulales) and Zygomycota (Endogonales). Rainfalls 700-1200 mm per year, sylvan-brown and calcareous soils are necessary conditions for their underground growth. HMs are mainly mycorrhiza-forming fungi. Previously, 6 species (*Tuber aestivum, Elaphomyces granulatus, Octaviania stephensii, Rhizopogon roseolus, R. luteolus, Cremeogaster klikae*) were reported in Armenia. In our studies (2000-2005) the favourable growth territories for HMs in north-eastern and south-eastern parts of Armenia have been uncovered. The collected ascomas have been identified by morphological and molecular methods using rDNA-ITS sequence data. The 11 species from Ascomycota (*Tuber scruposum, T. rufum f. ferrugineum, T. rapaeodorum, Tirmania pinoyi, Picoa juniperi, Elaphomyces muricatus, E. granulatus, Hydnotrya tulasnei*) and Basidiomycota (*Hymenogaster griseus, H. olivaceus, Octaviania stephensii*) have been described. Among them, 9 species (except *O. stephensii* and *E. granulatus*) have been originally reported for Armenian mycobiota. The *Fagus orientalis, Carpinus betulus, Tilia cordata, Corylus avellana, Pinus kochiana* and *Cornus mas* are the main host-plants for Armenian HMs. They prefer sandy-clayish, humus-rich, from slightly acidic to strong alkaline sylvan-grey soils. SMB thanks the NATO (#980764) and DAAD (#548.104401.174) for support.

9.7 Macrofungi from *Castanea sativa* Mill. and *Quercus pyrenaica* Wild.: Evaluation of mycorrhizal vs nonmycorrhizal fungi biodiversity – Project AGRO 689

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The present work concerns results from the Project AGRO 689 on evaluation of macrofungi biodiversity associated with chestnut and oak trees in the northeast of Portugal (Trás-os-Montes), a region where these species have important social and economical roles. The study of relative abundance of mycorrhizal and nonmycorrhizal species and of edible vs non edible mushrooms are also main goals of this work. Ecological and economical impacts of edible fungi harvesting, including the impact on mycorrhizal and nonmycorrhizal species, are also under evaluation. Field work took place from October 2004 to December 2005. Total macrofungi were collected weekly during autumn and spring and monthly during the rest of the year, in five plots of 100m2 each per habitat. Identification and quantification of carpophores were made. Along the two years, 17 species belonging to 11 genera were collected in chestnut tree habitat. The distribution of species by functional groups shows a dominance of mycorrhizal species (59%). During the same period, oak tree habitat showed 59 species belonging to 27 genera. In contrast with chestnut tree habitat, the distribution by functional groups in oak habitat showed a slight dominance of saprotrophic species (49%). The reduced macrofungi biodiversity here shown will be discussed taking into account the particular climatic conditions in the northeast of Portugal during the main growing seasons of the current study.

9.8 A comparison of the effects of fire and clearcutting on the mycorrhizal colonization and growth of *Pseudotsuga menziesii* var. glauca seedlings

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This study aims to compare the impact of wildfire severity with clearcutting on Douglas-fir regeneration. The study consists of five treatments that compare a range of disruptions to the soil organic layer and host vegetation: high severity burn, low severity burn, clearcut, screefed clearcut, and undisturbed forest. Seed beds were sown at four replicates of each treatment in late spring of 2004. Germinants were assessed monthly for growth and mycorrhizal colonization. Germination was highest in clearcuts and low severity burns, intermediate in the high severity burns, and lowest in the undisturbed stands. After two growing seasons, high severity burn seedlings had the highest mean shoot biomass while the low severity seedlings had the highest foliar % N and P. Seedlings growing in the undisturbed stands had the highest percentage of ectomycorrhizal roots and highest ectomycorrhizal diversity while the lowest of both occurred in the high severity burns.

9.9 Patterns in activity, richness, and community composition of arbuscular mycorrhizal fungi in a Sonoran Desert riparian area, Verde River, Arizona

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In the semi-arid riparian zones of the western United States the effects of river regulation on overstory *Populus* populations is well understood, but less is known about impacts to other aspects of riparian ecosystems including the herbaceous community and their associated arbuscular mycorrhizal fungi (AMF). In this study, floodplain soil texture and chemistry, herbaceous cover and richness, and AMF richness and colonization were compared between unregulated and regulated reaches of the Verde River, Arizona and along a lateral gradient from the river margin to the uplands. When compared to the unregulated reach, the regulated reach had sandier soil and lower nutrient concentrations along with lower herbaceous cover and a higher proportion of annual species. Despite these differences, AMF colonization, richness and community composition were similar between reaches. AMF species richness declined with stand age and distance from the active channel and was positively related to perennial species cover and richness and gravimetric soil moisture. Distance from the channel, forest age, annual species cover, perennial species richness, and exchangeable potassium all play a role in structuring the AMF community in this riparian area. Most AMF species were found across a range of soil conditions, but a subset of species tended to occur more often in hydric areas. This group of riparian affiliate AMF species includes several not previously encountered in the surrounding Sonoran desert.

9.10 Mycorrhizal status of main spontaneous or introduced forest trees in the Algerian North-east

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In Algeria, often, serious imbalances of the forest ecosystems caused by the badly led exploitations, the repeated fires, the overgrazing involve the disappearance of the state timbered on vast territories. Within the framework of the programs of plantations and restoration of these degraded forests, we try to contribute to these programs by tests of controlled mycorrhization in forest nursery. We present there, the first stage of our work which consisted in establishing the mycorrhizal status of main spontaneous or introduced forest species into Algerian North-east. The study was carried out in three great different forest areas and related to all the species of Pine, oaks and other leafy trees existing in these areas. The results show that all the species observed have either ectomycorrhizas or arbuscular endomycorrhizas or both at the same time. In addition, the introduced species of the Acacia genus or Casuarina carry arbuscular mycorrhizae and also nitrogen fixing nodules. It should be noted the remarkable diversity of ectomycorrhizal morphotypes associated to the pines and to the oaks and also that of the endomycorhizals fungi spores occur in the rhizosphere of other species. Lastly, it is significant to note the omnipresence of the mycorrhiza of Cenococcum geophilum in all the stations, during every season and at the majority of the ectomycorrhizal trees.

9.11 Natural recovery of an abandoned ash disposal island in Venice: AMF colonization of spontaneous vegetation

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In this work we studied the mycorrhizal status of spontaneous vegetation and the diversity of arbuscular mycorrhizal fungi (AMF) in an artificial island in Venice. The island hosted an urban solid waste incinerator till 1984, producing 50000-60000 m3 of ashes, which were disposed all over the island surface. The soil, containing high levels of heavy metals (Cu, Pb, Zn, As), represents a source of pollution for the lagoon and the city. Since furnace demolition the island was abandoned and spontaneously colonized by natural vegetation. Our aims were: 1) to characterize the vegetation; 2) to assess the mycorrhizal status of the more representative plant species; 3) to evaluate the diversity of AMF population; 4) to isolate and maintain AMF in the ex-situ culture collection of Pisa University. Two-thirds of plant species were mycorrhizal and showed an AMF root colonization ranging from 13 to 50%. AMF occurring in mycorrhizal roots were characterized by partial SSU rDNA analysis. AMF biodiversity studies would contribute to a better understanding of the adaptive strategies of AMF populations in metal contaminated soils. Such indigenous and presumably stress-adapted AM fungi, isolated and maintained in culture, may represent a useful tool for improving restoration of degraded ecosystems.

9.12 Mycorrhizal fungal species identity alters trade-offs in plant allocation

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Several theories have been suggested for how plants allocate resources, many involving the relative ratio of carbon availability to nutrient availability, primarily nitrogen. We ask whether the plant mutualist arbuscular mycorrhizal fungi alters plant allocation patterns, and if allocation patterns vary with species identity. We grew replicates of seven genotypes of *Plantago lanceolata* with three species of arbuscular mycorrhizal fungi, *Glomus* sp., *Archaeospora trappei*, and *Scutellospora calospora*, and subjected half of these plants to herbivory by the *Junonia coenia* larvae. We then measured defense response, tolerance to herbivory, growth and reproduction. Our analyses show that chemical defense in *P. lanceolata* is positively correlated with growth and reproduction, but trade-offs in tolerance, growth, and reproduction varied with mycorrhizal species identity.

9.13 Diversity of arbuscular mycorrhizal fungal communities associated with maritime sand dunes in Southern Brazil

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Coastal sand dunes are often characterized by high pH soils with low available nutrients, and extremes in moisture and temperature. Many sand dune plants have been shown to rely heavily on arbuscular mycorrhizal fungi (AMF) for nutrient acquisition, and these fungi may play a role in dune stabilization. We examined the sporulation of AMF communities associated with two dominant beach grasses, *Spartina ciliata* and *Sporobolus virginicus*, along a latitudinal gradient in Southern Brazil. We chose eight study sites on the Atlantic coast, in the states of Paraná, Santa Catarina, and Rio Grande do Sul. Study sites ranged from those that were heavily populated resorts to several that were more remote. A total of 18 AMF species were found at all sites, dominated by members of the genus *Scutellospora*. While the presence and abundance of spores of several AMF species varied by site, no significant differences were detected using a variety of diversity indices. No patterns among the sites were detected using multivariate techniques. *Spartina* supported more AMF species (4.9 per sample) than did *Sporobolus* (3.6 per sample). Similarly, total spore number was higher in association with *Spartina* (141 spores per sample) than with *Sporobolus* (44 spores per sample). AMF community differences among hosts at a single site often were as great as differences between sites. Our data suggest that plant host species is the most important determinant in AMF community structure in Brazilian sand dunes.

9.14 The influence of ectomycorrhizal community structure on the incidence of *Phytophthora* diseases in chestnut

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High mortality of sweet chestnut (*Castanea sativa*) caused by ink disease (*Phytophthora* spp.) has been reported in different areas of Europe, in some cases limiting the establishment of new groves or the conservation of old ones. In this work, the community composition of ectomycorrhizas was investigated in chestnut stands with and without the incidence of *Phytophthora* infestation. The ectomycorrhizal community composition was investigated using morphotype analysis and PCR techniques. Clear differences were shown in the ectomycorrhizal community composition between infected and non-infected sites, suggesting a relationship to disease incidence. No major differences were found between the sites in terms of soil chemical properties and site aspect. Although this study does not allow conclusion to be met about causality, it clearly shows the importance of ectomycorrhizal community composition and disease incidence.

9.15 Scots pine trees influence fungal diversity in understorey ericaceous roots

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Boreal forest and heathland cover 20% of the Earth's land surface at northern latitudes. Ectomycorrhizal fungi of forest trees, and ericoid mycorrhizal fungi of heathland shrubs, including *Calluna vulgaris* and *Vaccinium vitis-idaea*, play key roles in ecosystem processes and biogeochemical cycles, but have previously been assumed to be restricted to their respective plant partners. *C. vulgaris* plants were collected every 8 m along 80 m transects in open heathland, mature Scots pine forest and a transition zone of sparse natural pine regeneration between the two. In addition, *V. vitis-idaea* plants were collected along the forest transect. A combination of molecular approaches was used to characterise fungal endophyte community structure in surface sterilised hair roots. Fungal 'species' richness and diversity (H\') were slightly lower in the transition zone ($P < 0.05$) and there were clear differences in fungal endophyte community structure and composition across the heathland : forest gradient. We show that fungal endophyte diversity in *C. vulgaris* and *V. vitis-idaea* hair roots is influenced by the presence of trees, and that common ectomycorrhizal fungi of pine are found in the hair roots of forest understorey ericaceous roots supporting the possibility of shared mycelial networks between ericaceous and coniferous hosts.

9.16 The fungal endophyte of the mycoheterotrophic *Rhizanthella gardneri* (Orchidaceae)

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Rhizanthella gardneri Rogers is a critically endangered orchid restricted to two isolated regions of south-western Australia. *R. gardneri* is an entirely subterranean mycoheterotrophic species that purportedly forms a tripartite relationship with a mycorrhizal fungus that links with an autotrophic shrub of the *Melaleuca uncinata* complex. Through molecular sequencing and morphological characterisation, we have confirmed the fungus is, as originally described, a member of the rhizoctonia-forming family Ceratobasidiales (Heterobasidiomycete), probably belonging to the genus *Thanatephorus*. This is unusual as mycoheterotrophic orchid species almost exclusively associate with homobasidiomycetes. The orchid - fungus relationship was also found to be very specific throughout all of the geographically-isolated populations. Further, we confirmed the ability of this fungus to form ectomycorrhizas with species of the *M. uncinata* complex, a phenomenon not seen in other species of the Ceratobasidiales family.

9.17 Ectomycorrhizal fungal communities from serpentine and non-serpentine Mediterranean oak forests

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This study focuses on the ectomycorrhizal (ECM) fungal communities of a serpentine *Quercus rotundifolia* forest and a non-serpentine *Quercus pyrenaica* forest in northeastern Portugal. These forests are often adjacent, and though very different in soil type and vegetation, host some common fungal species (Branco, unpublished). Serpentine soils are extreme environments characterized by a unique combination of chemical and physical stressors including low levels of nutrients, high levels of heavy metals, and drought. Soil cores from 20 oak trees were collected in each forest. ECM roots were analyzed morphologically and molecularly by terminal restriction fragment length polymorphism analysis (TRFLP) of the nuclear rDNA Internal Transcribed Spacer (ITS) region. Based on morphological data alone the serpentine oak forest was less diverse, a finding consistent with the trend detected previously with fruitbody data (Branco, unpublished). However, a combined morphological and molecular analysis indicated that both ECM communities were similarly species rich. This result might be a consequence of a significant number of cryptic species not distinguishable by morphology, under-sampling, DNA amplification of contaminant fungi, and/or inherent problems with the ITS-TRFLP technique. Further sampling and refinement of the techniques employed will continue to unravel the structure of ECM communities of these two interesting habitats.

9.18 Mycorrhizas in disturbed, non-disturbed and under-recovery areas in the Parque Biológico Sierra de San Javier (PSSJ), Tucumán, Argentina

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Most plants are associated with mycorrhiza fungi. This work confirms this association and its types (endo and ectomycorrhizas) in PSSJ. The park belongs to the Tucumano-Boliviana Yunga and has a humid subtropical climate. Three areas were studied: disturbed, non-disturbed, and under-recovery, both at 700 and 1,300 masl. The aims of this work were: to identify the types of mycorrhizas present, the fungal species, and to reproduce the endomycorrhizal fungal species in trap plants. Soil and root samples were taken and a study of mycorrhizal capture with eight species of trap plants was set up in a greenhouse. Six months later the spores were collected by means of wet sieving and identified with specific key. Forty native and exotic plant species were identified; among them were healing species, natural propagators of mycorrhizas, and facultative mycotrophic species. Twenty-nine species of endomycorrhizas, belonging to Glomerales and Diversisporales were identified and the presence of Dark Septate Endophytes (DSE) was detected. To our knowledge, this is the first report on the presence of Glomeromycota and DSE at the PSSJ. The ectomycorrhiza Scleroderma aff. verrucosum was also identified. The most efficient trap plants turned out to be Sorghum sp. and Melilotus sp. Further studies will be carried out in order to have a deeper insight on the impact of the application of these fungi both in recovery projects of valuable tree species and in restoration projects of disturbed areas.

9.19 Sampling strategy influences mycorrhiza type richness but not the difference in mycorrhiza diversity between a beech and a Scots pine forest

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Biodiversity of ectomycorrhizas in two forests in north-eastern Germany representing the endpoints of transformation from Scots pine (Pinus sylvestris L.) to beech (Fagus sylvatica L.) stands was examined along a 30-core transect (87 m, 3 m lag distance). Presence of morphotypes was recorded for each core and for four different soil horizons (Of, Oh, Ah, Bv) of the two test forest stands. In the beech forest, morphotype richness (30 types) was 50% higher than in the Scots pine forest (20 types). At both sites three or four morphotypes dominated the community. To demonstrate the effects of a reduced sampling effort, Shannon diversity, evenness, and type richness were calculated for 10-core transects from the 30-core data for this approach: three short 10-core transects (27 m, 3 m lag distance) covering the first, second, and third part of the 30-core transect, and one long 10-core transect (81 m, 9 m lag distance). All 10-core transects detected the same dominating types but missed some rare types, so that richness and Shannon diversity were reduced in comparison to the 30-core transect. For the long 10-core transect reduction was lowest and the diversity difference (13 morphotypes for Scots pine and 22 morphotypes for beech) corresponded to the result of the 30-core transect. Thus, for site comparisons of type richness and diversity with low sample numbers, long transects with large lag distances would be preferred over short transects with small lag distances.

9.20 The change of the use of soil affects the composition of species of arbuscular mycorrhizal fungi in a temperate forest

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The open avocado growing areas, in the temperate forest zones in Mexico, means an alteration in the original soil condition, unknowing how this last, affects the composition of Arbuscular mycorrhizal fungi (AMF). The main objective of this investigation was to compare the composition of AMF species in an avocado agrosystem with the one in a natural system (Temperate Forest). The obtained results showed that the number of species was higher in raining season for both ecosystems, registering 15 species in agrosystem and 14 species in the forest. In the mean while, for the dry season 8 and 11 species were found. The isolated species belong to the next families: *Acaulosporaceae: Acaulospora scrobiculata, Acaulospora spinosa, Acaulospora laevis, Acaulospora sp1, Entrophospora infrequens; Gigasporaceae: Gigaspora sp1, Gigaspora aff. candida, Scutellospora scutata, Scutellospora verrucosa, Scutellospora sp1 y Scutellospora sp2; Glomaceae: Glomus clarum, Glomus geosporum, Glomus viscosum, Glomus rubiforme* and *Glomus sp1*. From the species presence analysis in both systems and seasons, it was observed that *Glomus rubiforme* is an exclusive specie of the agrosystem in raining season, *Entrophosphora* is exclusive of the forest (both seasons), *Glomus sp1* is exclusive of raining season (both systems) and *Scutellospora sp1* is exclusive of the dry season.

9.21 Belowground mutualisms and plant invasions: influence of disturbance and soil history

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It is unclear what influence soil mutualists (ex: mycorrhizal fungi and N-fixers) have on plant invasions. More specifically, it is unknown if disturbance influences invasion by changing plant-soil mutualism interactions. In this study we tested whether fire-induced changes in soil communities influence the role of mycorrhizas and rhizobia in exotic and native plant establishment. We compared plant-soil microbial interactions for the exotic *Acacia longifolia* (generally forming arbuscular mycorrhiza, ectomycorrhiza and N_2 fixing nodules), and the natives *Pinus pinaster* (ectomycorrhiza) and *Cytisus striatus* (arbuscular mycorrhiza and N_2 fixing nodules). Plants were grown in soil communities from recent fire-disturbed soils, and in soil with a history of conspecifics or heterospecifics. We found differences in growth responses among plant species due to the presence of soil microorganisms, disturbance and host soil history. Further mycorrhizal and rhizobial characterization will help interpret our data. However, so far the data suggest that the presence and composition of soil mutualists may influence exotic plant invasions.

9.22 Plant-soil feedback in serpentine and prairie grasslands

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Feedback between plants and soil microbial communities can play an important role in structuring the species composition of plant communities. Based on evidence that negative feedback occurs between the C_4 grasses found in a serpentine grassland in Eastern Pennsylvania (USA) and their associated arbuscular mycorrhizal fungi (AMF), we conducted a greenhouse experiment to determine the generality of these results for whole soil communities. We used soils and seeds of the same four grass species (*Andropogon gerardii, Schizachyrium scoparium, Sorghastrum nutans, Sporobolus heterolepis*) collected from this same serpentine site, another serpentine site in Maryland (where *S. heterolepis* is absent), and two prairie sites in Iowa. The experiment involved training soils with each of the different grass species for one year and then growing a second generation of plants in the trained soils. Negative feedback occurred if a particular plant species in the second generation performed least well in soil trained by conspecifics than in soil trained by other species. Of the 15 cases examined, negative feedback was found in eight and positive feedback in one. Feedback occurred both in prairie and serpentine soils. We also determined the composition of the AMF spore communities in soils trained by the different plant species and scored roots for infection by mycorrhizal fungi and pathogens. We use these results to discuss possible mechanisms generating the feedback.

9.23 Effects of tillage on arbuscular mycorrhizal propagules when white lupin is used in a short rotation sequence

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Arbuscular mycorrhizal fungal (AMF) propagules in soil are affected by management practices mainly tillage and crop rotation. White lupin is a non-mycorrhizal host but due to having a lot of beneficial effects on soil characteristics it is habitually used by local farmers. The goal of this work was to study the influence of tillage on AMF propagules left in the soil, when lupin-wheat is used as a short rotation system. After crop harvest AMF propagules (spores and hyphae) were determined from third to fifth year in an on-going field experiment carried out in an Ultisol under non-tillage (NT) and conventional-tillage (CT) with plowing. Biodiversity of fungal spores was also determined. Total and metabolic active hyphae were not affected neither tillage system nor pre-crop and remained nearly constant. On the other hand, spore number was almost not affected by the crop used in the rotation under NT but were higher under CT after lupin cropping. This unexpected result was higher at fourth year of the beginning of the field trial experiment coinciding with the second season of using lupin as pre-crop. However, richness and Shannon-Wiener index of fungal spores decreased after lupin cropping in both tillage systems. The high spore number found at fourth year showed a higher dominance of just some AMF morphotypes which explains the lower fungal spore diversity. The findings encourage us to persevere in continue studying this lupin effect in long-term.

9.24 Molecular characterization of arbuscular mycorrhizal fungi in an agricultural soil and in potato roots

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The symbiotic association between AMF and plants has been described to be non specific but recently some data indicated a certain specificity between host plant and AMF. It is known that management regimes can influence the fungal communities in the soil. In the present work, the populations of AMF in the roots of *Solanum tuberosum* L. and in the surrounding soil were characterized in order to check for possible differences in their community structure. We carried out a survey in four areas close to Castelnuovo Scrivia (Alessandria, Italy), identified according to soil structure. DNA was isolated from bulk soil and from roots and it was amplified by nested PCR using AMF specific primers of the large ribosomal subunit (LSU rDNA). PCR products were cloned and randomly selected clones were sequenced. The phylogenetic analysis allowed the delineation of 11 OTUs of AMF with high bootstrap values, all belonging to the genus *Glomus*. It was observed that *G. intraradices* was weakly represented in the soil but it was the most abundant AMF present in the *S. tuberosum* roots in all the areas of site. These data suggest that there is a preferential establishment of symbiosis between *S. tuberosum* and some AMF species, in spite of their low abundance in the soil.

9.25 Above- and below-ground views of the ectomycorrhizal community in a *Quercus ilex* stand in northern Spain

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The species diversity of the ectomycorrhizal community was studied on a 225 m² plot in a natural *Quercus ilex* forest, by sampling of ectomycorrhizal fruit bodies and ectomycorrhizas, to determine the correspondence between the above- and below-ground views of species composition and abundance. The study area was the Nature Reserve of Monte del Conde, which has been proposed to be included in the Sites for Community Importance (SIC) and for Special Areas of Conservation (SAC) for the European Community. In autumn 2003, 374 fruit bodies belonging to 52 taxa were collected. *Clavulina cinerea*, *Entoloma rhodopolium* and *Tricholoma scalpturatum* were the dominant species. Below-ground, 25 ectomycorrhizal samples were collected on each sampling, with a 10 x 4 cm soil core in autumn and spring, from autumn 2003 to spring 2005. Morphological and anatomical analyses of ectomycorrhizal root tips were performed, yielding more than 45 types. *Cenococcum geophilum* and thelephoroid fungi were dominant species on the root system. The results show that the fruit bodies survey does not reflect the real ectomycorrhizal community diversity. Therefore, it is important to sample both fruit bodies and root tips to achieve an accurate estimate of the ectomycorrhizal fungal community in forest ecosystems.

9.26 Beech forests in Navarra (Spain) and sustainability. Relevance of mycorrhizal fungi for ecosystem preservation

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The most extensive beech forests in Spain are located in Navarra, close to the Pyrenees. However, many of them have been deeply disturbed as a consequence of clearcutting practices. Clearcutting changes both ecosystem biodiversity and microclimate inside the forest -light, soil water and nutrient content and air and soil temperatures-. Mycorrhizal fungi are an essential component of terrestrial ecosystems that are able to improve mineral nutrition of host plants, behave as plant bioprotectors against diseases, favour growth of plants under stressful conditions, help the establishment of plantlets in revegetation practices and minimise soil erosion. In addition, the diversity of mycorrhizal microflora determines plant biodiversity and ecosystems productivity. Our aim is to provide useful information on dynamics and diversity of mycorrhizal fungi for preservation of beech forests. This goal could be divided into two partial objectives: (1) The study of both endo and ectomycorrhizal fungi in a mature beech forest not subjected to disturbance practices and (2) to study the effect of clearcutting performed 5 or 10 years ago on beech ecosystem. The research will include the study of the three main components of every mycorrhizal association: mycorrhizal fungi, host plant –especially beech- and soil. Results will contribute to better understand ecosystem functionality and, therefore, will help to choose the most adequate strategies focused on conservation of natural beech forests.

9.27 A checklist of ectomycorrhizal fungi associated with *Cistus*

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The genus *Cistus* comprises a group of about twenty shrub species found in wide areas throughout the whole Mediterranean region to the Caucasus. Being one of the main constituents of the Mediterranean-type maquis, this plant genus is peculiar in that has developed a range of specific adaptations to resist summer drought and frequent disturbance events, such as fire and grazing. In addition, it can form both ectomycorrhizas and vesicular arbuscular mycorrhizas. In the present study, information was gathered and critically sifted from both published literature sources and personal observations on the ectomycorrhizal fungi recorded with *Cistus* across its entire geographic range. Although the resulting data matrix was based primarily on accounts of sporocarp inventories in the field, existing knowledge on the features of *Cistus* natural and synthesized ectomycorrhizas was also included and discussed. In total, over 200 fungal species belonging to 40 genera have been reported so far to be associated with *Cistus*. An analysis of the pattern of ectomycorrhizal diversity and specificity revealed that members of the *Cortinariaceae*and *Russulaceae* make the most of both *Cistus*-aspecific and –specific mycobionts. Further studies are needed to expand our preliminary knowledge of the mycorrhizal eco-biology of *Cistus* and its fungal associates, focussing on topics like mycobiont diversity, host specificity, fungal succession, mycorrhizal influence on stress tolerance and impact of disturbances.

9.28 Influence of plant cover in the composition and structure of the AM fungal community in a semi-arid Mediterranean environment

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The influence of different plant species on the composition of arbuscular mycorrhizal fungal (AMF) community was investigated in a Mediterranean environment. A mesocosm system was established, using soil and plant species characteristic of a target Mediterranean area, as a basis for a time-course experiment. Four plants were grown in each experimental unit, to produce mesocosm either i) plant monospecific, ii) with all possible combinations of two plant species, or iii) with the four different species. An initial AMF community was designed using six species from Mediterranean areas (*Glomus clarum, G. constrictum, G. coronatum, G. intraradices, G. mosseae* and *G. viscosum*). Changes in the AMF community colonizing plant roots and rhizospheric soil were evaluated every three months during three years by nested PCR-TTGE of the SSU rDNA and by counting the spores number, respectively. The results obtained show a reduced impact of plant cover on changes in the AMF community composition. The population size and specific densities of *G. constrictum* increased under all types of plant cover tested, while other AMF species (*G. clarum, G. coronatum*) almost disappeared during the course of the study, or maintained a low specific density (*G. mosseae, G. viscosum*). A good correspondence among data of fungi actually colonizing the root and those sporulating in the soil was found. These results suggest a competitive advantage of *G. constrictum* in the experimental conditions studied.

9.29 AM fungi diversity associated with three woody legumes in the Reserva Natural de São Jacinto, Portugal

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Acacia longifolia is one of the main invasive species in the Portuguese coast, and represents a great danger to preservation of the native vegetation in the Reserva Natural das Dunas de São Jacinto (RNDSJ). This is a leguminous species native to Australia that was introduced into Portugal for sand stabilization. A great number of theories focusing mainly in the aboveground ecology of invasive species have been proposed, but cannot completely explain why *A. longifolia* proliferate and displace other native woody legumes. This work is part of a wider project that focuses on the belowground organisms, such as pathogenic organisms and mutualistic fungi in the search for an explanation of the invasive success of *Acacia longifolia*. The goal of the work presented here was to estimate and compare the AM fungal diversity associated with *A. longifolia* and the two native leguminous species, *Ulex europaeus* and *Cytisus grandiflorus* using PCR-DGGE. DNA was extracted from root samples collected from each of those species in two sites of theRNDSJ. A PCR targeted to the LSU was performed and the products analysed using DGGE. Our results did not show a higher diversity of AMF associated with the invasive species. In fact, the AMF community associated to the three species in each site was similar. The only differences occurred for *C. grandiflorus* in one of the sites. Further studies using different genomic regions and functional analysis are ongoing.

9.30 A study on the ectomycorrhizal fungal community associated with overharvested species *Tricholoma matsutake*

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In northwest Yunnan, China, the decline of *Tricholoma matsutake* due to overharvest is alarming because of the economic and ecological value of the species. Previous studies on mushroom overharvest mostly focused on the effect of harvest on productivity of the mushroom fruitbodies, but little is known about how such disturbances potentially affect the ectomycorrhizal fungal community. This study is aimed at understanding the importance of *T. matsutake* in the EMF community and its interaction with associated EMF species. A spatial hierarchical approach is being used. The study is first being conducted at a fine scale to compare matsutake-present and mutsutake-absent patches. Preliminary data suggest that the presence of *T. matsutake* may influence the EMF diversity and cause a composition shift of other EMF species in these small patches. Study at the scale of forest stands is in progress to investigate whether the abundance of *T. matsutake*, as well as its associated EMF species varies with forest age and other environmental factors. Findings from the study will help to establish ecosystem management strategy. This study is also the first one to investigate the below-ground mycorrhizal fungal diversity in northwest Yunnan, one of the world's biodiversity hotspots.

9.31 *Arum*- and *Paris*-type mycorrhizal colonisation – two extremes of a continuum

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Two major morphological classes of AM symbiosis differ in fungal structures. *Arum*-type AM colonisation consists of intercellular hyphae and intracellular arbuscules and *Paris* of intracellular hyphae, coils and arbusculate coils. It has been generally considered that the two classes are solely controlled by presence/absence of intercellular spaces within the root cortex of the plant. This cannot be correct because tomato produces both AM types with different fungi. A survey of 12 plant species colonised by 6 fungal species explored the diversity of *Arum*- and *Paris*-type structures. Whole roots and roots sectioned both longitudinally and transversely were stained and mycorrhizal structures examined. Formation of entry points on the plant root surface differed between fungi; some species formed appressoria prior to penetration, while others appeared to penetrate directly. Root squashes or longitudinal sections did not always reveal whether longitudinal hyphae were inter- or intra-cellular, whereas transverse sections gave precise locations. Both *Arum*- and *Paris*-type structures and intermediate forms were observed, depending upon the plant-fungus combination. There are certainly some plants that form 'robust' *Arum*- or *Paris*-AM independent of the AM fungus colonising them. Other plants produce a range of intermediate structures, forming a continuum influenced by not only the plant, but also the fungus and possibly the environment.

9.32 Persistence and eradication of exotic ectomycorrhizal fungi from Cabañeros National Park (Spain)

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Spanish authorities promote the eradication of eucalypts (exotic trees) from Spanish National Parks. Australian ectomycorrhizal fungi were spread with eucalypt plantations beyond Australia. We study the diversity of ectomycorrhizal fungi of the eucalypts still living in Cabañeros National Park, and the persistence of the exotic ectomycorrhizal fungi after eucalypt felling. We sampled soils from different type of plots. Our survey included natural forests, eucalypt plots, and former eucalypt plantations in which seedlings of native trees were planted. We used the T-RFLP and DNA sequencing of ITS regions of the nuclear rDNA for fungal identification in soil samples. We detected more than ten Australian ectomycorrhzial fungal species on roots of eucalypts still present in Cabañeros National Park. Many of the Australian fungi disappeared after eucalypt cutting, though some of them still persist (e.g. *Laccaria fraterna*). Native species were the main fungi in soil samples from oak seedlings planted on former eucalypt plantations, and the community of native fungal species colonizing roots of native tree seedlings was very diverse.

9.33 Species diversity of ectomycorrhizal fungi on *Abies alba* Mill. in the Black Forest (Germany) and the impact of great disturbance

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In the last decades, several hurricanes caused drastic damage on the forests of South West Germany. Regeneration of these areas is aspired through natural succession. In the Northern Black Forest, this may lead to an increased proportion of silver fir (*Abies alba*) in former Norway spruce (*Picea abies*) stands. The young silver firs in the wind throw areas are exposed to several stress factors, so ectomycorrhizal symbiosis is notably essential to maintain health and competitiveness. As a first step within the scope of an interdisciplinary project we examined the ectomycorrhizal species diversity on young silver firs on wind throw areas and adjacent natural and managed stands. The introductory questions were focused on: • Is there a difference in mycorrhizal diversity between young silver firs under normal conditions (natural/managed forest) and on the wind throw areas? • Which are the commonest mycorrhizal species colonizing young silver firs on the wind throw areas? Are these ubiquitous generalists or *Abies* specific species? Ectomycorrhizae were analysed morphologically and species discriminated via ITS sequencing. According to our results, we selected *Tomentella stuposa* to elucidate its population structure. The further purposes are to detect the allocation of different genets within an area and to determine a possible correlation between silver fir genotype and the associated fungal genet.

9.34 Fate of spore inoculated Douglas-fir seedlings out-planted in a green tree retention harvest unit

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Our study was designed to answer these questions: 1. Does spore inoculation of *Pseudotsuga* at the time of out-planting affect the survival and growth of seedlings? 2. If the inoculation is successful, do the inoculated fungi affect the species richness and community structure of ectomycorrhizal (EM) fungi? 3. How will these fungal treatments respond to different ground disturbance conditions? The fungal treatments consisted of 2 types of spore inoculation (one from local spores, one a commercial spore product) plus a non-inoculated control. The inocula were dominated by spores of *Rhizopogon* spp. The seedlings were planted into 3 types of disturbance treatments: burned areas, soils compacted by roads, and the relatively undisturbed matrix areas. Mortality and growth data were recorded. The roots of sampled seedlings were washed and assessed for ectomycorrhizae. Presence and numbers of *Rhizopogon* mycorrhizae were counted, and the presence of all other EM morphotypes was recorded. Results show higher mortality associated with the local spore treatment and lower mortality with the commercial treatment. The ground disturbance associated with the most mortality was the matrix, while the road treatment showed the least. Seedlings in the burn piles grew the most. However, EM diversity was much lower in the burn treatment despite the larger size of those root systems. The local inoculum treatment had the most *Rhizopogon* EM in the matrix and road plots, and the least in the burn areas.

9.35 Ectomycorrhizae of *Genea* and related genera from Hungarian broad-leaved forests

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Within the phylum Ascomycota detailed anatomical description of ectomycorrhizae (EM) are predominantly confined to truffle genera (e.g. *Tuber*, *Terfezia*, *Tirmania*) while other groups are underrepresented. That is why our work focused on the EM of *Genea spp.* and related genera (Otideaceae, Asomycetes). EM samples were collected from stands of *Populus alba*, *Quercus spp.* and *Fagus sylvatica* in different regions of Hungary. Morphology and anatomy were investigated by different microscopic methods (dissection microscope, Nomarski-DIC, PhC) and documented by microscopic drawings and photographs. Identification of the mycorrhizae was carried out by molecular techniques. Both the ITS and SSU regions of the nrDNA were sequenced and subsequently analyzed together with database-derived sequences by different phylogenetic methods. Since GenBank contains scarce data concerning *Genea*, we supported the identification by sequences obtained form Hungarian and Spanish herbarial sporocarps. Here we present the morphological-anatomical comparison and the phylogenetic relationship of the EM samples. Our results proved that the species of the genus *Genea* and its close relatives are abundant members of the EM communities in broad-leaved forests of Hungary. The observed morphological homogeneity of the samples contradicts the molecular diversity detected.

9.36 Survey of vesicular arbuscular mycorrhizas in central sand dune desert of Iran

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It is proved that vesicular arbuscular mycorrhizal symbiosis plays a key role in desert ecosystem. Anti-desertification efforts in arid central regions of Iran are going on in order to establish the native plants as anti-desertification elements. So it is intended to understand the role of the Arbuscular Mycorrhiza (AM) interactions in such ecosystem. This study was conducted to investigate the presence and distribution of AM colonization in desert sand dunes in Kashan (Central Iran). The roots of ten plants species were sampled and assessed for the mycorrhizal colonization infection. The fungal spores were extracted from rhizospher of each plant species using wet sieving technique. Based on spores characterization two fungal genera; *Glomus* and *Scutellospora* were identified. Results showed that four native Poaceae species; *Stipagrostis pennata*, *S. karelini*, *S. plumose* and *Aeluropus littoralis* were found associated with AM. Five native species: *Seidlitzia rosmarinus*, *Calligonum comosum*, *C. denticulatum*, *C. bungei* and *C. polygonoides* in addition to *Haloxylon ammodendron* which was introduced previously found nonmycorrhizal. Spore population ranged from 0-170 spores in $100g^{-1}$ sand. The maximum spore number was counted in the rhizospher of *A. littoralis*. Percentage of colonization ranged from 0-22%. The maximum percentage of colonization was observed in the roots of *A. littoralis*.

9.37 Glomalin contribution as mycorrhizal product to carbon sequestration in different forests of south of Chile

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The arbuscular mycorrhizal fungi (AMF) contribute indirectly to C sequestration in soils affecting its aggregation, plant physiology and botanical composition of the ecosystem. Recent studies have emphasized about the role of AMF in direct input of soil organic matter (OM) by glomalin. Due to the high level of OM in volcanic soil in the Southern part of Chile, it is expected to find a huge input from arbuscular mycorrhizas to soil C especially in temperate rain forest. How the level of glomalin change, according to the species present in forest, can brings signs about the importance of vegetal composition on glomalin production and the contribution to total soil C. Were studied five forests; 1) Pristine evergreen forest of *Laurelia philippiana*, *Saxegothaea conspicua* and *Nothofagus dombeyi*; 2) Evergreen forest with disturbance and the same species; 3) Deciduous forest of *N. obliqua* y *N.alpina*; 4) Deciduous forest with disturbance and 5) *Pseudotsuga menziesii* forest. It was analyzed the total glomalin (TG) and easy extractable glomalin (EEG), and evaluated the C and N concentration on these fractions and the soils to estimate their contribution to total soil C and N. Over all the forest plots, mean concentrations of TG were 55,4±2,4 mg g^{-1} in the top 20 cm of soil. The mean of EEG was 11,7±0,4 mg g^{-1}. The C in TG represents 11,9±2,5% of the total carbon in the top 20 cm of soil and the N 14,7±0,5%. Both total C and total N demonstrated a positive correlation with TG and EEG.

9.38 The ectomycorrhizal fungi of birch colonising *Calluna* moorland in Northern Scotland

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Colonisation of moorland by trees is slow, unpredictable and spatially variable. This is thought to be due to the difficulty of tree establishment in the phytotoxic conditions created by the moorland dominant, *Calluna vulgaris*, coupled with intense competition between *Calluna* and tree seedlings for nutrients. We hypothesised that successful colonisation of moorland by birch (*Betula* spp) would be associated with the formation of ectomycorrhizas on birch by fungi capable of tolerating the conditions created by *Calluna*, and competing successfully for nutrients. As a first test of this hypothesis we went to a field experiment established 25 years ago where birch seedlings were introduced into *Calluna* moorland. The birch trees now show a 5-fold variation in height growth with great variation in performance over distances of less than 10m. We took soil cores on a spatially explicit sampling scheme, and recorded birch growth and various environmental parameters at the sampling points. Ectomycorrhizas in the cores were counted and identified using morphological classification, supported by RFLP-typing and sequencing of the fungal ITS region. The relative abundance of different ectomycorrhizal species is discussed in relation to birch growth, geographic position and environmental factors.

9.39 Occurrence of arbuscular mycorrhizal fungi in petroleum-contaminated soils from Tabasco, Mexico

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Petroleum industry is an important activity in Mexico that represents environmental threats due to spill accidents during extraction, refining, conduction, and storage of crude oil and petroleum derivatives. Arbuscular mycorrhizal fungi (AMF) have been hypothesized to enhance plant adaptation and growth under petroleum-contaminated soils. Nevertheless, the ecology of AMF under chronically petroleum-contaminated soil has received little attention. Rhizosphere soil samples were collected from Activo Cinco Presidentes, Tabasco where high number of oil spills have occurred; thus, affecting grassland, fruit-tree plantations, and natural vegetation. Native plant species were sampled: *Echinochloa polystachya*, *Brachiaria mutica*, *Citrus aurantifolia*, and *Citrus aurantium*. Soil rhizosphere samples were propagated during six months under greenhouse conditions, and subsequently AMF-spores were identified. Petroleum hydrocarbons in soils affected both AMF-colonization and AMF-sporulation. Colonization among plant species ranged from 63 to 85 % whilst spore number ranged from 715 to 1,200 in 100 g soil. This suggests that AMF tolerate the presence of petroleum hydrocarbons in the rhizosphere. From grass species, four AMF-morphospecies were identified: *Gl. ambisporum*, *Gl. sinuosum*, *Ac. laevis*, and *Ar. gerdermanni*. For citrus trees, four AMF-species were also identified: *Sc. heterogama*, *Gl. ambisporum*, *Ac. scrobiculata*, and *Gl. citricola*.

9.40 Pulse crops influence arbuscular mycorrhizal fungi diversity and abundance in agricultural soils of southern Saskatchewan, Canada

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Water, N and P are often limiting factors for plant growth in the Brown Chernozemic soils of the semi-arid region of Saskatchewan, Canada. Conservation tillage in this area typically includes a combination of cereals, pulses, and oilseeds in the cycle. This two-year field study (2004 and 2005) on long term agricultural plots investigated the diversity and abundance of arbuscular mycorrhizal fungi (AMF) in a fallow-wheat-pulse (pea, lentil, chickpea) cropping rotation, with non-mycorrhizal canola included as a negative control. The objectives of this study were to: (1) determine if AMF inoculum potential and colonization in a durum crop is significantly affected by cropping history; (2) assess the impact of pulses in crop rotation on the biodiversity of AMF communities and their dynamics; and (3) measure the plant N and P and the available soil N and P pools under a durum crop, and assess any correlation with AMF communities. Preliminary results indicate that although soil N and P differences between treatments were insignificant, durum following pulses resulted in higher plant P uptake ($P=0.05$) compared to the non-mycorrhizal treatment. Mycorrhizal root colonization in durum roots following pea, lentil and chickpea resulted in significantly higher colonization ($P=0.05$) than in canola. Phospholipid fatty acid analysis was used to assess differences in the soil microbial community, combined with DNA analysis to identify the possible AMF species present.

9.41 Establishment of shrub species in a semiarid zone after inoculation with native arbuscular mycorrhizal fungi in the Mesquital Valley, Hidalgo, Mexico

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Shrubland ecosystems are dominants in the Mesquital Valley, Hidalgo State, Mexico. In this work, the arbuscular mycorrhizal (AM) fungal association was analyzed in twenty native plant species. For this, the source of AM spores was taken in a native soil of the study site at the Mesquital Valley; spores were successfully multiplied in pot cultures with *Lolium multiflorum* as host plant. Once germinated, emerging seedlings of *Prosopis laevigata*, *Acacia farnesiana*, *Mimosa biuncifera* and *Opuntia strepthacanta* were transplanted into growing substrate consisting of sterilized soil with AM fungal inoculum (5% v/v). Inoculated and non-inoculated seedlings were grown for 6 months under greenhouse conditions; plants were watered when necessary and fertilized with Long-Ashtong solution. The next plant variables were evaluated: AM root colonization, shoot weight and root/shoot ratio. After this, inoculated and non-inoculated plants were planted in field conditions of the Mesquital Valley. One year after planting, survival and growth were evaluated. Inoculated plants were significantly greater than non-mycorrhizal plants; also, AM treatment increases shoot biomass and survival percentage. Therefore, the use of native AM spores, as a source of biofertilizer inoculum, may be considered as a emergent strategy for the successful re-establishment of native plant populations.

9.42 Spatial distribution of ectomycorrhizal fungi on two different scales

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Spatial heterogeneity of physical and chemical soil conditions creates a mosaic of fungal colonisation in soil. Little is known about the distribution of ectomycorrizal fungi (EM) in relation to the physical and chemical habitat conditions and biological interactions on different scales. The aim of our study was to characterize spatial distribution patterns of EM-types on meso- and micro-scale. Investigations were carried out in three red oak stands and one sessile oak stand in Lower Lusatia, East Germany. On meso-scale a grid based sampling design was used to investigate root tip density, abundance and frequency of the EM-types as well as chemical and physical parameters (content of ammonium, nitrate, phosphate, sulfur, pH and EC). On micro-scale the position of EM-types in a soil monolith was investigated. In addition, interactions of EM-types were analysed on both scales. Spatial investigations showed that the EM-types were unevenly distributed. On both scales EM-types showed specific distribution patterns. On meso-scale the majority of EM-types occurred in clusters, while on micro-scale most of the EM-types showed a randomly distribution. Investigated soil parameters did not influence the spatial distribution of EM-types. But most EM-types showed significant preferences to soil layers. Within the organic horizon many EM-types even preferred certain organic layers (particularly the F-layer). The results showed different degrees of species interactions.

9.43 Mycorrhizal diversity in green mediterranean orchids

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In the first stages of their development, all orchids share a heterotrophic phase and rely on symbiotic fungi for C supply ("mycoheterotrophy"). Whereas some orchids remain aclorophyllous in the adult phase, most species become green, although their actual photosynthetic efficiency may be reduced under some environmental conditions. In contrast with the high mycorrhizal specificity of achlorophyllous orchids, which are specialized exploiters of the ectomycorrhizal symbiosis, mycorrhizal specificity of green species remains controversial. We have investigated mycorrhizal diversity of green Mediterranean orchids in both forest understorey (*Limodorum* spp., thriving in woodland shade) and meadow habitats (*Serapias vomeracea*, *Ophrys fuciflora*,*Orchis purpurea* and *Anacamptis laxiflora*). Both a culture-dependent and a culture-independent, PCR-based identification approaches were adopted. Our published work on *Limodorum* plants collected over a wide geographical area indicates mycorrhizal colonization by a single dominant fungus belonging in the ectomycorrhizal genus *Russula*. By contrast, our new data on meadow orchids indicate that all species are colonized by either *Ceratobasidium*, *Tulasnella* and/or *Sebacina* species, thus exhibiting broad mycorrhizal diversity. These findings support the idea that specificity of orchid mycorrhizal symbiosis may decrease along a photosynthesis gradient, reflecting reduced dependency on external, fungus-derived carbon.

9.44 Mycorrhizal studies in pristine forest ecosystems of southern Chile

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The native temperate forests of southern Chile represent a globally significant reserve of biosphere which are classified as a biological "hotspot" for conservation. The biotic components regulate the internal circulation and output of nutrients giving stability to the ecosystem. The chemistry of the precipitation in southern Chile reflects one of the closest approximations to pre-industrial atmospheric conditions in the world. Microbial processes in soils, metabolic processes in trees and the overall functioning of these forest ecosystems are considered to be better adapted to N and P limitation. *Nothofagus* and several relict conifers represent the dominant tree species in the region. The mycorrhizal status of 245 species of vascular plants in 17 different native forest communities were investigated. Results showed 85 % of species, (trees, shrubs, vines and herbs) with mycorrhizal dominated by AM. The total 9 endemic conifer species were found to be colonized by AM and only the *Nothofagus* genus were documented with ectomycorrhizae. Controlled greenhouse assay of several seedlings species with mycorrhizal were permormed and the results of plant mycorrhization confirms as an alternative to reforestation programs and mitigate the potential ecological effects of deforestation and forest frangmentation. (Grant: Fondecyt 1050313).

9.45 Design and implementation of the míscaro (*Tricholoma flavovirens*) mycorrhizal mushroom monitoring project

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Míscaro (*Tricholoma flavovirens*) is very common in maritime pine (*Pinus pinaster*) forests in Beira Litoral, Portugal, and it is the most commonly harvested mycorrhizal mushroom in these forests. Timber production is the main management goal of these forests but míscaro harvesting is a traditional activity for local inhabitants. However, commercial harvest of this mushroom has increased substantially in recent years and has prompted concerns about its sustainability. The emergence of this new trend in míscaro harvesting comes at a crucial time for the region, in both economic benefits and changing attitudes towards forest management. Its development coincides with recent local efforts to diversify the products one can obtain from pine forests. The MÍSCARO project aims to: i) better understand the reproductive mechanisms, ecology and population genetics of the míscaro; ii) compare míscaro productivity across different pine forest management regimes and iii) evaluate potential harvesting impacts on subsequent fruiting. These research goals entail unique practical considerations. In this work we examine issue and concerns, discuss monitoring challenges, and suggest appropriate sampling design and procedures. Although intended for the MÍSCARO project, this discussion and recommendations can be widely applicable to monitoring edible forest mushrooms elsewhere. MÍSCARO is co-financed by FEDER through the FCT program POCI 2010 (POCI/AGR/57669/2004).

9.46 Comparison of the mycorrhizae and vascular flora in *brûles* of producer and not producer holm oaks in *truffièrs* in Navarra (Spain)

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Despite the numerous studies on the biology and the development of truffle, important aspects on its ecology and on the environment in which it develops are still unknown. The ecosystem of *truffièrs* constitutes a special habitat in which the plants that grow inside the burnt area or *brûle* are very influenced by the inhibiting substances produced by the micelium of the fungi, so the few species that can grow in these areas have peculiar characteristics, for example they are smaller than usual. However, the appearance of *brûle* does not guarantee the presence of black truffle carpophores, since it can appear as a consequence of the presence of other mycorrhizal species, some of them also of the genus *Tuber* (*Tuber aestivum*, T. *brumale*) but also AD type or *Scleroderma* sp. The study has been carried out in holm oaks with well developed *brûles* in two *truffièrs* of different ages in Navarra (Northern Spain). During the periodical visits to the *truffièrs*, we observed a change of the flora as the plantations evolve and mature. This encouraged us to start a study of both vascular flora and mycorrhizae, of black truffle and of other competitors. Thus, we have related the presence or absence of black truffle carpophores, the presence of its mycorrhizae and other mycorrhizal species in the roots and the characteristics of the vascular flora present in the studied trees, in order to know the different ecological characteristics of *brûles* in both producer and not producer trees.

9.47 Arbuscular mycorrhizal fungi of aged *Eucalyptus* in calcareous soils

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In previous studies, we have well established that roots of aged eucalypts are colonized by arbuscular mycorrhizal fungi (AMF). Occurrence of these fungi within roots of aged eucalypts has been controversial for a long time. In this study, three *Eucalyptus* plantations were surveyed. One of the three stands present a calcareous soil. The understorey in this stand shows a very low plant diversity. The two others present a non calcareous soil and an understorey made of many shrubs and herbaceous plants. The survey of the mycorrhizal status was carried out during three consecutive years. Percentages of total roots colonized either by ectomycorrhizal fungi or by arbuscular mycorrhizal fungi were determined. Results obtained showed that root lengths colonized, species richness and diversity of arbuscular mycorrhizal fungi were higher in *Eucalyptus* roots collected from the calcareous soil.

9.48 The importance of resupinate ectomycorrhizal fungi to establishment of coniferous seedlings on fallen logs

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To clarify the effects of ectomycorrhizal fungi on coniferous seedlings on fallen logs in boreal coniferous forests, ectomycorrhizal fungi of *Picea jezoensis* and *Abies sachalinensis* seedlings growing on fallen logs were investigated. In addition, most dominated ectomycorrhizal fungal isolates from fallen logs were inoculated with their seedlings in vitro. Total of 14 fallen logs were selected and 98 and 112 sample seedlings of *P. jezoensis* and *A. sachalinensis* were collected, from two natural coniferous forests of Hokkaido northern Japan. Morphological and molecular identification were performed for those ectomycorrhizas. Over 80 % of total ectomycorrhizas were identified as resupinate fungal *Tylospora fiblillosa* and *Tylospora asterophora* by ITS sequences of the seedlings from both sites during study season. The ectomycorrhizal colonization in seedlings inoculated with *T. asterophora* or *Amphinema byssoides* was observed only on the low pH and poor nutrients media. After 120 days of incubation, the growth of these seedlings was significantly enhanced as compared with that of non-ectomycorrhizal seedlings. The results suggest that the ectomycorrhizal resupinate fungi, especially *Tylospora* spp. would be closely associated with the coniferous seedlings on fallen logs, and this ectomycorrhizal colonization may help them to adapt to acidic and poor-nutrient condition, and thereby support regeneration of the boreal coniferous forests.

9.49 Interactions between arbuscular mycorrhizal fungi and native and non-native invasive plant species after wildfire in the Cascade Range of Oregon, USA

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Suppression of wildfire for the last 100 years has led to large organic matter accumulations, contributing to high severity wildfires throughout the western United States. Small severely burned areas of soil associated with the consumption of large down wood or stumps in direct contact with the soil are common after high severity wildfire. Excessive heating and oxidation of the soil matrix changes the top mineral layer to various shades of red. Such soils, largely void of biological activity immediately following a fire, are thought to increase the potential of invasion by non-native plants. We compared the soil microbial community and growth of 3 native and 3 non-native invasive plant species in paired samples of severely burned and less severely burned soils from a recent wildfire in the Cascade Range of Oregon. Ordination results of phospholipid fatty acid (PLFA) analysis showed that soil microbes were most abundant in the less severely burned soils. Similarly, colonization by arbuscular mycorrhizal fungi (AMF) was greatest in plants grown in the less severely burned soils. Despite dramatic differences in AMF colonization, shoot biomass of several of the native and non-native invasive species did not differ between severely burned and less severely burned soils. Understanding the interactions among burn severity, soil microbial communities, and growth of native and non-native plants will assist forest managers with post-fire recovery.

9.50 Diversity of arbuscular mycorrhizal fungi in spores, roots or soil is differing at a grassland stand (of Central Germany).

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Most studies on AMF species composition solely rely on analysing either mycorrhizal roots or spores. Recent publications showed that different AMF species follow specific colonization strategies, leading to produce variable proportion of intra- and extraradical mycelium. Consequently studies on the AMF communities should consider different AMF structures. We report such a survey in which we used a molecular approach to study AMF species composition on spores, intraradical mycelium in roots and extraradical mycelium in soil of an intensively farmed meadow in Central Germany. We detected remarkable differences in AMF species composition at the family level in the different fungal structures. The *Glomus* group Ab, was dominant in roots and spores, which is consistent with previous works, but was clearly less detected in the extraradical mycelium. For this structure, *Paraglomus* was dominant, though this genus was never found in roots or spores, even not when using a group specific primer set.

9.51 Community structure of ectomycorrhizal fungi colonizing on eight codominant host species in mixed conifer broad-leaved forests in Japan

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Ectomycorrhizal fungal (EMF) communities were investigated in two mixed conifer broad-leaved forests (a secondary and a primary forests), codominated by the variety of tree species. Root systems that were traced to the trunks were collected from eight tree species: *Abies homolepis, Tsuga sieboldii* (Pinaceae), *Betula maximowicziana, B. grossa, Carpinus japonica* (Betulaceae), *Fagus crenata, F. japonica and Quercus crispula* (Fagaceae). Using morphological traits and terminal-RFLP analysis of rDNA ITS regions, we found 205 RFLP types in 13,850 root tips. The observed (estimated) richness of EMF in the secondary and the primary forests were 121 (207) and 137 (209) types, respectively. These values are higher than those reported in most other forests, indicating hyperdiversity of EMF species in these mixed forests. Most of the frequently observed EMF were generalists, detected in two or more host tree families. The EMF richness on *Betula* species, which are early successional species, was significantly lower than that on Fagaceae. The EMF richness on *A. homolepis* in the primary forest was higher than that in the secondary forest, in which *A. homolepis* trees were still small and infrequent. The ordination analysis (DCA) of EMF community also showed that host species belonging to the same families were included in clearly distinguishable clusters. These results indicate that EMF community may depend on the ecological characteristics and the taxonomic status of the host.

9.52 Mycorrhizal communities in a chronosequence of Scots pine stands in North-West Poland

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Diversity and abundance of mycorrhizal fungi were investigated in a Scots pine stands of different age in NW Poland. Root samples were collected from stands of 7, 15, 33, 45 and 80 years. A total of 94 000 mycorrhizal roots were extracted from 120 root samples (24 per age class) and were separated into mycorrhizal morphotypes according to their morphology. Total of 39 morphotypes were found, with only four common for all age classes. The highest species diversity were found in 33 years-old stand, consisting of 20 fungal morphotypes, while the lowest in the youngest stand (12 morphotypes). The relative abundance of *Suillaceae* generally decreased with stands age, from 50% in 7 years-old stand to almost complete disappearance in 33-years-old stand, while reverse tendency were found for *Russulaceae* (4% in 7- to 65% in 80-years-old stand). Abundance of other basidiomycetes, treated collectively, also increased with stands age, from 5% in 7- to 20% in 80-years-old stand. *Cenococcum geophilum* and other ascomycetes were common and frequent in all age classes, with considerable variation in abundance. Mycorrhizal root tip density increased with stand age up to 45 years-old stand (from 2 to 12 root tips per cm^3), and then decrease in the oldest stand (8 tips per cm^3), while live/dead root tips ratio remained constant through all age classes. Results of study suggests, that complex of factors related to forest age, contribute to diversity and composition of mycorrhizal communities.

9.53 Community of arbuscular mycorrhizal fungi in *Moringa* spp.

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Moringa is woody plants mainly distributing in India, East Africa and Madagascar. *Moringa* was known in the ancient, but multipurpose use of the plants, such as edible fruit, cooking oil, animal forage, medicine and water purifier was recently rediscovered. *Moringa* has water-storing organs like bloated trunks or tuberous roots, which enable the plants to adapt to arid environments. They could be, therefore, planted in arid regions. *Moringa* has symbiotic association with arbuscular mycorrhizal (AM) fungi, but little is known for the community of AM fungi in natural and introduced environments. There have been many studies to show that AM fungal association enhanced the plant drought tolerance. In this study, community of AM fungi was examined for eight individuals of *M. hildebrandtii* and two individuals in *M. drouhardii* in Madagascar, and 18 individuals of *M. oleifera* in Uganda. Root samples were collected from those individuals, and DNA was extracted from the samples. Partial 18S rDNA of AM fungi was amplified by PCR using primers NS31 and AM1. After cloning of the PCR products, RFLP and phylogenetic analyses were carried out for those clones. The analyses showed that AM fungi nearly related to *Glomus intraradices* were generally dominant in *Moringa* spp. both in Madagascar and Uganda. The AM fungi might be indispensable for *Moringa* spp. to grow in arid-stressed environments.

9.54 Morphological characteristics and molecular diversity of *Tomentella* ectomycorrhizae in deciduous forests

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Tomentella species (Thelephoraceae, Basidiomycetes) are among the most abundant ectomycorrhizal (EM) fungi in the mycorrhizosphere in needle and deciduous forests. Although identification of these EM is mainly carried out using molecular tools, more valuable results can be got by combining microscopic and molecular methods. In this presentation, *Tomentella* EM collected during a five-year project from broad-leaved forests in Hungary are demonstrated. EM were investigated using both morphological-anatomical (Nomarski DIC, PhC microscopy and SEM) and molecular methods, and identification was approached by comparing their nrDNA ITS sequences with fruitbody-sequences from the GenBank. From *Populus alba*, *Quercus spp.* and *Fagus sylvatica* roots, about 80 specimens of 10 different tomentelloid EM morphotypes have been isolated. Six of these are formed by the species *Tomentella galzinii*, *T. ferruginea*, *T. pilosa*, *T. sublilacina*, *T. subtestacea* and *T. stuposa*. The morphological-anatomical characterization and the result of the phylogenetic analysis of these EM are presented here. Comparison of some anatomical characteristics (mantle, cystidia, rhizomorphs) suitable to separate *Tomentella* EM and their taxonomical value are discussed. Results confirm that tomentelloid EM are constant and abundant members of the EM communities in temperate-continental broad-leaved forests.

9.55 Patterns of AMF host specificity in a serpentine grassland

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Feedback between mycorrhizal fungi and their plant hosts can influence the species composition of the plant community. Feedback relies on some level of host plant specificity among the mycorrhizal fungi. Specificity could involve differences among host plants in the composition of fungal communities, the abundance of individual fungal species, or intraspecific fungal variation. We examined the mycorrhizal communities associated with four C_4 grasses in an East Coast (USA) serpentine grassland (*Andropogon gerardii*, *Schizachyrium scoparium*, *Sorghastrum nutans*, *Sporobolus heterolepis*) using both fungal spore composition found under plants in the field and fungal species frequency revealed through trap cultures in the greenhouse. Fungal communities did not differ significantly among host plants. At the population level, at least two of the ten common AMF species showed clear patterns of host specificity in the field, but others were equally abundant across different hosts. Intraspecific variation was examined by analyzing partial 18S rRNA gene sequences in individual spores. Preliminary data reveal multiple DNA sequences within single spores of *Gigaspora gigantea* as well as genotypic differences among spores. Understanding fungal host plant specificity at the community, population, and genotypic levels will together enhance our understanding of the role of AMF in plant-soil feedback.

9.56 Diversity of ectomycorrhizal macrofungi in mesocosms of 14 tree species

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The role of different tree species in maintaining specific biodiversity of other organisms in forests is still poorly understood. An interdisciplinary project was undertaken in 2004 to investigate the effect of site conditions created by 14 tree species on other groups of organisms, including ectomycorrhizal fungi. The study was carried out in common garden experimental plots, in 33-year-old monocultures growing on two soil types. Fruitbodies of ca. 60 species of ectomycorrhizal fungi were recorded. The plots planted with a variety of trees differed in species number and composition and sporocarp production. Sites with *Quercus robur*, *Betula verrucosa*, *Larix decidua*, and *Pinus nigra* were the richest. Fruitbodies of 25 species were found only in deciduous plots and 10 species only in coniferous ones. Different patterns of fruitbody distribution were identified depending on host specialization of the fungus and tree species. Most mycorrhizal fungi, e.g. *Paxillus involutus*, were multi-host species and occurred under various trees. A few were associated only with one tree species, and their fruitbodies were limited to its sites, e.g *Lactarius quietus* under *Quercus*, *Leccinum scabrum* and *L. versipelle* under *Betula*. Total sporocarp biomass was the highest in *P. involutus* and *Xerocomus badius* (most productive under *Larix* and *Pinus nigra*, respectively). Parallel analyses of mycorrhizae were conducted enabling a joint above- and below-ground view of ectomycorrhizal fungal populations.

9.57 Elevational effects on ectomycorrhizal inoculum: results from a soil bioassay_

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Species turnover within ectomycorrhizal (EM) fungal communities may facilitate host species establishment along environmental gradients. In particular, variation in soil moisture along elevational gradients may drive changes in the composition of EM communities. We tested the influence of elevation and watering regime on the abundance and species composition of EM fungal inoculum. Ectomycorrhizal inoculum was sampled at low (350-750m asl) and high (1300-1400m asl) elevations, two ends of a gradient along which soil moisture was hypothesized to play a particularly important role in structuring both plant and mycorrhizal fungal communities. We planted non-mycorrhizal Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) seedlings into these soils and watered them at various rates to obtain gravimetric soil moisture conditions ranging from <10 to 30%. Seedlings grown in soil collected from low elevations had lower richness of EM morphotypes and had colonization levels that were less than half of those grown in soil collected from high elevations. These responses coincided with changes in soil characteristics. We also observed an inverse relationship between EM colonization and soil moisture. Contrary to our prediction, elevation and watering regime had no effect on EM community composition when measured by abundance. Our findings suggest that pioneer EM fungi, such as would be expected on seedlings establishing after disturbance, occupy a broad range of soil moisture conditions.

9.58 Morphological characterization of *Epidendrum secundum* and *Zygopetalum mackaii* mycorrhizae, native orchids from Brazil

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Epidendrum secundum and *Zigopetalum mackaii*, native from State Park Serra do Brigadeiro, Minas Gerais State, Brazil, were studied to characterize the structure of mycorrhizae and associated mycorrhizal fungi. From August/2004 to July/2005, roots were collected from four different areas and microscopic examination revealed that all mycorrhizae were tolipophagic. In both orchid species, all the extension of the root system presented intact and degraded pelotons. Initial colonization occurs mainly through root hairs and sometimes via epidermic cells, while cortex colonization occurs via passage cells of the exodermis. Pelotons occupy all cellular space and their degradation starts by hyphal deformation, followed by a uniform reduction of peloton volume. Colonization intensity was higher in the dry season. The species growing on rocks showed higher colonization frequency and higher mycotrophic activity. A total of 28 isolates were obtained, all belonging to the genus *Epulorhiza*. The isolates were separated into three groups according to cultural and morphological characteristics. All groups occur both in *E. secundum* and *Z. mackaii*. This is the first report on the mycorrhizal association of *Z. mackaii*.

9.59 Morphological and molecular characterization of *Pisolithus* occurring in Hokkaido Island - Northern Japan

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Pisolithus basidiocarps were found occurring under different forest trees, five of them on volcanic area. Sample of basidiocarps were collected and characterized morphological and molecularly. Although presenting different morphological characteristics of basidiocarps, such as peridium colour and uneven aspects, and growing under different host, Gymnosperms and Angiosperms, all isolates presented spores diameter with 14 ± 1.79 um (including spines), coarsed, crowded and blunted spines and in number of until eight basidiospores per basidium. DNA sequencing also showed high conspecificity. These results suggest low variability of *Pisolithus* occurring in this Island and also that same species is colonizing Gymnosperms and Angiosperms.

9.60 Arbuscular mycorrhizal fungi over a physical disturbance gradient

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Standard arable practice causes high levels of disturbance within soils and it has been demonstrated that arbuscular mycorrhizal (AM) fungal species diversity is lower in arable sites when compared with undisturbed sites. As agriculture becomes more extensive, inputs from agrochemicals are likely to decrease. It is likely crops will have an increasing dependence on a diverse AM community able to provide a wide range of functions. The cultivation of raspberry (*Rubus idaeus*), as a perennial crop, provides an opportunity to assess the diversity of AM fungi across a physical disturbance gradient under arable conditions. In addition, raspberry is one of few plants in the UK that can be found in both wild and cultivated forms allowing comparison between native and cultivated conditions. We have used a combination of molecular approaches to analyse AM fungal colonisation within root material. Further work will assess the functionality of differential fungal communities. We will present data from high throughput sequencing and T-RFLP designed to test the hypothesis that AM diversity increases with stand age and wild sites present contrasting populations compared to cultivated sites. Preliminary results suggest a clear shift in community structure between recently disturbed samples compared to older stands with a gradient of diversity, highest in older plants. We have also identified a seasonal pattern in colonisation and contrasting AM community structure in native plant material.

9.61 Disproportionate abundance between ectomycorrhizal root tips and their associated mycelia

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Despite the acknowledgement of the functional importance of ectomycorrhizal external mycelia, very little is known about the species composition of such structures in soil. In one study, the ectomycorrhizal external mycelia trapped into in-growth mesh bags were compared with the adjacent root tip community. Ectomycorrhizal fungi were identified from roots tips and mycelia by sequencing the internal transcribed spacer region. Boletoid species were significantly more frequent as mycelia than as root tips while russuloid and Cortinarius species appeared to be less dominant as mycelia than as root tips. Tomentella species were equally frequent as root tips and as mycelia. The differences between the root tip and the mycelial view was largely in accordance with the classification of ectomycorrhizas into ectomycorrhizal exploration types. The dominance of Boletoid mycelia in soil found further support in another study where 64 samples were taken in a regular grid with 2 m between sampling spots. In this study, Xerocomus pruinatus root tips were identified from 15 sampling spots but additionally, X. pruinatus rhizomorphs were detected from 29 more sampling spots where no X. pruinatus root tips were present. Ongoing studies are aimed at developing polymorphic markers to track down the belowground structure of X. pruinatus mycelia as well as Q-PCR of specific mycelia within single samples.

9.62 Frantic diversity of resupinate thelephoroid fungi on ectomycorrhizal tree roots

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We will demonstrate the extremely high species richness of the resupinate thelephoroid fungi based on four independent studies: two from Danish beech forests, one in an Estonian wooded meadow and one from a Californian oak forest. In these studies ectomycorrhizal (EcM) tree roots were sampled by using slightly different sampling design and efforts. Sampled EcM were segregated into different morphotypes and further into anatomotypes based on the structure of mantle, cystidia, emanating hyphae and rhizomorphs. One or two root tips of each anatomotypes were subjected to DNA extraction and sequencing. The ribosomal DNA internal transcribed spacer (ITS) sequences of the EcM fungi were obtained for all analysed root tips. Root tip sequences of the resupinate thelephoroid fungi were separated from the rest and were aligned against an appropriate data matrix of fruitbody ITS sequences. We selected 98.0% of ITS sequence identity as species criterion. We also created species accumulation curves for the EcM resupinate thelephoroid fungi to demonstrate species richness vs sampling effort at different sites and to estimate the number of unseen species. In three of the studied sites the resupinate thelephoroid fungi was the most species rich EcM clade exceeding 60 species per site in Estonian wooded meadow.

9.63 External mycelium of ECM fungi and bacteria in outer mycorrhizasphere supported by slow and fast growing Norway spruce (*Picea abies* L.) clones

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External mycelium is the most important functional part of ectomycorrhizal (ECM) symbiosis providing host trees with nutrients and water of vital importance. Mycorrhizosphere is also a habitat for interacting bacteria. In boreal forest communities of ECM fungi and bacteria are diverse and patchy although only a few dominating tree species exist. Trees are known to affect underground vegetation and further soil chemical, physical and biological properties but impact of individual trees on associated microbial community is not well known. A field study investigated ECM mycelium and mycelium associated bacterial communities of 11-years old Norway spruce clones and the relation between host growth and microbial community. External mycelium of ECM fungi was let to colonize sand filled mesh bags four months in clonal trial followed by estimation of fungal biomass. ECM community in mycelium was analysed with DGGE (denaturing gradient gel electrophoresis) and sequencing of ITS1 region and bacterial community with PLFA (phospholipid fatty acid) analysis. Production of mycelium was higher under the fast growing clones than under the slow growing clones and microbial communities of clones varied. Atheliaceae fungi and gram-positive bacteria were often associated to fast growing clones whereas ascomycetes and gram-negative bacteria were more common with slowly growing clones.

9.64 Molecular diversity of arbuscular mycorrhizal fungi colonizing the sporophyte of the rattlesnake fern (*Botrychium virginianum*, Ophioglossaceae)

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Arbuscular mycorrhiza (AM) is the most abundant mutualistic plant-fungus interaction type formed by fungi from the phylum Glomeromycota, with the members of almost all groups of plants including ferns. The purpose of the study presented here was to obtain information about the molecular diversity of the arbuscular mycorrhizal fungi colonizing the sporophytes of *Botrychium virginianum* (L.) Sw. (Ophioglossaceae) whose AM showed distinctive anatomical characteristics. During the study presented here, nine *B. virginiananum* sporophytes were sampled from the Hungarian population of this strictly protected eusporangiate fern. Group specific primers were used in PCRs targeting either partial SSU and ITS or partial SSU region of the nrRNA gene. The amplicons were cloned and sequenced. The *in vitro* drift of the methods was checked. The arbuscular mycorrhizal fungi colonizing *B. virginianum* show relatively high diversity. The sequences were separated into four main lineages during the phylogenetic analysis. Three lineages grouped into the *Glomus* Group-A. One of these was distinct from the sequences of known *Glomus* species, and grouped with sequences obtained from root level of different plants from distant geographic areas. The fourth main lineage clustered into the genus *Scutellospora* and grouped with *S. reticulata*. Although the distinct *Glomus* group might be interpreted as a kind of specific lineage, we can not exclude that this separation is only due to the lack of information.

9.65 Response of seven origins of *Pinus pinaster* Aiton from Spain to inoculation with *Pisolithus tinctorius* and *Paxillus involutus* in nursery

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P. pinaster Aiton is a very important tree in Spain because of its ecological and forest characteristics. This species shows different phenotypic and genotypic characters depending of provenance. Micorrhization and their effects depend, among others, on isolate of fungi used, the tree species used as hospedant and even their origin. In this work the response of several origins of *P. pinaster* Aiton due to ectomycorrhizal (ECM) inoculation was investigated. Seven origins of *P. pinaster* from Spain (Sierra Bermeja, Sierras de Segura-Alcaraz, Serranía de Cuenca, Sierra de Gredos, Meseta Castellana, Sierra de Oña and "piñeiro femia" from Northwest of Spain) were inoculated with *Paxillus involutus* and *Pisolithus tinctorius* using conventional techniques of forest plant propagation. The origins showed different behaviours regarding germination, length increase and anothers biometric variables evaluated, and could be grouped into two different groups by common characteristics: pines from mediterranean climates and pines from atlantic ones. The effect of ECM- inoculum was positive on some biometric variables although no differences were detected of ECM-colonization. Furthermore the morphotypes of the inoculated fungi were not identificated. ECM- colonization had a positive influence on the growth of the seedlings growing in ground beds in the field. However when seedlings were grown in containers in a cold frame, there was not correlation between ECM-colonization and growth.

9.66 Ectomycorrhizal community in a cork oak stand in sardinia (Italy)

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The aim of this work was to provide with a description of the ectomycorrhizal taxa present in a Sardinian cork oak stand. 2895 mycorrhized root tips from 12 soil samples were analysed. Discrimination of ectomycorrhizae was performed by: a) morphological description in order to form homogeneous groups; b) molecular characterization (RFLP pattern using *Alu* I, *Dpn* II and *Hinf* I, and sequencing of the ITS regions) to detect the presence of different species with similar morphotypes. In total 51 types of ectomycorrhizae were found and 43 of them were successfully sequenced. Their taxonomic position was determined comparing sequences with those in GenBank. Nine ectomycorrhizal fungi were identified at family level, 29 at genus level and 5 of them as species. *Russulaceae*, *Thelephoraceae*, *Cortinariaceae*, *Sebacinaceae* and *Tuberaceae* were well represented, whereas *Bolbitiaceae*, *Boletaceae*, *Pluteaceae* and *Sclerodermataceae* were occasionally present; *Cenococcum geophylum* Fr. was the most widespread taxon. The prevalence of *C. geophylum* and the first three families above mentioned is in accordance with what observed in many other ectomycorrhizal communities. As reported in literature, *Sebacinaceae* are among the most frequently encountered taxa in sclerophyllous forests. The high presence of *Tuber* (*Tuberaceae*) is in contrast with the general opinion that this genus avoids acidic soils like that of the cork oak stand under study.

9.67 Succinate dehydrogenase as a measurement of viability of arbuscular mycorrhizal propagules in *Coffea arabica* L. agrosystems affected by erosion

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The viability of arbuscular mycorrhizal fungi (AMF) propagules such as colonized roots, spores and extraradical mycelium was assessed via dehydrogenase activity (DHA). AMF-propagules were collected from four coffee agrosystems characterized by their erosion perturbation: No erosion (NE), Low (LE), Medium (ME), and high erosion (HE). The viability in mycorrhizal roots had significant differences among agrosystems. The highest viable values of root colonization (10.2% total hyphae, and 1% for both arbuscules and vesicles) and extraradical mycelium (35%) occurred at NP samples. In contrast, NP had the lowest values on spore viability (2.3-3.0%). Samples from ME resulted in low viability of root colonization (1-1.5% total hyphae, 1% vesicles, and 0% arbuscules); while viability of extraradical mycelium and spores, was 6.5% and 8.3%, respectively. In the case of samples from HP, viability of root colonization was not observed, though extraradical mycelium viability was low (2.5%); however, spore viability was higher (55%) than that observed at NP, LP, and MP samples.

9.68 Biodiversity of endophytic fungi associated with epiphytic orchid roots in Soconusco, Chiapas, México

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The diverse and mysterious orchid family includes 20–30,000 species and many of these plants are of great importance to the specialist and commercial floriculture industry. Orchid epiphytes are colonized by mycorrhizal fungi; mycorrhizae play a role in the nutrition and competitive abilities of seedlings and the mature plant. For conservation and commercial purposes, it is, therefore, of vital importance to quantify and characterize the diversity of micosymbionts present in the ecozone colonized by epiphytes. The roots were examined of six epiphytic orchid species collected from shade trees and coffee bushes in traditional coffee plantations at 900masl in Soconusco, Chiapas, in Mexico. The root tissues were cleared and stained to permit the observation of pelotons using light microscopy, suggesting the infection of tissue. We found that the roots of *Guarianthe aurantiaca* contained three fungi, *Notylia barkeri* four, *Trichocentrum oerstedii* five, *Platystele ovatilabia* five, *Stelis quadrifida* (synonyms *Anathallis racemiflora*, *Pleurothallis racemiflora*) one and *Erycina crista galli* eight. Species of *Rhizoctonia* were dominant amongst the root isolates. The DNA of each fungal strain was extracted and PCR-RAPD analysis was performed using OPC8 primers. Our results indicate a wide diversity of fungi present in the roots of these epiphytic orchids. Acknowledgements: CINVESTAV–IPN and CONACyT-SEMARNAT (2002-C01-0697).

9.69 Mycorrhizal mycobiota associated with European larch (*Larix decidua* Mill.) in Poland

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European larch is native to Europe and wide distributed in Poland, obligatory ectomycorrhizal (ECM) forest tree. Until recently our knowledge about mycorrhizal fungi colonizing root system of this tree was limited to the above-ground sporocarp inventories. Molecular methods based on polymerase chain reaction (PCR) and investigation of restriction fragment length polymorphisms (RFLP) are among the most useful approaches for more detailed identification of the fungi from mycorrhizal roots and can be target at several taxonomic levels. Two different approaches were performed to examine ECM mycobiota of European larch: morphological analysis of ECM roots and molecular techniques (PCR-RFLP and sequencing of ITS rDNA region). Numbers of individual mycorrhizal morphotypes (richness) differed depending on the age of larch trees. Young trees (1-4 years old) were mostly colonized by ectendomycorrhizal fungus *Wilcoxina mikolae* and ECM fungus – *Suillus grevillei*. Important role at the below-ground ECM community structure in larch forests fulfills *Cenococcum geophillum* and *Paxillus involutus*.

9.70 Effect of forest moss cover disturbance on arbuscular mycorrhizal colonization in *Pineetum myrtillosa* communities

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Arbuscular mycorrhizal fungi (AMF) were studied in Boreonemoral Pine forest. The purpose of present research was to investigate how a moss cover disturbance influences the mycorrhizal infection. Moss cover was removed and turned over. In such a way the mycorrhizal infection was caused in the root system of *Lerchenfeldia flexuosa*. It was found that the total AMF root colonization did not depend upon the climatic reasons in summertime, but from disturbance character. Percentage root colonization of AMF was significantly higher in the sampling sites where moss cover was turned over rather then simply removed away. Four species of AMF were collected from soil samples. All species was from the order *Glomerales*. One of them was identified as *Glomus intraradices*. Keywords: arbuscular mycorrhizas, pine forest, *Lerchenfeldia flexuosa*.

9.71 Molecular analysis of AMF colonising roots of *Gossypium hirsutum* and *Trifolium repens* inoculated with field soil by ARDRA

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Molecular methods have been successfully applied to study communities of arbuscular mycorrhizal fungi (AMF) colonising the roots of plants. This study was conducted to examine the effect of crop rotations on AMF communities in cotton growing systems. The diversity of AMF colonising the roots of two host plants (*Gossypium hirsutum* and *Trifolium repens*) grown in field soil was analysed using amplified ribosomal DNA restriction analysis (ARDRA). Partial (560bp) 18S rDNA fragments were amplified using universal eukaryote primer AMV4.5F and newly developed, AMF-targeted primer AM1130R. A peptide nucleic acid probe, PNA 1150R, was designed and included in the reaction to suppress non-specific amplification of host plant DNA. A total of 1920 clones were screened using three restriction enzymes (HinfI, Hsp92II and Taqlá). One representative from each RFLP type was sequenced. The majority of sequences (<95%) were Glomeromycotan in origin. *Glomus* group A, group B, *Diversispora* and *Acaulospora* sequence types were detected in the roots of the two hosts. Approximately 2% of cloned fragments were ascomycete in origin. The remaining clones were suspected chimeric sequences. These were excluded from the analyses.

9.72 Arbuscular mycorrhizal fungi spores in soils of a reclaimed site impacted by buried solid wastes in the México City Valley

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In the Mexican Republic, the urban solid wastes are usually buried and covered with soil. This practice generates severe environmental impacts and it is difficult to establish a new plant community on the site due to the substrate pollution. For this, the objective of this work was the establishment of plant species (shrubs and trees) on an ancient open air solid wastes disposal area, covered with exogenous soil. The site is located at the Nezahualcoyotl Municipality (19° 25' 13" – 19° 26' 00" NL and 99° 00' 45" – 99° 01' 20" LW; 2250 m above sea level); soil conditions were characterized and 250 plant individuals of 7 species were planted in the site. Ten years after, the results show that the total survival of the planted individuals was of 8% and that soil conditions changed to present now higher values in electric conductivity, organic matter content and heavy metals concentration. The spores number by 100 g of soil, associated to roots of survival individuals, was 46 for *Casuarina equisetifolia*, 48 for *Senna tomentosa* and 34 for *Nicotiana glauca*. *Glomus* was the most frequent genus of AM fungi. It was concluded that there was a natural selection of soil pollution resistant plants and AM fungi; also, it is possible to apply AM fungal inoculum, of the reclaimed site, to seedlings of resistant species in order to establish plant populations and to achieve the phytoremediation of this type of soils.

9.73 Respiratory acclimation and adaptation to temperature by ectomycorrhizal fungi

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The soil respiratory CO_2 flux is many times larger than that caused by fossil fuel burning. In a warming planet, scientists are concerned for the possibility of a positive feedback between global temperature and carbon dioxide fluxes from soil. Ectomycorrhizal fungi contribute greatly to soil respiration in forests, along with roots and other soil microorganisms. The physiological response of ectomycorrhizal fungi to temperature is poorly understood. If like in plants, respiration by ectomycorrhizal fungi is able to acclimate, or regulate its response to temperature, it might partially ameliorate the positive feedback between global temperature and carbon dioxide fluxes from soils. We found no evidence for short-term respiration acclimation to temperature for at least ten different isolates of ectomycorrhizal fungi in culture. However, since we sampled ectomycorrhizal fungi from across a latitude gradient, we were able to find evidence for long-term evolutionary responses to temperature. Thus, in a warming climate, ectomycorrhizal fungi may not be able to ameliorate the positive feedback between global temperature and carbon dioxide fluxes from soil in the short-term, but may be able to do so on a longer time scale. Nonetheless, if plant roots limit carbon fluxes to ectomycorrhizal fungi, they might influence the short-term respiration response to temperature by ectomycorrhizal fungi.

9.74 Is diversity of arbuscular mycorrhizal fungi involved in the growth of pioneer plants in acid sulfate soil ?

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Acid sulfate soil is formed by oxidation of sulfur-rich minerals. Revegetation of the soil is difficult due to low pH and poor nutrient availability. However, some pioneer plants in symbiosis with microorganisms survive and grow under such an extreme environment. The objective of the present study is to clarify the involvement of AM fungal diversity in the growth of pioneer plants in acid sulfate soil. The experimental sites are disturbed areas in Rankoshi-cho, Hokkaido Is. (sub-arctic) and Hazu-cho, Aichi, Honshu Is. (temperate) in Japan. The rhizosphere soils of *Miscanthus sinensis*, a common pioneer grass in Japan, were collected from 12 plots in each site, and *M. sinensis* was sown onto the soils and grown in a greenhouse for 2 months. Biomass of *M. sinensis* was measured, and DNA was extracted from the roots. About 700 bp fragment of 28S rDNA of AM fungi was amplified by using the fungi-specific primers and cloned, and 14-32 clones were randomly chosen from each plot and sequenced. *Glomus intraradices*, *Archaeospora gerdemanni* and *Paraglomus occultum* in addition to uncultured glomeromyocta were found from the Rankoshi-cho site, and *G. intraradices*, *Acaulospora mellea* were found from the Hazu-cho site. The involvement of the soil chemical properties (pH, available P, total N and C) and species richness of AM fungi in the growth of *M. sinensis* was assessed.

9.75 Colonization of air roots by arbuscular mycorrhizal fungi in decomposing *Araucaria angustifolia* (Bert.) O. Ktze. Trunks in Campos do Jordão, Brazil

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A great variety of plant species is present in *Araucaria angustifolia* forests, similarly a significant amount of munch and organic matter accumulates on the soil. Roots of *Araucaria* trees have been observed on the soil surface recovering decomposing trunks, up to 1 m high. Five areas have been randomly chosen for root and organic substrate collection on the trunk surface in a native *Araucaria* forest area, located at Campos do Jordão State Park, São Paulo, Brazil. The goal of the present work was to access the colonization of the surface roots by arbuscular mycorrhizal fungi (AMF) and to investigate if the spore production is enough for morphological classification. The identified AMF were further characterized by molecular analyses. All *Araucaria* root segments investigated showed mycorrhizal colonization. Colonization rates ranged from 31.62% to 54.2%. The number of AMF spores found in distinct locations ranged from 27 to 164 spores per 50 g of dry substrate. We have identified 18 AMF species, belonging to four genera. The predominant species depended on the location and *Acaulospora laevis* was the only species present in all arias. DNA was extracted from the AMF colonized roots and it was further submitted to PCR amplification using the NS31/AM primer pair. The obtained fragments were subsequently cloned and sequenced, allowing us to identify species of *Glomus* spp. and *Acaulospora* spp. colonizing the roots, along with other non mycorrhizal fungi.

9.76 Mycorrhization of a rare plant community on an alpine chalk gravel slope

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Arbuscular mycorrhizal fungi (AMF) appear to influence plant community structure and diversity. Hence it is of special importance to analyse natural fungal communities in order to be able to provide inoculum consisting of indigenous AMF, if needed. The aim of the study is to determine the diversity of AMF species colonizing plant species of the rare plant community Laserpitietum sileris developed on an alpine chalk gravel slope. In 2003, soil and root samples of 11 plant species were collected from the field site at three different stages of plant development. The study site is in a NATURA 2000 habitat in the county of Salzburg, Austria (Bluntautal, 47°34'N 13°8'E, 520m asl). AMF spores were isolated from field soil, counted and identified. The colonization intensity by AMF and dark septate endophytes (DSE) was determined. Roots of the same plant individuals were surface-sterilized, DNA was extracted and a PCR was performed for amplification of partial SSU ribosomal RNA genes. After cloning, RFLP was used to screen for polymorphism to select clones for sequencing. The results obtained so far show that the roots of all investigated plant species are colonized by AMF, forming specific structures over the whole vegetation period. The majority of the plant species is colonized by DSE as well. Different AMF species could be detected by spores and will be related to the molecular data, which indicate that the dominant AMF species colonizing these plants belong to the genus *Glomus*.

9.77 Morphological and molecular identification of *Kobresia myosuroides* mycorrhizae from a glacier forefront in the Austrian Alps

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Unknown fungal lineages have recently been detected in snow-covered tundra soils with *Kobresia myosuroides* (Vill.) Fiori & Paol. (*Cyperaceae*) vegetation (Schadt *et al.* 2003). This makes fungal communities associated with the sedge *K. myosuroides* especially interesting. It is long known that *K. myosuroides* forms ectomycorrhiza in arctic and alpine areas (Fontana, 1963), but mycorrhizal communities of this unusual ectomycorrhizal host plant are still poorly understood. Therefore, we are studying the mycorrhizal associations of *K. myosuroides* with special emphasis on its seasonal dynamics. Our sampling area (about 20.000 m2) on the glacier forefront of the Rotmoosferner (Austria) lies within the moraine ridge of 1920 (2200 m.a.s.l). Five sampling plots (1 m2) were selected within this area. Mycorrhized root tips of the fife plant samples (5 x 5 cm) per plot were pooled, and 100 root tips were randomly selected. They were classified into morphologically similar groups (morphotypes), several representatives of each morphotype were examined using molecular techniques (PCR of the rDNA ITS region, RFLP, sequencing). Eleven morphotypes could be differentiated during the vegetation period 2005 (Jun., Aug., Sept. sampling), but our molecular approaches showed that several fungal mycobionts exhibit the same morphotype. Fungi forming inconspicuous fruitbodies such as corticoid basidiomycetes (*Sebacina, Tomentella, Thelephora*) or *Cenococcum* dominate in this primary successional habitat.

9.78 Selection of DSE1 hosts and effect on wheat, tomato and soybean growth

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DSE (dark septate endophytes) are a group of anamorph fungi that colonize root tissues causing different effects in several plant species. We isolated DSE strains from superficially sterilized rye grass roots and tested the response to different inoculation forms using various horticultural and grain-producing plants. The best results were obtained using vermiculite saturated with malt extract broth as the fungal carrier. After 28 days, the percentage of colonization was the following: Corn :30.3%, tomato: 29,6%, wheat: 34.6, barley: 47%, oat:<5%, rye grass:22%, egg plant:19.3% and soybean: <5%. We chose three of the species tested: wheat and soybean (which are grain-producing and extensive crops) and tomato (which is a horticultural crop). After 90 days, we measured different growth parameters and observed an advanced flowering and greater growth in height in wheat (colonization index= 76.7%), and greater growth in height and dry weight in tomato (colonization index=49.33%). Differently, no significant changes in the parameters measured, together with a small colonization of the root (<5%), were observed in soybean. We concluded that: a) different plants can be colonized by the same species of DSE, b) each plant reaches a different colonization percentage and c) the effect of this endophyte varies with each plant species.

9.79 Effect of *Glomus intraradices* on alfalfa growth and survival in contaminated soils treated with biopile and thermal desorption

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Arbuscular mycorrhizal fungi (AMF) enhance plant survival and growth in polycyclic aromatic hydrocarbon (PAH) contaminated soils and may contribute to PAH degradation. AMF also help the plants to cope with high heavy metal concentrations. There is a potential benefit for using AMF for the phytoremediation of multi-contaminated sites and of residual contamination or poor nutritionnal status of remediated soils. We investigated the effect of AMF and nutrients on plant growth and on the fate of pollutants in an industrial site contaminated with both PAH and heavy metals treated or not with biopile and thermal desorption. In a pot experiment, the three soils were planted or not with alfalfa (*Medicago sativa* L.), inoculated or not with *Glomus intraradices* and fertilised or not. After 2 months, plant survival and biomass, AMF colonization, PAH concentrations in soil and ETM concentrations in plants were measured. AMF and bacterial communities in roots and rhizosphere were also analysed with a PCR-TTGE technique. Alfalfa survival and growth was lower in biopile and thermal desorption treated soils and the diversity of the bacterial communities differed in comparison to the non treated soil. The fertilization and especially AM inoculation improved the survival, the growth and the nutrition of alfalfa. The effect was less pronounced on the fate of PAH and heavy metals. The AMF could indeed act on the structure of the microbial communities in the rhizosphere.

9.80 Edge effects in a tropical rain forest: implications on arbuscular mycorrhizal fungi communities.

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Deforestation is the main cause of forest fragmentation, changing the landscape to patches of secondary vegetation, natural forest and livestock farm. In the transition zone between the forest and livestock farm area, there is an edge effect where light, humidity and some soil conditions change. In this study, we analyzed if the edge effect is related with richness and spores abundance of arbuscular mycorrhizal fungit in a tropical rain forest in Veracruz, Mexico. Soil bulk density and temperature had higher values in livestock farms. Humidity values increased inside the forest, whereas pH was lightly acid in the livestock farm. 25 AMF species were reported, belonging to the following genera: *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*. The highest richness and spores abundance values were observed in middle part of the edge. *A. scrobiculata*, *A. apendiculata* and *G. caledonium* were wide distribution along the edge; *G. magnicaule*, *G. etunicatum* and *A. mellea* were found only in the forest and *A. foveata* and *G. fasciculatum* appeared only in the livestock farm. We conclude that there are AMF species with more tolerance to environmental stress in the livestock farm area.

9.81 Sporocarps occurrence after the 2000 eruption on the volcano Usu, Hokkaido, Japan

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We investigated sporocarps occurrence at the volcano Usu, devastated by thick volcanic deposition in 2000. In 2004 and 2005, we determined species richness and composition of macrofungi in the devastated area and its surrounding area where natural vegetation survived and recovered quickly. A total species richness of macrofungi was 86. Species richness was quite larger in surrounding area than devastated area, sixty-nine and 9 fungal species, respectively. In devastated area, saprophytic fungi such as *Coprinus* spp. and *Lyophyllum* spp. were mainly observed. ECM sporocarps did not be observed in 2004, however, four ECM fungi, *Laccaria amethystea*, *Laccaria bicolor*, *Inocybe lacera* and *Hebeloma mesophaeum*, which seemed to associate with seedlings of 3-4 years old willows, were newly observed in 2005. In the surrounding area, various saprophytic fungi such as Collybia and Mycena were mainly observed. Most of ECM fungi observed in 2004 were also occurred in 2005 with new occurrence of 5 ECM fungi. Those ECM fungi associated with survived mature trees. Only 8 species, such as Coprinus spp. and H. mesophaeum were commonly observed in both areas. The species composition of macrofungi between the devastated and surrounding area was largely different. Difference of several factors such as presence of litter layer, composition and maturity of woody plant species and moisture content of ground between both areas may affect the colonization of macrofungi.

9.82 The extraradical mycelium of different native AMF influences the coexistence of two plant species in a highly alkaline anthropogenic sediment

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Species of arbuscular mycorrhizal fungi (AMF) can produce different amounts of extraradical mycelium (ERM) with differing architectures and have different efficiencies in gathering phosphate from the soil. These differences often result in differential growth responses of plants and this may be an important contributor to plant species coexistence. The effects of the development of the ERM of AMF on the coexistence of two plant species were investigated. The dominant shrub (*Salix atrocinerea*) and herbaceous (*Conyza bilbaoana*) plant species found in a highly alkaline anthropogenic sediment were studied in symbiosis with four native AMF species that represented the majority of all the AMF diversity found at the sediment. Different AMF species did not influence total plant productivity, but had a great impact on the individual biomass of each plant species. The AMF species with greater extracted ERM lengths preferentially benefited the plant species with a high mycorrhizal dependency (*C. bilbaoana*), while the AMF species with the smallest ERM length benefited the plant species with a low mycorrhizal dependency (*S. atrocinerea*). Seed production of *C. bilbaoana* was only observed in plants inoculated with either of two AMF species or the mixture of the four AMF. The community composition of native AMF and the length of the mycelium they produce spreading from roots into the surrounding soil can determine the coexistence of naturally co-occurring plant species.

9.83 Are black truffle systems under risk of recession due to a warming climate?

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High soil temperatures are known to prevent the formation of *Tuber melanosporum* fruiting bodies. Because of greenhouse-gas-induced climate change, summer average temperatures are expected to increase 4°C-4.5°C in the Mediterranean basin area. We aimed to know the influence of soil temperature and soil water content on the symbiotic relationship between *T. melanosporum* and *Quercus ilex*. One-year old holm oak seedlings inoculated with *T. melanosporum* were grown in containers with a mixture of natural soil and perlite (4:1 v/v) and placed in large container resembling field conditions, covered from rainfall by a semi-tunnel. We exposed the containers to four soil temperature regimes and to four soil water content regimes from July to October. Larger biomass production was obtained with greater soil water contents. Under moderate soil water deficit, the root tip colonization by *T. melanosporum* decreased with increasing soil temperatures. Nevertheless, under strong soil water deficit, only the lower temperature treatment enabled the largest presence of *T. melanosporum*. In a warmer temperature scenario, holm oak could keep its growth contrary to its partner *T. melanosporum*, especially if higher temperatures occurred with great water inputs. Climate change conditions could displace present *T. melanosporum* systems to greater latitudes or altitudes. Mulching and other host species could be considered to reduce the effects of a possible increase of soil temperature.

9.84 Evaluation and valorization of biological diversity in Edough mountain (N.E, Algeria)

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The Edough mountain makes up the northern segment of alpine chain in northern, Algeria. It was an old island connected to the continent by pliocenis sediments which covered the depression whose middle is occuped by Fetzara lake. The originality of this forest consists on simultaneous presence of *Quercus canariensis*, *Pinus maritima*, *Prunus avium*, *Castanea sativa* and *Ilex aquifolium*. About 350 species had been determined, among which different biogeographical origins, essentially tropical and european ones, some relics species attest that. So it is urgent to protect them. It will be interesting to study the adaptation of rare and exotic species. An important human activities take part in the degradation of forestry ecosystems. Some rustics species well adapted can be used to reforest the area and other have medicinal, aromatic and culinary properties. Preservation and sustainable use of biological diversity, requires the protection of habitats and vulnerable ecosystems. It had become important to go thru deeper knowledge of natural environments and ecological possibilities, with an evaluation and valorization of plant genetic resources.

9.85 The impact of ecosystem degradation on arbuscular mycorrhizal fungal diversity in the rhizosphere of plant species of singular ecological value

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Disturbance of the vegetation cover in natural ecosystems usually has a negative impact on AM fungal population (number and diversity), a constraint for the successful reestablishment of native plants. This is particularly relevant in the case of shrub communities, characteristics of semiarid Mediterranean ecosystems. To investigate these aspects, five representative communities of shrub species, known to form AM, were selected in the Natural Park of Sierra de Baza, Granada, southeaster Spain. For each community, plots where the vegetation cover was optimal and plots where diversity and structure of the optimal vegetation cover appear degraded were chosen. Rhizosphere samples were taken from all the target species and AM spores isolated and characterized (morphology). Species richness, isolation frequency, relative abundance, and spore density were calculated. The Shannon-Weiner biodiversity index, which incorporates evenness and the number of species, the two main components of diversity, was used. Molecular techniques based on the sequence analysis of the SSU rRNA were further applied. The main conclusion is that the degradation of the vegetation cover affected negatively the diversity of AM fungi. Some 10/12 AM fungal taxa per well-preserved communities have been characterized both morphologically and molecularly. These ecosystem-adapted fungal ecotypes are available as a source of AM-inoculants for revegetation of degraded area in the target ecosystem.

9.86 Effects of wildfire on arbuscular mycorrhizal fungus associations in *Araucaria-Nothofagus* forests soil, Chile

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Temperate forest ecosystems of Chile are N limited and biological processes of soil are key for ecosystem stability. Mycorrhizal status is clearly dominated by arbuscular mycorrhiza fungus (AMF) at those ecosystems. Wildfires affect simbiotic associations of AMF, increase losses of disponible N from organic soils and enrich ∂^{15}N pattern in soil profile and plants tissue. Fire damage on AMF is normally responsible for changes in ∂^{15}N signals in associated plants and resistant AMF should provide advantages for seedlings recruitment avoiding ecosystem threshold shifting. Spore study from burnt and unburnt soils was conducted after a wildfire in the Tolhuaca National Park, Chile (38° S; 72° W). Experimental assay at greenhouse was conducted for evaluating morphological and biomass development and AMF colonization rates in roots of Araucaria araucana seedlings on burned, unburned and sterilized soil. Fire-resitant spores of AMF were found in burnt soil. Significant differences were observed to seedling development under treatments at the greenhouse assay, mainly root biomass and the general quality index. Frequency and intensity of AMF colonization in A. araucana roots were clearly higher at unburnt soil and correlation to the morpho-physiological aspects of seedlings was observed. Signals of $‰^{15}$N increased significatively in detailed soil profile and plants at burnt area and the relation to AMF demaged is discussed. Programa Bicentenario/CONICYT/UdeC (Chillán) is aknowledged.

9.87 Species diversity of mycorrhizal fungi associated with alpine plant communities from a glacier forefront in the Austrian Alps

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Presence and species diversity of mycorrhizal fungi can be assessed in various ways. One is to collect data on the occurrence of fruit bodies: they indicate the presence of a taxon in the soil, whereas the opposite may not be true. Studies on fungi on glacier forefronts are rare because these sites are difficult to access, and alpine fungi are difficult to identify. We studied the fungal species diversity at the Rotmoosferner glacier forefront (46°50´N, 11°03´E), a primary successional site located in the Austrian central Alps (2280 to 2450 m a.s.l.). First ectomycorrhizal (ECM) plants such as *Salix herbacea*, *S. retusa*, *Polygonum viviparum* and *Kobresia myosuroides* appear after about 70 years of soil development. They grow in a patchy distribution together with *Poa alpina*, *Alchemilla* spp. and *Carex bicolor*. Fruit bodies belonging to 187 fungal species were detected. 42 % of them are ECM, 58 % are saprobial. Seven ECM genera of Basidiomycota and one of Ascomycota were found. The most species-rich genus was *Inocybe* (35 spp., 46 % of ECM fungal species). Also *Cortinarius* species were frequent (22 spp., 29 %). The other ECM genera were *Hebeloma* (3 spp.), *Russula* (5 spp.), *Lactarius* (4 spp.), *Laccaria* (3 spp.), *Helvella* (3 spp.), and *Thelephora* (1 sp.). Sequences generated from this voucher material will be used for comparison of the above-ground ECM species composition with the respective belowground mycorrhizal frequencies.

9.88 Belowground ectomycorrhizal communities in three Norway spruce stands with different degrees of decline in the Czech Republic

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In an effort to understand the constraints of natural regeneration in declining spruce forests in the Giant Mountains, the ectomycorrhizal communities associated to three stands with different degrees of decline were investigated by morphological and molecular techniques. On roots of adult trees and seedlings the ectomycorrhizal species richness was lowest in the most damaged site, however, less drastically than seen in a fruitbody survey. Although less species-rich, roots were fully mycorrhizal even on the site with the highest degree of decline. *Tylospora fibrillosa* was the most abundant species on adult trees. It made up over 60 % of root tips on the most damaged site, twice as much as on the other two sites. This species, as well as *Thelephora terrestris*, were efficient colonizers also of roots from seedlings, in particular in the most damaged site. Since highly defoliated trees assumably transfer less carbon to ectomycorrhizal fungi, we hypothesize that species being able to survive under these conditions can utilize other carbon sources than those mediated by the living plant. The *Corticiaceae* and *Thelephoraceae* are an important component of the belowground ectomycorrhizal community in most temperate and boreal forests, but the role they play might even be more crucial in stressed forest ecosystems. Based on our results we suggest that there must be other reasons than missing ectomycorrhizal inoculum that constrain natural regeneration in these declining forests.

9.89 *Medicago* species affect the diversity of arbuscular mycorrhizal fungi associated with roots

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The symbiotic association between arbuscular mycorrhizal fungi (AMF) and plants was described to be non specific. However, AMF were reported to influence plant community diversity and productivity. In contrast, the effect of plant genotypes belonging to closely related species on AMF diversity has not been explored so far. The aim of this work was to assess the impact of four different *Medicago* species, *M. polymorpha*, *M. murex*, *M. laciniata* and *M. truncatula*, on the diversity of AM fungal community. The intensity of mycorrhizal colonization was evaluated microscopically. A nested PCR targeting partial sequence of the variable domains D1 and D2 of the large ribosomal subunit (LSU rDNA) genes was performed on DNA extracted from bulk soil and root tissues. PCR products were used to construct LSU rDNA libraries. Randomly selected clones were sequenced and their phylogenetic analysis allowed the design of Operational Taxonomic Units (OTUs)-specific PCR primers, applied for quantitative PCR. A significant difference in the intensity of root colonization by AMF was recorded among the Medicago species. The phylogenetic tree gave high bootstrap values, allowing the delineation of 12 OTUs of AMF sequences, all belonging to the genus *Glomus*. Results of quantitative PCR showed that the abundance of the four selected OTUs associated with the four Medicago species differed significantly. These data suggest that the genetic structure of the AMF community is affected by the plant species.

9.90 Arbuscular mycorrhiza influences plant community structure in succession on spoilbanks

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The experiment simulated a plant succession stage when perennial grasses (e.g. *Calamagrostis epigejos*) invade communities of annuals with different mycotrophy (e.g. *Atriplex sagittata* and *Tripleurospermum inodorum*) on coalmine spoil banks. Communities of these three model species were planted in large-volume microcosms either in the presence of pre-established mycelium network of three arbuscular mycorrhizal fungi (AMF) species (individually and in a mixture) or without AMF. Different AMF species had significantly different effects on specific plant species' growth, which resulted in changes in plant community structure. While in non-mycorrhizal conditions the non-mycotrophic plant species *A. sagittata* contributed nearly 70% to the total plant biomass, in the presence of the 3 AMF mixture the contribution of this species was only about 10%. Different effects of AMF on tiller formation by *C. epigejos* suggest that some AMF could have greater potential to promote the replacement of annuals by perennial grasses than others. It can be concluded that not merely the presence but also the identity of AMF present on spoil banks can affect the outcome of coexistence of plant dominants, the community structure and the progress of plant succession. Financial support of the Grant Agency of the Czech Republic (grants 526/04/0996 and 206/03/H137) and Grant Agency of the Academy of Sciences of the Czech Republic (grant AV0Z60050516) is gratefully acknowledged.

9.91 Influence of ectomycorrhizal (ECM) communities on the growth of *Pinus montezumae* on volcanic soils

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Tree growth depends on abiotic and biotic factors that together are known as site quality. We meant to study fungal species composition and their possible influence on the growth of *Pinus montezumae*, along a volcanic chronosequence. The forests of the Sierra del Chichinautzin were highly disturbed by tree logging o fires; reforestation with pine seedlings inoculated with native species of ECM fungi, and thus adapted to site conditions, would enhance seedling survival and more rapid growth. The first year objectives were: 1)To identify the species of ECM fungi that were associated with *P. montezumae* on volcanic soils with different development states. This characterization was done through morphological determination of fruiting bodies and mycorrhized fine roots, sampled during the rainy season. 2) To correlate fungal communities with soil factors and pine growth rates. We demonstrated that communities of ECM fungi varied according to the site. Some of the sampled species were shown to be typical of a site. Species like *A. muscaria*, *L. bicolor* and *G. floccosus* were only found in the youngest site, whereas *C. olla*, *A. echinocephala* and *G. triplex* were only sampled in the intermediate site, and *H. crispa* and *C. sp.* in the oldest site. Differences were also observed in species richness and diversity. The Chichinautzin volcano, with the lowest site quality (shallow soils, lowest organic matter content, etc) was shown to be the site with the highest species variety.

9.92 Spatial distribution of arbuscular mycorrhizal fungi in the rhizosphere of the salt marsh plant *Inula crithmoides* L. along a salinity gradient

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The influence of flooding and salinity on the arbuscular mycorrhiza (AM) fungal spore and root colonisation level in *Inula crithmoides* was studied. One transect divided into five plots was established from shoreline (border) to inland in La Mata lagoon, Alicante (Spain). Soil salinity and flooding are two environmental factors which are at first unfavourable for AM fungi development; therefore, it has always been believed that plants developing in these environments are not generally colonised by these symbiont fungi. High salinity level and flooding led to and decreased spore number in soil, and a decrease in the percentage of mycorrhization. In our study, spores are negatively correlated with EC, and they also lower when soil flooding increases. The number of mycorrhizal fungal propagules resulting from the Most Probable Number (MPN) experiment was very low. *Glomus geosporum* was the most frequent fungal species in all plots. With regard to the mycorrhization percentage, an inverse relationship exists with salinity in such a way that the higher colonisation values appear in salt marsh zones with a lower electrical conductivity. On the other hand, this percentage is positively correlated with a higher percentage of colonisation at pH values around 9. This might suggest that pH plays a role when the symbiont relation of arbuscular fungi is produced.

9.93 *Bromus tectorum* associations with arbuscular mycorrhizal fungus community change: causation or correlation?

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Non-native plant species may alter arbuscular mycorrhizal fungi (AMF) communities in ways that benefit themselves in competitive relationships. Studies show that *Bromus tectorum* L. invasion is associated with shifts in AMF community composition and decreased AMF richness. Using two experiments, we examined whether *B. tectorum* directly changed AMF communities or whether *B. tectorum* invasion was correlated with areas low in AMF. In one experiment we compared mycorrhizal inoculation potential of native plant soils when surrounded by either *B. tectorum* or native vegetation. Mycorrhizal inoculation potential was lower in the *B. tectorum* dominated areas for both species. In a second experiment we examined how *B. tectorum* and three native plant species would condition two sources of soil. *Bromus tectorum* did not affect the AMF or final plant biomass differently than other species studied. These results suggest that *B. tectorum* does not directly affect the mycorrhizal community in the short term.

9.94 Diversity and function of ectomycorrhiza during transformation of forests

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During the transformation of forests biological niches are created that are thought to result in enhanced biological variety. In the present study diversity and function of mycorrhizas were investigated in a pure stand of Scots pine (*Pinus sylvestris* L.), in a chronosequence of Scots pine stands that were underplanted with beech (*Fagus sylvatica* L.) and in a pure beech forest. Mycorrhizal diversity was higher in the pure stand of beech compared to the pure stand of Scots pine. Beech trees in the mixed forests had dominating morphotypes that were very similar to the ones dominating in Scots pine. Therefore, it seems plausible that mycorrhiza coenosis of Scots pine is influencing the coenosis of beech in Scots pine stands being transformed into mixed stands of Scots pine and beech. However, trees in the pure beech stand were mycorrhized with other types. These types were distributed in even dominances. Along the chronosequence, frequency per soil volume and nutrient amount of beech mycorrhizas increased. Nutrient amounts were higher for mycorrhizas of older beech than for mycorrhizas of older pine. This indicates that older beech trees are able to accumulate specific nutrients in the upper mineral soil and the forest floor layer. This observation supports the hypothesis of the so-called ‚base-pump effect‘ of the older beech trees.

9.95 The influence of monoterpenes on Scots pine ectomycorrhizal communities

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Little is known about the effect of genetic variation between mature trees in the field on ectomycorrhizal communities. Scots pine (*Pinus sylvestris*) shows high intraspecific variation in the monoterpene content of its foliage, and this is known to be under strict genetic control. Because monterpenes have the potential to be biologically active in a number of ways in soil/plant/fungal interactions, we chose to examine the effect of monoterpene profiles on ectomycorrhizal communities of individual trees. Six pairs of trees with high and low foliar concentrations of 3-carene were selected in a native Caledonian pine forest. In each pair the trees were within 10m of each other and more than 10m away from any other pair. Cores were taken from beneath the canopy of each tree and ectomycorrhizas were counted and identified using a combination of morphological and molecular methods. Mycorrhizas were allocated to individual host trees using microsatellite markers (Saari et al., 2005). The data were examined for effects of host or canopy type on ectomycorrhizal community structure. There were no effects of 3-carene in isolation but there were more subtle effects of overall monoterpene composition. Reference: Saari, S. K., Russell, J., Campbell, C. D., Alexander, I. J. and Anderson, I. C. 2005. Pine microsatellite markers allow roots and ectomycorrhizas to be linked to individual trees. New Phytologist 165, 295-304.

9.96 Diversity of arbuscular mycorrhizal fungal isolates derived from a boreo-nemoral forest

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Previous studies have shown that plant roots in managed and unmanaged areas can host dissimilar arbuscular mycorrhizal (AM) fungal communities. AM fungal species may differ in properties like mycelium growth rate, amount of spores they produce, spore germination rate and colonization rate of plant roots. The objective of this study was to isolate AM fungi from Estonian boreo-nemoral forest soil and to compare the molecular diversity of AM fungi in plant roots and the spore community in soil. Spores from unmanaged (90-100 years old stand) and managed (15-30 years old stand) forest soil were isolated from trap-cultures set up with natural soil inoculum and grouped according to their morphological characters. Representative spores of each group were identified by sequencing of small subunit RNA gene fragment. To date we have isolated ~10 spore morphotypes, belonging to families Glomaceae; and Acaulosporaceae. From plant roots, 30 sequence groups have been identified belonging to the same two families. Our data confirm previous results that AM fungal diversity analyses based only on spores in soil or only on root-colonising fungi give an incomplete view about the diversity in a given ecosystem. The observed difference in number of spore morphotypes and sequence groups may arise from differing sporulation manners of AM fungi, spatial or temporal growth patterns of fungi, or methodological reasons.

9.97 Analysis of the AM fungi colonising the roots of representative shrubland species in a desertification-threatened Mediterranean ecosystem

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Mediterranean climate together with anthropogenic degradation activities are constraints for plant development which can promote degradation of the soil-plant systems. Degradative processes are known to reduce the inoculum potential of arbuscular mycorrhizal (AM) fungi, a key ecological factor in governing the cycles of major plant nutrients and hence in sustaining the vegetation cover in natural habitats. Within the framework of the revegetation programmes currently being developed in our Department, several approaches are followed to analyze the diversity of AM fungal population associated with key plant species from target areas of typical Mediterranean ecosystems in Southern Spain. In all cases, the final aims are to select the more appropriate AM fungi for plant inoculation with regard to revegetation strategies. One of these approaches is based on analyzing the AM fungal community actually colonizing the roots of representative shrublands species (*Retama sphaerocarpa, Genista* spp., *Rosmarinus officialis, Lavandula latifolia* and *Thymus* spp.) characteristics from the Parque Natural "Sierra de Baza", Granada. For this purpose, DNA was extracted from field roots and the AM fungal DNA was PCR amplified by nested PCR using the primer sets NS31-NS41 and NS31-AM1. PCR products were cloned and a total of 50 clones from each root sample was analysed by single stranded conformational polymorphism (SSCP). Results from the sequence analysis of the different clones will be discussed.

9.98 Arbuscular mycorrhizal fungi and plant ecological diversity in a tropical rain forest of Mexico

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Tropical rain forest (TRF) has a high plant diversity, several factors have been explored to explain it, among them, biotic interactions such as competition, and predation have recognized as key structural factors; however recent studies point out mutualism as a more relevant biotic factor, for explaining vegetal diversity, than once was thought. In TRF little is known about arbuscular mycorrhizal fungi (AMF) and plant diversity relationship, despite its relevance as a mechanism to regenerate or restore original vegetation. Every year, thousands of hectares of tropical rain forest are lost by deforestation, leaving a mosaic of different size patches of the original vegetation surrounded by grasslands, if this fragmentation represents a reduction of AMF diversity, recovery potential of small patches will be lower than that of the large ones. In this way, our main goal was to characterize AMF diversity in different size patches, and to relate it to the observed plant diversity. Seven different size patches were selected, a total of 70 5x5m quadrats were sampled for plant species, and soil. Spores were separated, mounted, identified, and counted. Twenty five AMF species were found, *Glomus* was the best represented genus. Dead spores were more abundant in small patches, however spore number, and AMF diversity were not directly related to patch size neither to plant diversity. Results are discussed in function of land use, size patch, and AMF inoculum quality and functionality.

9.99 ITS primers for the identification of three *Suillus* spp. specific to *Pinus cembra*

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Host specificity varies particularly among the fungi, ranging from generalists to fungi associating with a plant genus only. Suilloid fungi belong to the largest monophyletic group of ectomycorrhizal fungi that is essentially restricted to associations with a single plant family. Three *Suillus* spp., *Suillus placidus* (Bon.) Sing., *S. plorans* (Roll.) Sing. and *S. sibiricus* (Sing.) Sing. are restricted to fife-needled *Pinus* spp., *P. cembra* being the most important host plant in Alpine regions. *Suillus* spp. are known for their typical, tubercle like mycorrhizae. However, also *Rhizopogon* spp. form tubercle like mycorrhizae, and a reliable distinction of involved *Suillus* spp. is often impossible based on morphological characters. Therefore, we design specific rDNA ITS primers for the detection and identification of these three *Suillus* spp. in environmental samples. We tested these primers with fruit bodies, and with tubercle mycorrhizae of *Pinus cembra* plants of different age classes (1-17 years, 25-30 years, 35-40 years and >100 years) originating form nurseries and natural stands. With our three specific primer pairs, we present a fast and easily method for the identification of tubercle like *Suillus* mycorrhizae on species level. Tubercle like mycorrhizae not amplifiying with our specific primers were sequenced, and identified with BLAST searches. Our results demonstrate, that besides *Rhizopogon* spp., also *S. granulatus* can form tubercle like mycorrhizae with *Pinus cembra*.

9.100 Occurrence of arbuscular mycorrhizal fungi associated with some crop plants.

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The spores of arbuscular mycorrhizal fungi were isolated from rhizosphere soil after every thirty days from fields of ten different crop plants from Baglan soils of Maharashtra India. The results obtained incase of three annual plants i.e. grapevine, pomegranate and sugarcane showed maximum number of spores per 100 gm soil in December (165, 125 and 93 respectively) and minimum in May (42, 24 and 31 respectively). Similar kinds of results were obtained from percentage root infection in all the three plants. The other plants were seasonal which includes maize, pearl millet, black gram and green gram. In maize and pearl millet maximum number of spores per 100 gm soil were reported in September i.e. 51 and 46 respectively whereas black gram and green gram showed maximum spores in August (93 and 66 respectively). In onion, wheat and chickpea maximum number of spores were reported in February (72, 122 and 167 respectively). There was similar trend for percentage root colonization incase of all these seasonals. Thirty species of five genera were reported from rhizosphere soil of all above crop plants. The genus *Glomus* was dominant with sixteen species. Five species of *Sclerocystis*, six of *Acaulospora*, one of *Gigaspora* and two species of *Scutellispora* were reported from all the six localities.

9.101 Diversity and community composition of ectomycorrhizal fungi on *Pinus densiflora* seedlings of disturbed and undisturbed area in Mt. Songni

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This study was conducted to compare diversities and species compositions of ectomycorrhizal (ECM) fungal communities on seedlings of *Pinus densiflora* between a disturbed site where top soils were removed and remained for about 10 years and an undisturbed pine forest site in close proximity to the disturbed site. The seedlings were randomly collected from both sites and the percent ECM colonization, number of morphotypes present, and total number of ECM root tips were examined for entire root systems of the seedlings. The identity of ECM fungal taxa was determined based on morphotyping and molecular analysis through PCR-RFLP with a fungal specific primer pair ITS1F/ITS4 and sequence analysis. The root: shoot ratio and total number of ECM root tips were significantly higher in the disturbed site than in the forest site. However, RFLP and sequence analysis showed higher number of fungal species in the forest site than in the disturbed site, and species compositions of ECM fungal communities were different in both sites. The ECM fungi were mostly unidentified species of ECM fungi in the disturbed site while basidiomycetous fungi such as *Russula vesca* and *Thelephoraceae* sp. in the forest site.

9.102 Culture repository of arbuscular mycorrhizal fungal isolates from wheat agroclimatic regions of India

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The climatic, edaphic and socio-economic diversity of the Indian crop production scene is dotted with many cropping patterns and presents a range of diversity in agriculture, and hence resulting in division of the country into 15 agroclimatic regions. As the performance of arbuscular mycorrhizal (AM) fungal isolates vary with the agro-climatic regions, the AM fungal isolates were collected from five different wheat growing areas (Budaun, Badshahpur, Ghaziabad, Gual-Pahri, Pachmari, and Palwal) of India. 139 trap cultures from five different regions were established and are being maintained via successive trap culture methodology using different host plants, *Allium cepa* (onion), *Tagetus* spp. (marigold), *Daucus carotus* (carrot), *Medicago sativa* (alfalfa), *Trifolium alexandrianum* (berseem) *Gossypium* spp. (Cotton), *Vetiveria zizanioides* (Vetiver), *Vigna radiata* L. Wilezek (mungbean), and *Sorghum* spp. From each region, five fields were selected for sample collection, which were raised for trap cultures. Trap cultures are being monitored for all culture cycles for spore count, intraradical root colonization, infectivity potential, and species richness. In the present study, direct field sampling indicated richness of 1 to 6 species per sample. Comparable numbers of species were recovered from one cycle of trap cultures. The trap cultures are being maintaining and tested for their future exploitation, such as wheat-pulse rotation studies.

9.103 Effects of prescribed fire and wildfire on fungal communities in ponderosa pine forests in eastern and central Oregon, USA

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Decades of fire suppression have resulted in high fuel levels in dry forests in eastern and central Oregon. To alleviate the risk of stand replacing wildfire, forest managers are applying prescribed underburning treatments. Little knowledge exists about the impacts of fire restoration treatments and wildfire on the belowground ecosystem. Post-fire aboveground ecosystem recovery is dependent on functioning soil fungal communities. In a series of studies, the community composition and response of fungal species richness to seasonal prescribed burning, various combinations of thinning and burning, and a recent wildfire was investigated with molecular techniques (RFLP & TRFLP). Prescribed treatments significantly reducing duff depth negatively impacted species richness of ectomycorrhizal fungi and after wildfire, soil fungal diversity was lower in plots with severely burned soils compared to paired plots of less severely burned soil. The distribution of a few abundant species in a majority of the prescribed burning treatments as well as in soils severely burned by wildfire demonstrates that some fungal species survive or rapidly reestablish after disturbance by fire. Understanding how mycorrhizal and other soil fungi respond to prescribed fire and wildfire will assist forest managers in selecting fuel-reducing restoration treatments that maintain critical soil processes and promote forest recovery.

9.104 Ectomycorrhizal community structure in a xeric *Quercus* woodland as inferred from rDNA sequence analysis of pooled EM roots and sporocarps

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Seasonally dry *Quercus* woodlands are key components of California's wild landscapes, yet little is known about their associated ectomycorrhizal (EM) fungi. Previous research suggests that EM fungi are most diverse and abundant in mesic ecosystems. However, sporocarp collections and rDNA sequence data from EM roots of *Quercus douglasii* in California yielded 163 EM species, suggesting that EM fungal communities of seasonally arid biomes are extremely diverse. We detected a large number of Ascomycota and hypogeous species, both on EM roots and as sporocarps. Because of the erratic weather conditions, we expected seasonal and annual variation among EM fungi on roots sampled in winter and spring of 2003 and 2004. However, evidence suggests that the belowground EM community was relatively stable. Hierarchical cluster analysis indicates that soil cores from within a 25 cm radius were similar in terms of EM species composition regardless of sampling date. We found no evidence of EM taxa specifically adapted to winter or spring. Furthermore, only one common EM species varied widely between the two years and several rare EM species were detected within the same 25 cm radius on successive sampling dates, suggesting they persisted in or re-colonized small, localized areas. We compare and contrast these results with those from other studies and discuss implications for further studies of EM community ecology.

9.105 The diversity and community composition of arbuscular mycorrhizal fungi in Inner Mongolia grassland

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This study was carried out in a long-term experiment site of the Inner Mongolia Grassland Ecosystem Research Station, the Chinese Academy of Sciences. Four plots, i.e. Plot 1 (*Leymus chinense* plot), Plot 2 (*Stipa grandis* plot), Plot 3 (restored degraded plot) and Plot 4 (grazing plot), were selected, and 32, 26, 23, 24 soil samples were randomly collected from the four plots, respectively. A total of 20 taxa belonging to six genera of AMF were isolated, of which 12 taxa in *Glomus*, four in *Scutellospora*, one in each *Acaulospora, Entrophospora, Gigaspora* and *Pacispora*. Among the 20 taxa, 15, 11, nine, and nine taxa were isolated from the Plot 1, Plot 2, Plot 3 and Plot 4, respectively. *Glomus geosporum* was the dominant species and *Glomus* was the dominant genus in the four plots. The spore density (spore per 100 g air dry soil) of AMF from high to low was in Plot 1 (21.1±2.6), Plot 2 (16.9±3.6), Plot 3 (11.8±2) and Plot 4 (8±1.6). The species richness (species per 100 g air dry soil) of AMF was the highest in Plot 1 (2.6±0.2) and was the lowest in Plot 4 (1.4±0.2). The Shannon Wiener diversity indices of AMF in Plot 1, Plot 2, Plot 3 and Plot 4 were 1.65, 1.65, 1.28 and 1.28, respectively. The Sorenson similarity indices of AMF between Plot 1 and Plot 2 was 0.629, Plot 1 and Plot 3 was 0.583, Plot 1 and Plot 4 was 0.417, Plot 2 and Plot 3 was 0.7, Plot 2 and Plot 4 was 0.5, and Plot 3 and Plot 4 was 0.556.

9.106 Three plant species co-occurring in two Swiss alpine meadows harboured distinct AMF communities in their roots

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The aim of this study was to characterize the communities of arbuscular mycorrhizal fungi (AMF) in the roots of different plant species from two species-rich alpine meadows and to determine whether possible differences can be explained by host preference or spatial heterogeneity. Soil cores, each containing *Gentiana verna* or *Gentiana acaulis* and up to eight species of their surrounding plants, were sampled. AMF within the roots of each plant were analyzed by nested PCR, restriction fragment length polymorphism analysis and sequencing of ribosomal small subunit and Internal Transcribed Spacer regions. Sequences of AMF were analyzed phylogenetically and used to define monophyletic sequence types. The differences among AMF communities in the root samples were evaluated using multivariate analysis. The composition of AMF communities was strongly influenced by the host plant species whereas it did not significantly differ between the two sites. The most frequent sequence type GLOM A-1 corresponded to *Glomus intraradices*. Detailed analyses of three common and co-occurring plant species (*G. verna, G. acaulis, Trifolium* sp.) revealed that their AMF communities differed significantly. The results indicate that within a relatively small area with similar soil and climatic conditions, the host plant species can have a major influence on the AMF community composition in its root.

9.107 Effect of benomyl and propiconazole on arbuscular mycorrhizal colonization and P uptake of *Maianthemum dilatatum* in the forest floor

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Fungicide application decreased colonization of arbuscular mycorrhizal (AM) fungi P concentration of plants in grassland, indicating contribution of AM fungi to P uptake in grassland. Although AM colonization has been detected in many plants grown in forest, it is not clear whether they contribute to P uptake and growth of plants in the forest. We investigated effect of fungicides on AM colonization and P uptake of plant in a forest floor. Benomyl (B), propoconazole (P), which sterilize the basidiomycota, and water (C) application established in June 2003 in floor of Tomakomai Experimental Forest (42° 40'N, 141° 30'E). *Maianthemum dilatatum* was collected from each plot in July 2004. AM colonization, shoot P concentration and dry weight were determined. DNA was extracted from roots and amplified by PCR with AM1-NS31. AM colonization of *M. dilatatum* was 67% in C, 33% in B and 60% in P. Two AM fungal species (*Glomus mossea* and *Glomus sp.*) Glo9 in C and four species (*Glomus mosseae*, *Glomus intraradices*, *Glomus sp.* Glo9 and *Glomus sp.* Glo26) in P were identified. Three species (*Glomus mosseae*, *Glomus sp.* Glo9 and *Glomus sp.* Glo26) were detected from one root of *M. dilatatum* in P. No AM fungal sequence was detected in B and no basidiomycota sequence was detected in P. Colonization of AM and other fungi in the root of *M. dilatatum* and their contribution to P uptake are discussed.

9.108 Level of phosphorus influences the movement of hyphe of arbuscular mycorrhizal fungi

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Considering the linkage of plants with the extraradical hyphae and inhibition of AMF at high P levels, a glasshouse experiment using *Zea mays* L. was performed to assess the movement of AM hyphae through a phosphorous gradient. Three levels of P i.e. 0,10, and 15 kg/ha as KH_2PO_4, were used in a sandy loam (avail.P, 4.5 kg/ha P_2O_5) placed in the middle chamber of a three compartment system. Soil based inoculum of *Glomus intraradices* was applied to only the middle chamber; a nylon mesh allowed movement of only AM hyphae to the two side chambers but not roots. In control treatment, no AM inoculum was used. Each treatment was replicated five times. At 15 kg/ha level in the middle chamber, AM infection in the side chambers maintained at 0 and 10 kg P & remained uninoculated, exhibited 50.2% and 44.4%. Likewise having 10 kg P/ha in middle chamber, AM infection in uninoculated side chambers was, 19.33% (15 kgP/ha) and 32.03% (0 kg P/ha). Only 11 & 24% infection was recorded in side chambers at 15 & 10 kgP/ha, respectively when 0 P kg/ha was applied in middle chamber. This suggested greater movement of extramatrical hyphae towards lower P dose from higher P level but not vice – versa, which concludes that P availability away from the point of AMF inocula considerably influences movement of extraradical hyphae and resulting AMF infection. This observation is of relevance in agricultural production systems where AM inoculation is practiced without consideration of its spread laterally.

9.109 Establishment of mesquite plants (*Prosopis laevigata*) inoculated with arbuscular mycorrhizal fungi under water stress in greenhouse conditions

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Mesquite (*Prosopis laevigata*) is a "nurse plant" that form "resource islands" in semiarid ecosystems in Central México; the adult plant (shrub or tree) favors plant succession in natural communities, nevertheless, low mesquite establishment is a limiting factor in restoration programs. For this, the objective of this work was to determine the effect of arbuscular mycorrhizal fungi (AMF) on establishment of mesquite plants under water stress conditions. The experimental design was an analysis of variance of two factors: soil irrigation and mycorrhizal treatment, with two levels each one: wet and dry watering (W, D) X AMF inoculated and non-inoculated pots (M+, M-). We used 20 repetitions in each treatment. The source of AMF spores, P. laevigata seeds and soil was a semiarid ecosystem at the "Valle del Mezquital", in the Hidalgo State. Mesquite seedlings were grown on stelirized soil for 6 months under greenhouse conditions. The results show that Reproductive Growth Rate and Water Use Efficiency (dry biomass/irrigated water) of AMF treatments was significantly higher than non-mycorrhizal plants. Also, the caulinar water potential of mycorrhizal plants was lower than their controls; moreover, establishment and survival of mycorrhizal plants were higher than their witness. Therefore, it was concluded that AMF inoculation of mesquite seedlings is a useful tool for increase the plant establishment in restoration programs for degrated semiarid ecosystems.

9.110 The effects of defoliation and acorn deprivation on the development of oak seedlings and the mycorrhizal level of their root system: a field study

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Comparing the short-time (6 weeks) and long-time (5 months) results by examination of 200 fine root tips per newborn oak seedlings (*Quercus robur* L.) indicate that the mycorrhizal level on the roots was higher in autumn than in summer and deprivation of the acorns significantly decrease the growth and survival of the seedlings, whereas the mycorrhizal level of the fine root tips was unaffected. Deprivation of the acorns only reduce the mycorrhizal level of the fine roots when it was applied together with defoliation. Defoliated (up to 2 leaves) seedlings had significantly lower mycorrhizal level on the root system, although their growth and the survival rate were unaffected. The results suggest that diminution of the mycorrhizal level on fine roots of the oak seedlings did not significantly effect the survival rate. On the other hand, the acorn deprivation, via different mechanisms, dramatically lessen the chance of the survival of the seedlings, and the comparatively high mycorrhizal level was not able to compensate this effect. Further morphological and molecular examination need to detect if there was any change in the fungal species composition on the mycorrhizal root tips. According to this, it seems, that beside the well know effect of edafic and microclimatic factors, the impact of acorn deteriorater and/or consumer organisms on the fine scale spatial pattern of the oak seedlings in nature can be considerably higher than it was supposed previously.

9.111 Site effect on mycorrhizal community structure of four temperate tree species

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This research was conducted to explore the role of site factors on mycorrhizal community structure of four tree species: *Picea abies*, *Larix decidua*, *Pseudotsuga menziesii* and *Quercus robur* growing at two different forest sites in three replicates. The experimental stand is located in central Poland (52°14.87' N, 18°06.35' E, 150 m a.s.l.) containing 34-year-old monoculture plots of 14 temperate forest tree species grown at two adjacent forest type sites: coniferous forest site and mixed forest site. We have examined the frequency and the relative abundance of particular morphotype and we have identified fungal species forming mycorrhizas using sequencing of ITS rDNA. Mycorrhizal species diversity was greater on coniferous forest site for all examined tree species than on mixed forest site. Mean number of ectomycorrhizal species was the highest for *Q. robur* (7.8), 4.2 for *Picea abies* and *Larix decidua*, and 3.2 for *Pseudotsuga menziesii* (ANOVA effect: $R^2=0,65$, $p=0.009$). Soli pH was among the most important factors influencing diversity of ectomycorrhizal species. The results indicated significant effects of site conditions and tree species on mycorrhizal species community structure.

9.112 Below ground detection and quantification of rare pinewood mycorrhizal fungi

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The overall aim of this research is to provide information to assist in the conservation and management of rare stipitate hydnoid fungi that form ectomycorrhizal associations with Scots pine. Research based on sporocarp production indicates that stipitate hydnoid fungi have declined in Europe and many species are thought to be threatened by extinction. In the UK, a grouped Species Action Plan has been developed to provide a management framework for 14 rare species from the genera: *Bankera*, *Hydnellum*, *Phellodon* and *Sarcodon*. However, we know that the absence of above ground sporocarps does not necessarily indicate the absence of an ectomycorrhizal species below ground. The development of a useful management strategy for these fungi relies on a better understanding of their ecology, including information on their below ground distribution and persistence. An ITS sequence database was established from sporocarps of 12 species. Species-specific primers were developed within the ITS1 and ITS2 regions and the specificity of the primer pairs was tested using PCR and BLAST searches. The primers were used in conjunction with real time PCR to compare the relative abundance of DNA from 2 species below ground with their above ground sporocarp distribution. The ability of four species to persist below ground at locations where sporocarps were previously, but are no longer, recorded was also investigated.

9.113 The significance of plant gender in mycorrhizal symbiosis

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A basic assumption in plant life-history theory is that resources are not unlimited and that there are trade-offs in resource allocation between plant reproduction, growth, maintenance and longevity. Mycorrhizae are beneficial for the plant as they increase the supply of limiting nutrients, but they also represent a cost in terms of carbon. In sexually dimorphic plant populations, females and males/hermaphrodites plants do not have similar resource requirements: female plants usually invest relatively more resources in reproduction than male/hermaphrodite plants. Therefore, as plant sexual morphs differ in their pattern of resource allocation, it is logical to assume that they differ in their mycorrhizal relationship. The few data available indicate that the two sexes in dioecious and gynodioecious species have similar levels of arbuscular mycorrhizal colonization under natural conditions. However, the response of the two sex morphs differs when exposed to experimental conditions reducing the amount of C available to different plant functions. We hypothesise that the females increase C allocation to mycorrhiza when seed production is nutrient limited, but reduce resource allocation to mycorrhiza when plant reproduction is C limited. Consequently, plant C allocation to mycorrhizal symbiosis depends on plant sex and resource limitation of plant reproduction.

9.114 Diversity in arbuscular mycorrhizal fungi in El Palmar National Park (Entre Rios, Argentina)

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Among the fungal biota from soils, arbuscular mycorrhizal fungi represent an important component by their ubiquity and microbial biomass. They are directly involved in essential processes at the plant-soil interface. AM fungal associations are key factors determining diversity in ecosystems. The purpose of this study was to determine fungal species richness, diversity and community structure in soils from the El Palmar National Park, Entre Rios province. Samples were collected from scrubland, gully, palm forest, grassland, and gallery jungle. Arbuscular fungi were isolated from soil by wet sieving and decanting and sucrose-gradient centrifugation. Forty one species were recorded in the whole area. Species dominance was used to calculate diversity index and evenness. The scrubland and grassland evidenced the highest diversity index (3,75 and 3,76 respectively). Similar values were observed in gully and palm forest (3,40 and 3,35 respectively) whereas the lowest value was found in the gallery jungle (3,14). The community structure was composed of 5 families of Glomeromycota: Acaulosporaceae, Archaeosporaceae, Gigasporaceae, Glomeraceae and Pacisporaceae. All these families were found to be present in scrubland, whereas 4 families were observed in gullies and grassland, 3 families were present in palm forest, and only 2 of them in the gallery jungle. Those families that displayed a greater number of species in most sampled sites, were Acaulosporaceae and Glomeraceae.

9.115 Mycotrophy of preceding crops and peat amendment affected mycorrhizal function in soil but not yield of subsequent strawberry

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Mycotrophy of previous crops has been shown to have an impact on AMF, and the growth and productivity of succeeding crops. To study this we established a two phase field experiment on silty clay including three years of preceding crops (phase 1) and three years of subsequent strawberry (phase 2). The preceding crops had different degrees of mycotrophy, including mycorrhizal (strawberry, rye, timothy, onion, caraway) and non-mycorrhizal (turnip rape, buckwheat, fiddleneck) ones. At establishment of preceding crops and of strawberry, half of the area was treated with well humified peat (300 m^3 ha^{-1}, pH 4) to the depth of 20 cm. As a mean of three years of cropping in unamended soil, the mycorrhizal crops strawberry and caraway maintained mycorrhizal effectiveness most effectively, while the values were lower in the non-host crops buckwheat, turnip rape and fiddleneck. In addition, the numbers of AM spores detected in soil were considerably greater during three years of strawberry cultivation. In soil under caraway, there were also high numbers of AM spores. In soil amended with peat, the situation was partly the opposite of that from the unamended soil. Mycorrhizal effectiveness was the highest in rye and onion and the lowest in strawberry and caraway. Although mycorrhizal status of preceding crops and peat sometimes significantly affected AMF traits, neither AMF colonisation in roots of subsequent strawberry nor yields of strawberry were affected.

9.116 Mycorrhizal fungi diversity in dryland salinity-affected soils in New South Wales (NSW), Australia

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The large-scale removal of native vegetation and the sowing of annual crops/pastures has been a major contributor to dryland salinity (i.e. non-irrigated land) in Australia. These shallow-rooted agricultural plants require less water and have caused excess rain water to leak into the groundwater system. Water uptake by these plants is reduced and subsequently the watertable rises, bringing salts to the surface. Dryland salinity is a devastating environmental problem that reduces land productivity and biodiversity; however, little is known about the effects of mycorrhizas on plant growth or mycorrhizal fungi diversity in soil affected by salinity in Australia. The aim of this investigation is to improve the understanding of the genetic and functional diversity of mycorrhizas in saline soil. This may enable better sustainable resource management, successful rehabilitation of degraded ecosystems and may increase agricultural productivity in the future. Field sites within NSW have been selected with varying saline levels and with adjacent vegetation areas unaffected by salinity. Denaturing gradient gel electrophoresis is being employed to analyse the genetic diversity of mycorrhizal fungi isolated from spores, roots and bulk soil. Morphology studies are being performed in conjunction with the genetic investigation. Functional diversity (e.g. the role of mycorrhizas in plant accumulation of organic solutes) and infection efficiency of the isolated mycorrhizas will be investigated.

9.117 Initial development of ectomycorrhizal fungi communities

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The Lausitz region, East Germany, is characterized by many open mining casts. After the mining process, substrates without fungal diaspores are located on the surface. The primary succession of ectomycorrhizal communities was investigated in different ways. On some sites, which were not recultivated, undisturbed natural colonization was possible. Here, agar plates to sample fungal diaspores were used and sterile trees were planted as trap cultures. Up to three years old trees growing in older undisturbed forest stands, posed as reference for the trap cultures. On other sites, afforestation took place with three years old trees from nurseries. Such trees normally were planted after cutting off the fine roots, thus the trees were not mycorrhized while planting. Here the development of mycorrhizae within a year was investigated. One year after planting, the trees from the nursery developed mycorrhizae with up to six fungal species. The trap cultures showed a comparable mycorrhization but the mycorrhizal frequency and the species diversity differed widely with the tree species. The community composition differed from site to site, but all species were known from older sites in the region. The initial communities consisted of a limited set of functional groups of species. Fungi, which prefer growing in the organic soil layers, were lacking, as well as species with rhizomorphs. Species representing the short distance exploration type and the contact type dominated the communities.

9.118 Effects of the invasive species *Solidago canadensis* on the mycorrhizae of native plant species

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We conducted three experiments to examine whether and how invasive species effects on mycorrhizae of native species. An invasive species, *Solidago canadensis*, and three native species *Echinochloa crusgalli*, *Kummerowia stipulacea* and *Ageratum conyzoides* were used in the experiments. An indigenous arbuscular mycorrhizal fungi (AMF) community composed of five *Glomus* species was constructed. In the first experiment, AM fungi spores were inoculated to sterilized soil in which *S. canadensis* was planted. In the second experiment, extracts from rhizome of *S. canadensis* were added to native plants under the constructed AMF. In the third experiment, *S. canadensis* coexisted with each native species under the constructed AMF either with or without activated carbon to separate the allelopathic from non-allelopathic effects. The results showed that *S. canadensis* altered AMF community that *Glomus geosporum* and *Glomus etunicatum* tended to become dominant species. Extracts from rhizome of *S. canadensis* inhibited AMF colonization of three native plant species. However, when coexisted with *S. canadensis*, AMF colonization of *E. crusgalli* was promoted compared to monoculture without activated carbon. Colonization of *A. conyzoides* was enhanced when activated carbon was added while colonization of *K. stipulacea* was increased regardless of activated carbon. Our results suggested that *S. canadensi* may affect mycorrhizae of native plants through altering AMF community and exuding allelochemicals.

SESSION 10

Anthropogenic impacts on mycorrhiza diversity and functioning

Do crop genotypes have residual effects on Glomeromycota communities in agro-ecosystems?

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Plant and fungus are closely interdependent in the arbuscular mycorrhizal symbiosis and there is growing evidence that plant diversity can have an impact on fungal diversity in natural and semi-natural ecosystems. In agro-ecosystems, plant diversity is reduced and a same annual crop can be grown for several years in the same field using identical agricultural management practices. Whilst single crops and associated crop management can influence the soil microflora, less is known about eventual genotype effects of cropping on AM fungi in agricultural systems. A long-term field experiment was conducted with various maize genotypes grown, with their associated treatments, for seven years in the same plots. Using molecular tools, the diversity of the AM fungi was investigated in the soil and in the roots of a maize plant used as trap culture, in order to detect whether a residual effect had developed on the composition of the AM fungal community after the seven year cropping trial. A similar experiment was performed in a green house where various genetically modified maize plants were grown for several growing cycles, and their impact on AM fungal community composition compared to that of the nearly isogenic parent maize lines.

Anthropogenic influences on soil structure: glomalin and arbuscular mycorrhizae

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Soil structure is an important feature of terrestrial ecosystems, since it controls elements of many biogeochemical cycles and the functioning of soil biota. This soil property is strongly influenced by mycorrhizae. We provide and overview of the pathways (at various hierarchical levels) by which mycorrhizae can affect soil aggregation, with an emphasis on mechanisms residing at the mycelium-scale. Here, glomalin and glomalin-related soil protein (GRSP) has been invoked as an important contributor to certain aspects of AMF-mediated soil aggregation. In light of the recent description of the gene for the arbuscular mycorrhizal fungus-produced protein glomalin, we present a conceptual framework that could be useful in testing AMF effects on soil aggregation in the context of anthropogenic change. We conclude with a critical evaluation of GRSP as a tool for evaluating AMF contributions to soil aggregation in general, and under changing environmental conditions in particular.

CO_2-enrichment and the ectomycorrhizal symbiosis: consequences for community structure, hyphal dynamics and nutrient transport gene regulation

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Rising atmospheric CO_2 can affect forest fine root production, C availability and nutrient demand. Here we examined whether such plant-level responses to CO_2-enrichment may impact EcM community dynamics, foraging, and nutrient exchange. Our experiments were conducted at the FACTS-I research site in Duke Forest NC, USA, where intact *Pinus taeda* forest plots are maintained at either ambient or elevated CO_2 concentrations. EcM communities were assessed by sequencing the ITS rDNA gene region from mycorrhizae. EcM hyphal biomass and composition were determined with PLFA analysis and ITS/LSU sequencing of clone libraries, respectively, derived from nylon mesh sand-filled bags incubated in the field. We also quantified gene expression levels from field-collected EcM using real time PCR for *P. taeda* monosaccharide and NH_4 transporters, and fungal and *P. taeda* 18S rRNA. EcM richness and diversity were not affected by CO_2, but increased CO_2 altered the relative abundances of particular taxa, increased prevalence of unique species, and led to greater community dissimilarity among plots. Fungal 18s rRNA expression was greater for EcM in elevated CO_2 plots, suggesting a possible increase in EcM mantle biomass. We also detected trends in *P. taeda* monosaccharide transport expression consistent with greater C flux to EcM in elevated CO_2 plots. CO_2 x N interactions were also observed, underscoring the importance of considering CO_2 effects in concert with other environmental perturbations.

The exotic, invasive hemlock woolly adelgid (*Adelges tsugae*) affects ectomycorrhizal fungal communities and soil conditions in eastern U.S. forests

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The hemlock woolly adelgid (HWA; *Adelges tsugae*) was introduced to western North America from Asia in 1924, then migrated to the eastern U.S. in the 1950's and now is found throughout the northeastern U.S. Infestations of HWA have been associated with mortality and, hence, reduced populations of its host tree species, eastern hemlock (*Tsuga canadensis*). Ectomycorrhizal (EM) fungal communities can be sensitive to changes in host tree species composition and other environmental variables. By comparing HWA-infested hemlock stands to healthier stands, we found that mortality associated with infestations of the HWA has resulted in altered EM fungal communities and soil conditions in mixed coniferous and deciduous hardwood forests in the northeastern United States. We also examined EM fungal communities and soil conditions in stands dominated by mixed oak species (*Quercus* spp.) to examine the broader forest EM fungal community. EM fungal root tips were identified by morphotyping and DNA sequencing. EM tip density and richness was lowest in HWA-infested stands and highest in oak-dominated stands. Forest types were generally similar in the composition and abundance of their dominant EM species. Soil NO_3^- concentrations were highest in HWA-infested stands. Results of this study suggest that N cycling has been altered in these stands and that spatial variation in EM density and richness may be linked to variation in both host community composition and soil chemistry.

Influence of restoration practices on underground and aboveground biodiversity of heavy metal polluted areas

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Plant-based technologies in industrial areas are mostly limited to phytostabilization, involving the use of plants to stabilize the contaminated tailing material, in order to minimize leaching and wind or water erosion.The mycorrhizal status of plants commercially available, that were seeded on the industrial wastes, and those that were transfered from wet or dry places was studied. Field and laboratory experiments were carried out to evaluate whether introduction of plants assisted by well selected microbiota could help to increase above- and under-ground diversity. Chlorophil a fluorescence was measured to estimate the impact of microorganisms on plant vitality. Selected plants were analysed by Total Reflection X-ray fluorescence method. Commercially available grasses are often devoid of well established mycorrhiza and their populations dramatically decrease with time. Much more stable vegetation communities arise from plants that appear on the wastes spontaneously. The plants need appropriate below-ground ecosystems, especially at difficult sites. Plants originating from xerothermic grasslands were found to be very useful in wastes revegetation, but only if the mycorrhizal fungi were provided. Fungal strains either increased or decreased heavy metal uptake by the plant depending on the origin of a fungus. Strong influence of the pre-adaptation of the fungus in polluted soil on the ability of the plant to avoid uptake of potentially toxic elements was found.

Can we develop a general predictive model of mycorrhizal fungal community response to changing atmospheric chemistry?

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Human activities continue to drive dramatic changes in concentrations of atmospheric CO_2, oxidants, and nitrogenous and acidic compounds. A true test of our understanding of the impacts of these chemicals, both singly and in interaction, on mycorrhizal fungal communities is whether we can develop predictive models of community structural and functional response. We present evidence for consistent effects of altered atmospheric chemistry on mycorrhizal fungal communities, and suggest areas of research that would provide the greatest potential for advancing our understanding of the causes and consequences of these community changes. Predictive models, whether empirical or mechanistic, will need to accurately portray fungal species sensitivity and response to changes in carbon and nutrients status of different hosts, and nutrient/acid status of soils. A major challenge is parameterizing these models, which requires development of host species-specific response functions for whole mycorrhizal fungal communities. Multiple approaches to obtaining these parameters include statistical analyses of community data over pollution gradients, field manipulations of pollution or nutrient levels, and more controlled lab experiments that independently manipulate fungal communities and pollutants. Combinations of all of these approaches are necessary to achieve a full understanding of the mycorrhizal fungal community structural and functional consequences of changing atmospheric chemistry.

10.1 Response of mycorrhizal plants of *Capsicum annuum* L. cv San Luis to biotic and abiotic stress

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Physiological and biochemical responses to stress (*Phythophtora capsici* or drought) were determined. Additionally fruit quality was evaluated when the plants were subjected to water stress. Inoculated plants showed a higher drought resistance showing an increase in the photosynthesis rate and a decrease in water lost. Regarding fruit quality, size (weight) was not affected under stress conditions while the color was significantly influenced by the fungi and drought treatments. On the other hand, mycorrhizal plants showed differences in the reactive oxigen species production and the enzymes that regulate this stress response giving to arbuscular mycorrhizal plants a higher stress resistance.

10.2 Growth response of *Pinus patulata* Schl. and *P. ayacahuite* Schl. to inoculation with ectomycorrhizal fungi and *Bacillus subtilis* BEB-DN

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Pinus patula Schl. and *Pinus ayacahuite* Schl. were inoculated and co-inoculated with ectomycorrhizal (ECM) fungi *Pisolithus tinctorius*, *Lactarius* sp., *Ramaria* sp., *Amanita* sp. and, a plant growth promoting rhizobacteria PGPR: *Bacillus subtillis* BEB-DN. Pines responded to inoculation compared to non-inoculated control plants. Co-inoculation of *P. patula* with *B. subtilis* and *P. tinctorius* or *Ramaria* sp. resulted in an increase of P concentration. Pines inoculation with *Ramaria* sp. or *B. subtilis* BEB-DN showed a faster growth compared to those in the non-inoculated control. The greatest ECM colonization was found when seedlings were inoculated with *Ramaria* sp. *P. ayacahuite* ECM colonization was increased by co-inoculation with *B. subtilis* and *Amanita* sp. or by single inoculation with *P. tinctorius*. A significant increase in P content was also found, while chlorophyll concentration decreased. Growth increased inoculated with *P. tinctorius* or *B. subtilis*. The results suggested that inoculation of *P. patula* and *P. ayacahuite* with *B. subtilis* BEB-DN are recommendable, but not as mycorrhizal helper bacteria. The contrasting responses of both pine species indicate host selectivity. Desirable co-inoculants strains should be selected previously to their incorporation into nursery practices in order to increase seedlings growth and quality of pine species. Acknowledgements: CONACYT-SEMARNAT-2002-CO1-0129, CONAFOR-2004-CO4-01.

10.3 Effect of urban waste compost amendments on arbuscular mycorrhizal fungi (AMF) community structure in maize roots of an arable field

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Composting has emerged as a valuable route for the disposal of urban waste, with the prospect of applying composts on arable fields as organic amendments. Urban waste composts (UWC) could contain low concentrations of pollutants and could contribute to their accumulation in agricultural soils. Little is known about the impact of a long term deposition of such composts on microbial (bacterial and fungal, including mycorrhizal) community structure in soil. The aim of this study was to assess the effect of two particular composts, a green waste and sewage sludge compost (GWS) and a municipal solid waste compost (MSW), on AMF community structure in roots compared to control (non amended). The study was conducted on the "Qualiagro" site (Feucherolles, France), an arable field where these composts were amended every two years during six years. During the last year, the experimental site was planted with maize and roots samples were collected at two sampling times (June and October). DNA was isolated from the roots and a part of the AM fungal 18S rDNA was amplified by PCR using specific primers (Helgason et al, 1999). PCR products were separated using a TTGE technique. The different fingerprints obtained were compared to estimate the impact of UWC on AMF diversity in roots. Results will be discussed during the ICOM5 in the way to consider AMF diversity in roots as a bioindicator of pollution level in arable soils.

10.4 The pecan truffle (*Tuber lyonii* syn. *T. texense*) – First observations from Missouri, USA, placed in the context of the literature

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In September 2005, we found six *T. lyonii* Butters truffles clustered beneath a six-yr-old hybrid hazel (*Corylus* L.) in Howard County, MO, USA. This constitutes the first record of a *Tuber* sp. in Missouri, and a new host genus record for *T. lyonii*. The truffles were identified microscopically as *T. lyonii*. ITS sequences obtained from two of our truffles were approximately 90% similar to *T. lyonii* ITS sequences representing two distant collections, from Georgia and Texas. We have deposited specimens in the Oregon State University herbarium. Our truffles were found 0-2-cm beneath the surface of a well-drained silt loam soil of loess origin on an east-facing shoulder slope, with a surface soil pH of 6.85-7.15. The site had supported an unmanaged and weedy stand of alfalfa (*Medicago sativa* L.) for several decades prior to plantation establishment. We discuss our collections in the context of the literature on *T. lyonii*, and suggest directions for future research. Molecular as well as microscopic diagnostic tools are needed for reliable identification of *T. lyonii* ectomycorrhizae: to confirm suspected host associations and geographic distribution; to support ecological studies of *T. lyonii* establishment and fruiting; and to distinguish component species within the current morphological description of *T. lyonii*. A reliable system for production of well-colonized planting stock is also needed to support efforts to cultivate this potentially valuable truffle species.

10.5 Diversity and efficacy of arbuscular mycorrhizal fungi in rehabilitated quarries in the Mediterranean area

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The implication of AMF in the outcome of revegetation processes is evaluated. Two quarries were selected for revegetation with autochthonous shrubs, one where stored top soil was repositioned before planting and the other with no added soil. Six Mediterranean shrubs where planted, previously inoculated with *Glomus intraradices* BEG 72, or not inoculated. After 8 months growth in the quarry with added top soil all control plants sampled presented the symbiosis and there were no differences in plant growth. Molecular probes show that the most widespread fungi were *G. intraradices*, and *Glomus microaggregatum* although other fungi were present in the roots of the control plants. In the quarry where no top soil was added 60% of the non inoculated plants sampled presented the symbiosis. All inoculated plants grew better. *Glomus intraradices* was the only fungus detected in the roots of the inoculated plants whilst in the roots of the control plants other fungi were found. Our results show that adding stored top soil is a good system to enhance mycorrhizal colonisation in quarry restoration; otherwise it is important to inoculate the plants used with an effective fungus. Molecular probes show that in the Mediterranean ecosystems studied, with eroded soils and high pH, the AMF species diversity is very low and *G. intraradices* is present in a high percentage of the samples, indicating the resilience and adaptation to these conditions of this species.

10.6 Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems

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Shrub abundance is expected to increase with enhanced temperature and nutrient availability in the Arctic, and associated changes in abundance of ectomycorrhizal (EM) fungi could be a key link between plant responses and longer-term changes in soil organic matter storage. This study quantifies the response in EM fungal abundance to long-term warming and fertilization in two arctic ecosystems with contrasting responses of the EM shrub *Betula nana*. We used ergosterol as a biomarker for living fungal biomass in roots and organic soil and ingrowth bags to estimate EM mycelial production. Natural abundance of ^{15}N and ^{13}C confirmed the EM-saprotrophic divide in fungal sporocarps and validated the EM origin of mycelia in the ingrowth bags. At both sites, fertilization increased EM mycelial production and fungal biomass in the soil, and warming increased both measures at one site. This was partly caused by increased dominance of EM plants and partly by direct stimulation of EM mycelial growth. We conclude that cycling of carbon and nitrogen through EM fungi will increase when strongly nutrient limited arctic ecosystems are exposed to a warmer and more nutrient rich environment. This has potential consequences for belowground litter quality and quantity and for accumulation of organic matter in arctic soils.

10.7 Does AMF soil community mediate plant-root endophytic fungi interactions?

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This study tested whether plant-root endophytic fungal feedback (mycorrhizal and non-mycorrhizal) occurs between woody Mediterranean plants and indigenous soil fungal communities, and whether changes in soil AMF community alter plant-root endophytic fungal interactions. We selected two biotic factors relevant for plant production for restoration: (i) AMF communities from soils used for restoration trials (undisturbed; disturbed; inoculated disturbed), and (ii) rhizospheric soil communities, obtained by pre-cultivating the soils with host plant species (myrtle, carob, lavender). No differences occurred in soil AMF diversity associated with conspecific and heterospecific soil communities, regardless of the AMF soil source. This dynamic feedback was not related to root endophytic fungi. The growth response of myrtle plants to the heterospecific soil microbial communities varied depending on the first generation of plant species (lavender and carob) used to pre-culture the soil community. We conclude that plant growth responses are more dependent on plant-soil-microbe interactions than on the addition of commercial AMF inoculum. These results also suggest that the addition of single species commercial AMF inoculum to disturbed AMF communities may cause problems, since AMF allocthonous communities remain active for a long period in the soil.

10.8 Field mapping of *Amanita ponderosa* sporocarp production during six consecutive years

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Amanita ponderosa Malençon & R. Heim is an endemic species in the Southwest of the Iberian Peninsula, and some regions in Italy and in North Africa. It forms ectomycorrizae with holm oak wood; its fruiting bodies have a great gastronomic value and have been exhaustively harvested in the region for decades. In this study we have mapped during several consecutive years the sporocarp field location of this species in a 3 ha holm-oak site located in the Natural Park Sierra de Aracena y Picos de Aroche, at Southwest of Spain. The data indicated that sporocarp production patches showed a very similar shape each year, but some spots showed differences seeming a crop alternance. This species only fructifies in spring, being the fruiting period from 6 to 8 weeks, what coincides with increasing air-temperature from mid February to April. Differences in fruiting period length and total sporocarp production are related to rainfall and temperature. Data suggest that the rainfalls in the end of summer and early autumn are crucial for the next spring fruiting. During the fruiting period sporocarp production was well correlated to soil temperature.

10.9 Transport of radiocaesium by arbuscular mycorrhizal fungi: from myths to reality

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Radiocaesium (Cs) is the most threatening radionuclide present in the environment. Indeed, after its deposition on soil, following above-ground nuclear testing and catastrophic events such as the Chernobyl accident, it can be taken up by plants and fungi and thus enter the human food-chain. Ectomycorrhizal fungi have been shown to take up and accumulate significant amount of Cs. However, studies on the capacity of arbuscular mycorrhizal (AM) fungi to accumulate and/or transport Cs led to contradictory results. Here we present results of several experiments performed using either root organ cultures (ROC) or arbuscular-plant (AM-P) *in vitro* culture systems on the accumulation and transport of Cs by AM fungi. Our results unambiguously demonstrated that AM fungi can take up, translocate and transfer Cs to their hosts, while Cs accumulation in the extraradical mycelium is limited. They also suggest that AM fungi can participate in the reduction of Cs root to shoot translocation by limiting its loading into the xylem. The effects of potassium (K) and phosphorus (P) on the transport of Cs by AM fungi is further suggested as K (a chemical analog of Cs) can compete with Cs for transport, and because K/Cs are suspected to be associated with P during their translocation and transfer. From these results, it can be concluded that AM fungi can participate in the acquisition of Cs by plants, and that accumulation within the mycorrhizal plants is mostly restricted to the roots.

10.10 Mechanisms of *Acacia longifolia* invasive success - Impacts on AMF communities

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The introduction of exotic invasive species in ecosystems is one of the driving-forces of global change that most affects biodiversity. There is some evidence that arbuscular mycorrhizal fungi (AMF) may play a role in invasion processes. Since AMF are known to be rather unspecific, they can easily establish symbioses with exotic plant species, and eventually mediate invasions processes, namely by interfering in the outcome of the competitive interaction between native and exotic species, promoting the growth of the later and indirectly inhibiting the former. On the other hand, establishment of invasive species may sharply modify ecosystem abiotic and biotic properties, including changes in soil microbial communities. In this work, we studied the impact of *Acacia longifolia* in the composition of the AMF community of coastal sand dune systems, by screening the AMF species present under *Acacia* and under some native species. We also studied the associated changes in microbial communities, by determining the physiological profile of these communities, using sole-carbon-source tests. Endophyte fungi were also screened. Possible implications of the obtained results in revealing mechanisms underlying *Acacia longifolia* invasive success are discussed.

10.11 Biological and functional diversity of ectomycorrhizal fungi altered by charcoal production in central Mexico

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The oak tree forest of Santa Rosa in central Mexico is affected by charcoal production on local scale. This process alters the physicochemical properties of soil and the species microbial diversity. We found and increase in soil pH in charcoal production sites, with a decrease in organic matter and inorganic phosphorus content. The richness of fungal species founded by fruit body harvesting was lower in sites without this forest exploitation, but the percent of ectomycorrhizal species present was higher there. A high number of promoter growth rhizobacteria associated to ectomycorrhizal roots of oak tree were isolated from unheated soil, but none from charcoal site. Ectomycorrhizal native isolates were metabolic tested for its ability to use different carbon sources under different conditions of pH and temperature, simulating these conditions on charcoal production sites and in unheated soil. The data collected from growth rate and activity, demonstrates plasticity in carbon source utilization altered by pH.

10.12 Diversity and functionality of arbuscular mycorrhizal fungi in secondary communities of tropical dry forest, Oaxaca, Mexico

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The aim of this work is to analyze the change in the arbuscular mycorrhizal fungi (AMF) community in secondary plant communities of different ages from abandoned agricultural plots in the tropical dry forest in Nizanda, Istmo of Tehuantepec, Oaxaca, Mexico. This work was carried out in fourteen plots of 0, 1, 3, 5, 7, 10, 14, 18, 22, 25, 27, 32, 37 and 40 years of abandonment and in one non disturbed plot of tropical dry forest. Soil samples were randomly selected; spores were isolated and identified and quantification was carried out. A most probable number (MPN) bioassay was also elaborated. We identified 24 species of AMF belonging to the following Genera: *Acaulospora*, *Entrophospora*, *Arqueospora*, *Glomus* and *Sclerocystis*, *Pasispora*, *Gigaspora* and *Scutellospora*. The lowest species richness values corresponded to the plots 18, 22, and 32 year old plots. The higher values were found in those sites of less time of abandonment. The diversity index showed the highest values in the 3 and 5 year old plots and the lowest in those 40 year old sites. The lowest number of viable propagules was found in the 27 and 37 year old plots, while the 5, 18 and 25 year old plots showed the highest values. These results point out the importance of environmental heterogeneity as a modulator of the response of the mycorrhizal fungi.

10.13 Survival of *Thelephora terrestris* ectomycorrhizas on Scots pine seedlings outplanted on post-agricultural land

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Thelephora terrestris (Ehrh.) Fr. is a very common ectomycorrhizal symbiont in conifer trees. The role of this ubiquitous fungus in nurseries and Scots pine plantations, particularly after replanting on post agricultural land, is still unknown. In laboratory the seedlings of Scots pine *(Pinus sylvestris* L.) were inoculated with *T. terrestris* (Tt/IBL/2) or not inoculated (control) and grown in a growth chamber for six months. Next year, in the spring of 2004 these seedlings were planted on poor sandy soil. After first growing season inoculated seedlings have been colonised by seven mycobionts and the non-inoculated by five ones. Of all mycobionts *T. terrestris* was the most common, colonising on inoculated seedlings about 53% of the root tips and about 13 % of non-inoculated ones. After second growing season the mycorrhizal diversity of inoculated seedlings was not changed but percentage of *T. terrestris* increased to 65 %. On the non-inoculated seedlings found the seven mycorrhizal morphotypes and *T. terrestris* colonised about 48% of the root tips. The results showed that mycorrhizas of *T. terrestris* were also formed by indigenous strain but the pre-inoculation increased their number. The DNA analysis confirmed presence of *T. terrestris* on roots two years after seedlings' outplanting.

10.14 Stability and effect of ectomycorrhizal inoculum on containerised oak seedlings outplanted in ecologically disturbed locals

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Containerised seedlings of *Quercus petrea* and *Quercus robur* are commonly used for afforestation in Central Europe. ECM fungi can increase the quality of seedlings, adaptability and stress resistance after outplanting on ecologically disturbed locals. The ectomycorrhizal inoculum of four fungal species: *Laccaria laccata* , *Paxillus involutus* , *Scleroderma citrinum* , *Coenococcum geophillum* and mixed inoculum were added to containerised seedlings. The inoculated plantlets were used for reforestation of clear-cut forest area, exhausted peat bog and highway embankment in following year. The locality and inoculum showed to be significant factors increasing annual growth of plantlets, collar diameter and survival of inoculum a year after replanting. *C. geophillum* colonized roots of plantlets on all locals intensively. *L. laccata* and mixed inoculum survived on plantlet roots from 60 to 70% in clear-cut locality and restored peat bog. *S.citrinum* and *P. involutus* outlived about 50% on roots of plantlets on all sites. Non-inoculated plants entrapped ectomycorrhizal fungi occurred naturally in experimental sites. Generally, ten of ECM morphotypes occurred on feeder roots of plantlets on all locals. Morphotyping combined with molecular survey including sequencing confirmed the presence of inoculated species and ECM species such as *Tomentella sublilacina* , *Telephora sp* ., *Genea sp* ., *Lactarius quietus* . *T. sublilacina* was predominant species on all observed plots.

10.15 Arbuscular mycorrhizal colonisation responds to seasonality and long-term phosphorus application in an Irish grazed grassland

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Arbuscular mycorrhizal (AM) fungi have not been studied in Irish grasslands (81% of Irish farmland). This study assesses the effects of long-term phosphate fertilisation on AM colonisation in three grassland plant species. Roots of *Holcus lanatus*, *Lolium perenne* and *Trifolium repens* were taken bimonthly during 2004 and 2005 from plots of a long-term phosphorus (P) application trial at Johnstown Castle, Wexford. Cattle-grazed plots have received 0 (P0), 15 (P15) and 30 (P30) kg P $ha^{-1}yr^{-1}$ (as superphosphate) since 1968. Roots were stained and AM colonisation quantified as root length occupied by hyphae, arbuscules, vesicles and coarse (CE) and fine (FE) AM endophytes. *T. repens* was heavily colonised, with 96% colonisation in the P0 plots reduced to 56% at P30. Colonisation of *L. perenne* fell from 61% (P0) to 26% (P30). *H. lanatus* was least colonised, with a mean of 34% in P0 and 9% in all P-fertilised plots. In *H. lanatus*, all P additions reduced AM colonisation by 75% whereas in *L. perenne* and *T. repens* AM colonisation fell further with increasing P. CE occupied 2-3 times greater root length than FE. FE was more sensitive to P addition than CE. Seasonal differences between CE and FE were also apparent, with FE being most abundant in March–May and CE peaking in July–September. These studies have shown that different components of AM colonisation vary seasonally and in response to P addition in Irish grazed grasslands.

10.16 Diversity of arbuscular mycorrhizal fungi in soybean roots cultivated after different preceding cropping systems

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The growth of crops was more enhanced in the fields where the host plants for arbuscular mycorrhizal (AM) fungi had been cultivated previously, compared with in the fields after non-mycorrhizal plants or no plant. The diversity of AM fungi in soybean roots was investigated to know the dominant species and the effect of different preceding cropping on the AM fungal diversity. In July and September 2004, soybean samples were collected from three different plots whose previous cropping had been carrot or spring wheat or bare fallow, and the root DNA was prepared. Firstly, PCR using taxon specific primers (TSP) in the region of the 18S rDNA could detect the AM fungi in the roots and classify them at family level. PCR analysis with TSP did not show remarkable differences depending on the different-managed fields. Glomaceae and *Glomus etunicatum* with relative species were detected in almost all samples. Gigasporaceae and Acaulosporaceae were sometimes decreased or not detected in the roots collected in September. Secondly, amplified fragments by the nested PCR using the primers specific to the 18S rDNA of AM fungi were cloned into TA vector and sequenced. Sequence homology search revealed that many of the AM fungi in the roots of soybean after carrot or spring wheat and most of the AM fungi in the roots after fallowing were similar or related to unidentified *Glomus* sp. (AB076344), which had been isolated from the grassland in Japan.

10.17 Belowground ectomycorrhizal community structure of a *Populus tremula* (L.) forest growing on a heavy metal contaminated site

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The belowground ectomycorrhizal community associated with *Populus tremula* growing on a heavy metal polluted (Zn, Pb, Cd) site in Southern Austria was investigated. Mycorrhizas of the organic horizon and from mineral soil were investigated separately. Characterisation of mycorrhizal root tips involved a combination of morphotyping and PCR amplification of the rDNA-ITS-region. Following sequence analysis of the PCR products, fungal symbionts were identified by BLAST searches in public databases (http://unite.zbi.ee and http://www.ncbi.nlm.nih.gov/) and from comparison with sequences from collected fruitbodies. A total of 69 fungal species was detected as mycobionts of *P. tremula*. They belong to 21 fungal genera of 5 ascomycete and 8 basidiomycete families. *Tomentella* was the predominant genus with the most species (19 species). We observed significant compositional differences between the ectomycorrhizal communities of organic and mineral soil horizons.

10.18 Growth responses of *Pinus densiflora* seedlings to different ectomycorrhizal fungal species in coal mining spoils

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Study was conducted to investigate the growth response of *Pinus densiflora* infected with different ectomycorrhizal (ECM) fungal species. Two species of ECM fungi were collected from an abandoned coal mining spoils in Samcheok and six species from a pine forest in Mt. Sokri, Chungbuk. ECM fungi of eight species were inoculated to the pine seedlings. After growth in pots containing coal mining debris for four months under a growth chamber, mycorrhizal formation were observed and the growth and survival of seedlings were measured. Both species of *Pisolithus tinctorius* and *Lactarius hatsutakeI*, which were collected from the coal mining spoils, formed ECM and those seedlings showed better growth than others. However, only one species (*Boletinus cavipes*) among six fungal species, which were collected from a pine forest in Chunguk, formed ECM and most of those seedlings were died. The results suggest that the native fungal species isolated from the coal mining spoils would more efficient for survival and growth of pine seedlings and thereby could be used for restoration of the abandoned coal mining spoils to be practiced by introducing *P. densiflora* as a native species.

10.19 AM fungi spore germination as a bioassay for soil biological quality- Results of an international ring test

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Mycorrhizal fungi are important components of the soil microbial community in plant-soil systems and represent a direct link between soil and plants. They fulfill most of the criteria for bioindicator organisms: ubiquitous in soils, sensitive to pollutants, ecological-relevant role in plant health and ecosystems. Two laboratory bioassays have been developed to include mycorrhizal fungi in evaluations of the potential toxicity of contaminants (chemical substances, wastes, sewage sludges) or of contaminated soils. *Glomus mosseae* (BEG12) is used in the two bioassays, the first one based on spore germination, and the second one on root colonization of *Medicago truncatula* by the same fungus. Ring tests were organized in France to assess the robustness of these two methods. The results led to standardize the spore germination and the root colonization assay (XP X 31-205-1 and -2) in France in 2004-2005. An international ring test regarding the spore germination assay is currently running, with laboratories from 6 different countries, in the perspective of developing an international standardisation to submit to the ISO. The spore germination assay consists of placing thirty commercially produced *G. mosseae* spores between two nitrocellulose membranes, held together by a slide frame, placed in a Petri dish between two layers of the test substrate. Spore germination is determined after two weeks. Results of the test will be presented.

10.20 Effects of mushroom harvest technique on subsequent American matsutake production

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The harvest of American matsutake (*Tricholoma magnivelare*) is a multi-million dollar industry in the Pacific Northwest, USA. There is controversy regarding how the resource should be managed, including concerns about the cumulative effects of picking in the same areas year-after-year and whether raking of surface soil layers to find mushrooms will reduce subsequent fruiting. The objective of this study was to evaluate the effects of several mushroom harvest techniques on matsutake production. A soil raking study was established in the Oregon Cascade Mts. using similar matsutake shiros. Six treatments were applied: 1) no harvest control, 2) harvest by gentle rocking and pulling, 3) raking of the litter layer, sporocarp removal, and careful replacement of the litter, 4) litter raking, sporocarp removal, and no replacement of the litter, 5) raking of the litter layer and into the top of the mineral soil (7-10cm total depth), sporocarp removal, and replacement of litter and mineral soil, 6) raking of the litter layer and into the top of the mineral soil, sporocarp removal without replacement of litter and mineral soil. Post-treatment mushroom production of the shiros has now been monitored for 10 years. Results indicate that careful mushroom harvest had no impact on matsutake production, while raking without replacement of the litter and soil layers reduced matsutake production and those effects persisted for nine years. We did not test damage to shiros caused by repeated raking.

10.21 Papaya cropping systems: Taxonomy and functional diversity of AM fungi

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Taxonomic diversity of arbuscular mycorrhizal fungi (AMF) in agrosystems is negatively affected by both agricultural and human activity. High input application is usually considered to enhance production and profitability of crops, although some undesirable effects may be caused such as soil disturbance and consequently, loss of soil fertility. In the present study, the diversity of AMF at two different papaya plantations with low and high technology were compared. AMF-species composition, dominance–diversity curves, Simpson's and Shannon's diversity index, and colonization percentage were evaluated. For both papaya plantations, low and high technology, the indexes were similar. Only 3 out of the 10 identified AMF-morphospecies, had high number of spores at high input technology papaya plantation when compared to that plantation with low input. Based on the dominance-diversity curves, richness of native AMF species did not significantly decline at the plantation with the high input technology. Even though no differences were found in AMF-diversity, differences on colonization percentages were observed. The highest colonization occurred at the low technology plantation (from 70.6 to 75.4%) than that estimated at the high input technology (from 28.7 to 41.7%) Results suggest that agrosystem management does not negatively affect the occurrence of AMF species, but their functionality.

10.22 Short-term effect of tree competition release on the ectomycorrhizal status of a declining Peduculate oak forest

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The effects of a sylvicultural treatment (thinning) on the ectomycorrhizal community has been studied for two years in a declining *Quercus robur* forest in Northern Italy. In April 2004, sixteen dominant oak trees were chosen as to represent two decline classes. Fine root status, ectomycorrhizal community composition and structure were seasonally described at these two decline levels. Each ectomycorrhizal root tip was morphologically characterised and molecular tools were used to identify the symbiotic fungi. In July 2004, four trees in each class were released from competition by felling all the neighbouring trees within a radius of 3 m. The aim of this research was to describe the ectomycorrhizal diversity and to determine if its short-term structure changes according to sylvicultural treatment and decline class. The ectomycorrhizal community is characterised by a high richness with a large number of rare species: 65 anatomotypes were found and only 14 were present both before and after treatment application, demonstrating how the community structures rapidly changes after disturbance. However, it is influenced by the treatment only early after thinning, while ten months later the Simpson's diversity index shows the same value for all groups of trees, demonstrating the resilience of the ectomycorrhizal community to disturbance, independently from the health status of trees.

10.23 Mycorrhization, growth and carbon isotope discrimination in a young plantation of *Quercus suber* L. seedlings from two origins

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In the framework of the EU Contract CREOAK (QLK5-CT 2002-01594), the impacts of nursery-mycorrhization of *Quercus suber* with *Pisolithus arrhizus s.s.* on the mycorrhiza diversity and ecophysiology of seedlings are under study in the experimental plantation established at the Mas Vilar (Montesquieu-des-Albères, Pyrénées-Orientales, France) that involves cork oaks "introgressed" with holm oaks (Pyrénées-Orientales) and "non-introgressed" ones (Sardinia). After 14 months of outplanting, the highest average number of branches with diameters < 1 mm was observed in "introgressed" cork oaks nursery-mycorrhized with *P.arrhizus s.s.* (PO-M). In addition, the average length of all the branches was significantly higher in PO-M than in the non-mycorrhizal treatments (PO-NM, SARD-NM), whereas it was intermediate in SARD-M seedlings. Such differences in the seedlings growth seem to be related to the nursery-inoculation effects since only roots of inoculated cork oaks showed *Pisolithus*-like mycorrhizae. Specifically, growth, leaf N content and carbon isotope discrimination (δ) data are been collected to study their changes as responses to water constraints and biotic factors, inherent to the ecosystem studied.

10.24 Fuel reduction treatments alter soil properties, nutrient availability and arbuscular mycorrhizal fungal communities

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Forest management practices designed to reduce fire risk, particularly thinning followed by burning slash piles, can cause below ground disturbance that creates favorable conditions for invasive plant species. Other fuel-reduction methods, such as mechanical mastication, that may cause fewer disturbances, are being tested. We compared soil properties, arbuscular mycorrhizal fungal (AMF) spore and extramatrical hyphal abundance, and plant community composition in replicated plots of pinyon-juniper woodland that have been exposed to mechanical mastication, pile burning or left unmanaged as controls. Preliminary results show that pile burns have the highest exotic to native plant ratio; significantly higher soil temperatures, lower soil moisture, higher pH, higher concentrations of NH_4^+ and NO_3^-, lower soil stability, and significantly lower extramatrical density and spore richness when compared to untreated areas. Two soil properties (temperature and moisture) were significantly different between the mechanical mastication and untreated areas; however plant composition and AMF abundance variables did not differ between masticated and untreated areas. Overall, mechanical mastication had lower impacts on belowground and aboveground communities compared to slash pile burning, and therefore may be a preferable method for fuel reduction.

10.25 Production of external mycelia by ectomycorrhizal fungi is sensitive to elevated N deposition to forests

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Elevated N deposition to forests is suggested to induce shifts from fungal- towards bacteria-dominated soil microbial communities. This is most likely due to reduced abundance of ectomycorrhizal (EM) fungi since increased N input influence these negatively (Wallenda & Kottke, 1998). Production of EM external mycelia was found to decrease in the field after N fertilisation (Nilsson & Wallander, 2003) and in nutrient gradients where N varied naturally (Nilsson et al., 2005). Organic layer C/N ratio and tree foliage N are often the best variables to predict the risk of N leaching in coniferous forests (Gundersen et al. 1998; Emmett et al. 1998). We performed a study in spruce forests in a soil organic C/N ratio gradient to test whether reduced abundance of EM mycelia could explain enhanced N leaching in forest soils with C/N ratios lower than 25. Both a soil fungal biomarker (PLFA 18:2w6,9), its mycorrhizal fraction (degraded during incubation; see Bååth et al. 2004), and EM mycelial production in fungal ingrowth mesh bags (Wallander et al. 2001) were positively correlated with soil C/N ratios. However, only EM mycelial production was negatively correlated with N leaching. These results, in addition to similar findings in oak forests along a N deposition gradient (Nilsson et al. 2006), suggest an important role of EM fungi to prevent N leaching. Fungal ingrowth mesh bags have a potential to be used as bio-indicators in environmental programmes monitoring effects of N deposition.

10.26 Effects of cadmium concentration and arbuscular mycorrhiza on growth, flowering and Cd accumulation in geranium (*Pelargonium x hortorum* L.H. Bailey)

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Cadmium (Cd) is a nonessential heavy metal, which is toxic at low concentration to plants, animals, and people. Significant differences in plant ability to accumulate Cd and in plant responses to Cd toxicity have been observed. The purpose of the present research was to study the influence of Cd concentration in growing substrate and mycorrhization of root system on growth, flowering, and Cd accumulation in geranium which as a bedding plant can be used for phytoextraction of Cd in urban areas. AMF colonization of geranium roots at the end of experiment, judged by the percentage of root lengths colonized by hyphae was high: 93%, 67%, 60%, and 50% for plants grown at 0, 10, 20, and 40 mg dm^{-3}Cd, respectively. The non-inoculated plants had no root infection. Cd in the applied concentrations (0, 10, 20, 40 mg dm^{-3}) did not affect decorative value of geranium, no chlorosis or necrosis were observed on leaf margins frequently visible on leaves of other bedding plants. Cd did not also influence fresh and dry weight, shoot number and flowering of geranium. Mycorrhization lowered plant height and shoot number and did not affect flowering time, as well as the number of inflorescences. Colonization of root system with arbuscular mycorrhizal fungi increased accumulation of Cd in shoots of geranium subjected to high Cd concentration in growing substrate and did not affect Cd accumulation in geranium grown under lower Cd concentration (0, 10, 20 mg dm^{-3}).

10.27 Three-years monitoring and comparison of ectomycorrhiza bimass in *Pinus densiflora* stands that are different in the forest management

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Our study objective was to monitor ectomycorrhizal biomass in a *P. densiflora* forest during three years and to compare the biomass in relation to different forest management. Four plots were set in a *P. densiflora* forest (ca. 50-year old): control (no treatment), cutting any shrubs (treatment A), thinning and the cutting (treatment B), and removal of litter layer as well as the thinning and cutting (treatment C). Sampling has started since May 2003 in the control plot, and the other plots were added in June 2005. Seven soil cores (each sample consists of upper and lower layers) were sampled monthly from A-layer soil in each plot. Ectomycorrhizas and the others of pine root were collected from each soil samples and measured their biomass. Mycorrhizal tip number was highly varied by month and the cumulative number was significantly different in each of the three observation years in the control plot. In the treatment plots, mycorrhizal tip number was significantly small in the treatment B and C plots. Mycorrhizal tip number in the treatment A plot was significantly smaller than that in the control plot in the first two months. These results suggest that although any forest treatments may decrease mycorrhizal tips soon after them, which will be recovered within a few months. About 80% of mycorrhizal tips were in the upper layer in each plot. Mycorrhizal dry weight/100 tips showed the decreasing trend in autumn in plots except for treatment C plot.

10.28 Influence of arbuscular mycorrhizal symbiosis on Ni-hyperaccumulator *Berkheya coddii* Roessler

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Recently, the presence of mycorrhizal symbiosis was demonstrated in the Ni hyperaccumulating plant, *Berkheya coddii*. This plant, growing on ultramafic soils in South Africa, is an excellent model for phytoextraction and phytomining. To assess the influence of mycorrhiza on growth and element uptake by *B. coddii* the plants were cultivated under laboratory conditions on sterilized ultramafic soil and inoculated with native mycorrhizal fungi, *Glomus intraradices* from non-polluted soil or left noninoculated. All inoculated plants had several times higher shoots' and roots' biomass than the nonmycorrhizal ones. Roots of plants inoculated with *G. intraradices* and with native fungi from *B. coddii* rhizosphere were similarly extensively colonized. All mycorrhizal parameters estimated from alkaline phosphatase activity assays were significantly different from the data obtained from aniline blue staining. Both fungal strains originating from ultramafic soil showed higher viability than *G. intraradices*. There were no differences in Ni concentration in leaves between nonmycorrhizal and mycorrhizal plants, but the Ni uptake per plant was 10 times higher in plants inoculated with fungi from *B. coddii* rhizosphere than in the nonmycorrhizal ones. Mycorrhizal colonization increased P and K concentration in leaves. In soil, in pots with strongly mycorrhizal plants in comparison with the nonmycorrhizal control, P and K levels were increased, while extractable concentrations of Ni were lower.

10.29 Herbicide effects on mycorrhizal and rhizobial symbioses in genetically-modified crops

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The adoption of genetically-modified, herbicide-tolerant (GMHT) crops has been swift in many parts of the world. For example, in 2005, U.S. growers seeded 26, 61, and 87% of corn, cotton, and soybean acres, respectively, with GMHT varieties (USDA, 2005, Acreage Report Cr Pr 2-5). Most GMHT varieties have been modified for tolerance to glyphosate, a broad-spectrum herbicide. As a result, glyphosate use exceeds all other herbicides in some cropping systems (e.g., over 80% of U.S. soybean acres were treated with glyphosate in 2002, up from 10 % in 1994 (Baucom and Mauricio, 2004, PNAS 101:13386)). Few studies have investigated the effects of glyphosate on arbuscular mycorrhizal symbioses, and glyphosate effects on rhizobial symbioses differ among studies. Here, soybean plants were inoculated with *Glomus intraradices*, *Bradyrhizobium japonicum*, or both, subjected to a foliar glyphosate application, and then harvested one, two, four, or eight weeks later. Nodule mass and N_2 fixation were greater on glyphosate-treated plants, but this effect was dependent on colonization by *G. intraradices*. Effects of glyphosate on root colonization by *G. intraradices* were dependent on glyphosate timing. Most effects were consistent over the duration of the experiment. The use of glyphosate may affect rhizobial and mycorrhizal symbioses in GMHT cropping systems, but consideration should be given to the role of the tripartite symbiosis in mediating this effect.

10.30 Resilient arbuscular mycorrhizal fungal populations occur within intensively managed tomato production systems in southeast Florida

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Farming practices are known to significantly impact arbuscular mycorrhizal (AM) fungal populations. Effects of highly divergent nutrient and pest managements on the incidence and infectivity of AM fungi were evaluated at a farm site in Ft. Pierce, FL, that had a prior history of raised-bed, methyl bromide tomato production. Five management programs in replicated 0.4 acre plots were implemented in a 3-yr transitional period prior to resumption of tomato cropping: 1) conventional production; 2) continuous disk fallow; 3) bahiagrass (*Paspalum notatum*) conservation tillage; 4) weed fallow; 5) organic management. Very high levels of soil phosphorus (160-300 kg/ha extractable P) existed in treatment plots due to the previous manager's fertilizer applications. Additionally, stressful conditions were prevalent during the second year of this study when two hurricanes crossed over the area (500 mm rainfall in Aug.-Sept.). Field tomato roots were most heavily colonized in the conservation tillage treatment during both years of the study, while more moderate, relatively uniform levels occurred among the other management treatments. Mycorrhizal infection potential (MIP) of soil was reduced during the second year in all treatments except for organic management. Soil from disk fallow plots had the lowest MIP while actively fallowed, but recovered following a full season of tomato cropping. A substantial number of spores extracted from trap crops in both years were parasitized.

10.31 Influence of *Glomus intraradices* on the mass balance of Cd in a pot experiment with *Medicago truncatula* in four contaminated soils

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Metallic trace elements (MTE) in soils reach problematic concentrations in some places, causing damage to vegetation and being dangerous for health. Metal behaviour in soils is strongly influenced by soil characteristics (pH, organic matter, mineralogy…) but also by biotic factors. Roots and microorganisms can modify chemically or physically the soils properties, changing the availability of pollutants. Widespread arbuscular mycorrhizal (AM) fungi have been shown to affect metallic elements transfer to plants, reducing the translocation from roots to shoots (Leyval & Joner, 2001). However, there is little data on the effect of AM inoculation on the complete mass balance of MTE in soil-plant-fungus systems. The present study aimed at evaluating the mass balance of Cd in a pot experiment using the model plant *Medicago truncatula* inoculated or not with a metal tolerant AM fungus *Glomus intraradices*. Four soils were used: two historically metal-polluted soils and two artificially contaminated with Cd salt (6 mgCd.kg^{-1}), presenting a range of bioavailable Cd (NH_4NO_3 -extractible) from 0.4 to 2.2 mg.kg^{-1} . The pot system allowed measuring the loss of metal by percolation throughout the experiment. After 5 weeks Cd concentration in plants and in leachates were quantified in four soil conditions and mycorrhizal status was compared. The results will be discussed within the context of bioremediation and phytostabilisation to reduce the risks due to soil contaminants.

10.32 Post flood population dynamics of arbuscular mycorrhizal fungi as affected by long term farming systems in the North Carolina coastal plain

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A long term farming systems study was set up in the coastal plain region of North Carolina, USA to study the effects of farming system practices on components of the agro-ecosystem, including the mycorrhiza fungal population. The farming systems consisted of: conventional, including till and no-till; integrated crop and animal; organic; successional; and forestry/ woodlot systems. This 200 acre study site and surrounding fields, had been in agricultural production for the past 100 years. Contrary to expectations, a baseline survey revealed a species rich ecosystem, with twenty species of arbuscular mycorrhizal fungi (AMF) sporulating in trap culture. These fungi have either adapted to, or are tolerant of agricultural inputs and disturbance. On September 16th in the first year of the study, a hurricane struck North Carolina, resulting in a flooding event. Soils were saturated for approximately three weeks. By the time the fields were dry enough to be planted to a winter cover crop, mycorrhizal host plants had been absent for ten weeks. The effect of the flood on the AMF population and it's subsequent recovery, as affected by the various farming systems became the focus of the study. AMF species richness was monitored over the following six years. The greatest post flood increase in AMF species richness occurred in the conventional tilled fields. Tillage processes may have facilitated the dispersal of AMF propagules, speeding up population recovery post disturbance.

10.33 AM fungi in heavy metal-contaminated soil in Brazil: occurrence and plant effects

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We report the main findings of our latest research dealing with AMF in heavy metal contaminated soil in Southeastern Brazil. In samples from a highly contaminated site (mg kg^{-1} soil: Zn = 16,000; Cu = 586; Cd = 39 and Pb = 125), we found a total of 21 species of AMF. *Glomus clarum*, *Acaulospora mellea* and *Paraglomus occultum* were the most common species recovered. Experiments with different hosts showed that AMF have varied effects on plant's growth. Both herbaceous and woody plants were quite sensitive to the excess metals in soil, but in general these fungi were of advantageous to plant growth under these conditions. Mycorrhizal plants usually had higher dry matter yield, reduced translocation and lower shoot metal concentrations. In an experiment testing the effects of the AM stimulating isoflavonoid (formononetin), it was found that this compound alleviated plant toxicity by lowering Zn and Cd and increasing Fe concentrations in corn shoots. We also tested the hypothesis that AMF host's protection against metal toxicity is nutritionally mediated by P uptake, but no evidence was found to support this. AMF isolates have highly variable ability to enhance protection and metal extraction by the tropical grass *Brachiaria decumbens*. The most consistent fungal effect was found for Cu that is retained in higher quantities in mycorrhizal roots. Our results indicate that inoculation of tropical mycorrhiza hosts with selected fungi may enhance phytoremediation.

10.34 Carbon, nitrogen and phosphorus fertilization of white oak and sugar maple: effects on ectomycorrhizal and arbuscular mycorrhizal abundance

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A fertilization experiment with carbon, nitrogen and phosphorus was conducted on sugar maple and white oak to examine belowground processes affected by global climate changes. Increasing atmospheric carbon dioxide concentrations and nitrogen deposition are affecting biogeochemical cycles in hardwood forests, such as the temperate deciduous forest at the Morgan-Monroe Ameriflux research site in Southern Indiana, U.S.A. Sugar maple and white oak are two dominant tree species in this forest and were chosen for study due to differences in their primary mycorrhizal associations. Treatments were applied in a factorial design and were dissolved in water to simulate wet deposition. After two years of treatment, both ectomycorrhizal and arbuscular mycorrhizal abundance declined in response to nitrogen and phosphorus treatments. Ectomycorrhizal morphotype diversity decreased with phosphorus additions, but was not significantly affected by other treatments. White oaks increased fine root biomass and ectomycorrhizal abundance with carbon treatments, but sugar maples and arbuscular mycorrhizal abundance did not respond to carbon. Other parameters being examined are soil respiration, soil and fine root chemical content, and soil microbial biomass. Results from this study suggest that tree species, especially those with different mycorrhizal associations, may differentially respond to global climate changes that affect belowground processes.

10.35 Evaluation of arbuscular mycorrhiza as indicators for environmental impact studies

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Information about the biotic configuration that characterizes different used landscapes is essential to understanding functional consequences of the biodiversity in landscapes and for optimizing management strategies for conserving and using biodiversity in sustainable production. The diversity of soil flora and fauna, which contributes to sustaining soil quality and plant production, determines the extent of functional stability of soil ecosystems. Indicators are needed for systematically monitoring the consequences of constantly changing land uses on the diversity of soil organisms. The mycorrhizal symbiosis, a fundamental link between soil and plant is proposed as a part of a possible soil biodiversity indicator. Such an indicator will provide instruments for assessing at regional scales how various environmental and anthropogenic factors affect the soil biota and their population abundance. The potential use of mycorrhizal symbiosis as part of the biodiversity indicator requires research to define sensitive parameters to characterize the dependence of the mycorrhizal population on soil characteristics and land use. We studied the site specific potential of mycorrhizal fungi in agricultural used soils in Canada and Germany using molecular, morphological and functional tools. The abundance and molecular and functional diversity of the site specific AM-community will be discussed with respect to response to organic amendment applications on soil and changes in management.

10.36 Disturbance and land use effect on functional diversity of the mycorrhizal fungi

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The biodiversity of AMF in Mexico has not been intensively studied. Even though taxonomic studies have been made that show the presence of 29 % of the reported species in the world, but the knowledge about their functional diversity is poor. The aim of this study was to compare the impact of disturbance on functional diversity of AMF in different sites. Three sites were selected for this study according to disturbance gradient and land use: López Mateos (LM), San Fernando (SF) and Venustiano Carranza (VC) (with low, medium and high disturbance respectively). They are located in the "Los Tuxtlas "Biosphere Reserve in the state of Veracruz. The land use in each site includes, "milpa" and pasture. High percentages of colonization and viability of spores were found in the LM soils, 100 % of the pasture soils have a high MIP, the fungi consortium coming from that site promote the highest GRM with increases from 74.11 % to 194.59 % over the control treatment. SF soils have high percentages of colonization and viability of spores, only 20 % had a high MIP and their GRM promotes increases from 14.66 % to 120.36 % over the control. Soils from VC have the lowest percentages of colonization and viability of spores. Only 10 % of the soils have a high MIP and their GRM was not better than the control. These results seem to indicate that the soil disturbance or change in its use affects negatively the functionality of the AMF.

10.37 Below-ground ectomycorrhizal communities of Douglas-fir and paper birch along a chronosequence following clearcutting and wildfire in British Columbia

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Ectomycorrhizal (ECM) communities of Douglas-fir and paper birch were characterized in 5, 26, 65, and 100-year-old stands using ECM root tip morphology and ITS region DNA sequences. Stands disturbed by wildfire (all age classes) and clearcutting (two youngest age classes) were studied in the Interior Cedar-Hemlock forests of British Columbia. ECM community species richness on Douglas-fir was over three times higher in 65 and 100-year-old stands than in 5-yr-old stands; 26-yr-old stands were intermediate. In the 5-yr-old stands, the ECM community of paper birch had ~70% higher species richness than Douglas-fir. Roots of sprouting paper birch stumps may support mycorrhizae or inoculum that persist through disturbance. Overall ECM diversity increased substantially from 5 to 26-yr-old stands, but did not change substantially with further increases in stand age. ECM community composition and structure continued to change from 26 to 65-yr-old stands, including increases in Russula and Piloderma relative abundances. Similar ECM communities occurred on 65 and 100-yr-old stands. Available P was correlated with abundance of two dominant taxa, but available N and P, mineralizable N, and organic P were not related to ECM diversity or community structure. Host-specific ECM fungi were more dominant in the youngest stands, and host-generalists were more common on paper birch than Douglas-fir at younger ages. There were no significant differences between clearcuts and wildfire origin sites.

10.38 Mycorrhizal status of poplar species native to Poland (*Populus nigra*, *P. alba* and *P. tremula*) in natural and urban environment

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Most of tree species in the northern temperate forests are exclusively ectomycorrhizal (ECM), some form only arbuscular mycorrhiza (AM). Species in the genus *Populus* can be colonized by both, ECM and AM fungi. Although the interest in application of poplar species and hybrids in agroforestry and bioremediation of contaminated soils is increasing, the information on the mycorrhizal status of poplars, particularly in natural environments, are very scarce. The aims of this work were: 1) to study the mycorrhizal associations of mature trees (60-200-year-old) of three *Populus* species grown in a natural, protected environment (Ostromecko Nature Reserve) compared to the poplar trees grown in two urban sites influenced by different levels of antropogenic pressure, 2) to evaluate the influence of environmetal factor on the ability of the poplar species to form ECM and AM symbiosis. The results showed that regardless of the environment, *P. alba* formed only ECM, whereas *P. nigra* and *P. tremula* developed both, ECM and AM. The highest ECM diversity revealed *P. alba* followed by *P. nigra* and *P. tremula*. The ECM diversity, as well as the levels of colonization of roots by ECM and AM fungi were significantly lower at the urban sites than in the nature reserve. This suggests that the ability of the poplars to form ECM and AM is genetically determined, whereas the communities of mycorrhizal symbionts and the intensity of mycorrhizal colonization are influenced by environmental factors.

10.39 Effects of two sterol biosynthesis inhibitor fungicides (fenpropimorph and fenhexamid) on *Glomus intraradices*

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Fungicides are targeted to control phytopathogenic fungi. Therefore, their impact on beneficial soil fungi, among which the arbuscular mycorrhizal fungi (AMF), have been mostly neglected. However, these organisms play a key role in nutrient cycling, element mobilization and availability to plant, and plant tolerance to biotic stresses. Thus, there is a necessity to study the impact of Sterol Biosynthesis Inhibitor (SBI) fungicides on this group of symbiotic organisms. The effects of increasing concentrations (0.2, 2, 20, 200 mg l^{-1}) of fenpropimorph and fenhexamid on spore germination and germ tubes length of *Glomus intraradices* were investigated under root organ culture conditions. With both SBI fungicides, the percentage of germinated spores and length of germ tubes were strongly decreased at concentrations above 2 mg l^{-1}. Whatever the concentration of SBI fungicide tested, the impact appeared fungistatic and not fungitoxic. Indeed the non-germinated spores as well as the germinated spores that did not show germ tube growth at high concentrations of SBI fungicides were able to germinate and grow when transferred on a fungicides-free medium. From our data, it appeared that both SBI fungicides tested had no lethal effect on the AMF used. Therefore, it is questionable whether these fungicides could impact AMF in the field. As a side result, the in vitro system developed opens interesting avenues to study the sterol biosynthesis pathway, until now poorly understood.

SESSION 11

Biome-related functional ecology

Unearthing plant-to-soil carbon flux through AM mycelia in grassland: Field $^{13}\mathrm{CO}_2$ pulsing, mesh cores, collembolans and isotope-ratio PLFA analysis

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Annual carbon (C) flux through soil respiration is ten-times greater than fossil fuel combustion and recycles ~10% of atmospheric CO_2, but there is limited understanding of the importance of mycorrhizas in these fluxes. The largest, and fastest pathway of C flow from shoots to the soil occurs through roots into the rhizosphere. It has recently been shown that 4-6% of net photosynthate in grassland passes through roots directly to fungal networks of their symbiotic arbuscular mycorrhizal (AM) partners within 24h of fixation. We have investigated, in situ, the susceptibility of this important C-flux pathway to damage by the dominant fungal feeding soil invertebrate in an upland grassland. In the field, natural densities of the numerically dominant fungal feeding invertebrate Protaphorura armata (order Collembola) were extracted from the soil and added into alternate pairs of mesh-walled cores containing established mycorrhizal mycelial networks. The turf was then pulse-labelled with $^{13}\mathrm{CO}_2$. The collembolans reduced mycorrhiza-rhizosphere respiration of labelled C by 32%. The disruption of this pathway also impacts the soil microbial community with decreased 13C-enrichment of fungal biomarker phospholipid fatty acids. Our findings emphasise the need to consider multi-trophic interactions in empirical and theoretical studies of soil respiration.

Symbiont diversity as a driver of plant diversity and ecosystem functioning in grassland

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Symbiotic interactions between plants and micro-organisms are widespread and very abundant in nature. About 150,000 plant species associate with arbuscular mycorrhizal fungi (AMF) and more than 15,000 species of legumes form intimate relationships with nitrogen fixing rhizobia bacteria. In this talk, evidence will be presented showing that both AMF and rhizobia play a key role in grassland. These microbes influence plant diversity and can increase nutrient capture and ecosystem productivity by providing different limiting nutrients to the plant. AMF and rhizobia also can act as support systems for seedling establishment in nutrient poor grassland. Interestingly, many legumes form tripartite symbiotic associations with both rhizobia and AMF, and several legumes appear to rely on both symbionts to flourish in natural communities. The fact that specific AMF communities are found to be associated with the roots of nitrogen fixing legumes suggests that synergistic interactions may exist of which previously has not been thought.

Functional ecology of ectomycorrhizal symbiosis during early primary succession

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The last eruption of Mt. Fuji, Japan, left a vast barren desert, in which primary succession has been proceeding slowly. *Salix reinii* (Salix), the pioneer ectomycorrhizal (ECM) host in this desert, has established sparsely. *Salix* seedlings transplanted into bare ground or near the established non-host vegetation remained non-mycorrhizal throughout the first growing season, indicating the absence of dormant spores and difficulty of ECM infection. In contrast, transplants near established *Salix* were readily colonized by ECM fungi and showed improved growth. Another transplanting experiment, in which non-mycorrhizal *Salix* seedlings were transplanted with ECM mother trees that had been inoculated with one of 11 dominant ECM fungal species, confirmed that vegetative mycelia from the accompanying ECM trees were the prevailing source for ECM colonization on seedlings. This experiment, in which improved seedling performance was solely attributable to ECM fungi, also revealed a significant functional variance between ECM fungi. In addition to the conspecific seedlings, established *Salix* may contribute to the establishment of secondarily colonizing plants by providing compatible and accessible ECM fungi. Naturally established individuals of timber species, and even an herbaceous *Pyrola* species, were observed only near the established *Salix*, sharing common ECM fungi. These results indicate that ECM symbiosis is an important driving force of primary tree succession.

Mycorrhiza in alpine erosion control and slope stabilisation

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Soil erosion and landslides are major natural hazards in alpine ecosystems. Recent increases in the frequency of heavy rainfalls accelerated these processes and aggravated the problems of soil degradation. Hence, it is necessary to know and understand the factors that protect the fragile ecosystems based on long-term considerations. We tested whether mycorrhizal fungi contribute to the overall stability of alpine ecosystems and, in particular to soil stability and erosion control. Survival rates and root length of two alpine species, *Dryas octopetala* and *Salix herbacea* increased when inoculated with selected ecto-mycorrhizal fungi. Moreover, in artificial raining experiments, soil erosion and run-off were considerably reduced due to the positive impacts of ecto-mycorrhizal fungi on soil stabilisation. Laboratory experiments showed that soil aggregate stability was significantly higher in the presence of ecto-mycorrhizal fungi. Our results indicate that the stability of degraded soil against erosion and shallow landslides considerably increases due to inoculation of the appropriate plant material with selected mycorrhizal fungi. Based on conventional techniques and calculation models to assess slope stability, the "mycorrhizal effect" is reflected in an increase of the stable slope angle of up to 5°. Furthermore, we found that the soil aggregate stability is an appropriate parameter to include biological effects in slope stability calculations and risk management.

How much do AMF communities contribute to the soil stability of arid ecosystems?

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Ten years have passed since pivotal studies have shown the predominant contribution of AMF to the structure and stability of soil in mesic systems. However, the role of AMF in the stability of arid lands remains largely unstudied. Ironically arid soils suffer the greatest risk of degradation, soil loss, and desertification. In a landscape-scale field study in the southwestern US, we quantified AMF community characteristics, along with various other biotic and abiotic soil characteristics, to determine their relative contribution to soil stability in arid ecosystems. Using the Herrick slake test, we identified and examined sites along a gradient of soil stability levels. Several key patterns emerged: (1) Low stability soils exhibit less spore abundance, less hyphal lengths, less glomalin and an overall shift in AMF spore community composition compared to both moderate and high stability soils. (2) A disturbance threshold may exist hindering the passive re-establishment of AMF in low stability sites, but not moderately disturbed sites. (3) Surprisingly high AMF spore diversity (45 species) was discovered across the arid landscape, including many undescribed species. (4) The proportional contribution of AMF to soil stability compared to other biotic and abiotic components of arid ecosystems is explored using structural equation modeling. These results indicate a community-level relationship between AMF and soil stability never before demonstrated in arid ecosystems.

Mycorrhizal functioning in restored seasonal tropical forest, Mexico, following fires and hurricanes

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Tropical seasonal forests are widespread in the tropics, but remain the least studied tropical ecosystem. The Yucatan Peninsula, Mexico, has experienced major human and natural disturbances, including fires and hurricanes, which have resulted in a mosaic of secondary forests with increasing fire frequency. Forest and soil C were assessed across stands of different successional ages following fire, and we determined a threshold for total ecosystem C at 20 years. This indicates that some aspects of functioning may recover rapidly. We have undertaken several restoration studies in recently burned stands to determine the functioning of mycorrhizae in the recovery of these forests. We tested the responses of early- and late-successional tree species to early- and late-successional AMF inoculum. Plant response to inoculum varied by differences in the intensity of fire, residual soil organic matter, degree of herbivory, and fungal root pathogens. A stand-level restoration experiment was done by thinning stems < 2 cm dia. Changes in forest architecture resulted in higher aboveground biomass in the thinned plots and increments in root biomass, litter, mycorrhizal infection and glomalin concentration in the soil. Hurricane damage from October 2005 had different effects on thinned *vs.* control stands. We hypothesize that the outcome on belowground recovery will be dependent on initial stand conditions.

11.1 Leaf traits affect the colonization of decomposing leaves by arbuscular mycorrhizal fungi

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We have shown that arbuscular mycorrhizal fungi (AMF) spread within slowly-decomposing leaf litter of different plant species at montane sites in Colombia. Whether or not this phenomenon occurs in other ecosystems, and how leaf litter traits affect it are not known. We investigated this in a South Florida hammock. First, we examined the influence of decomposition rate on the extent of colonization of dead leaves by AMF. We buried mesh bags that singly contained dried leaves of five different species with different decomposition rates in randomized complete blocks harvested after 70, 100, 170, and 540 days. We found that all species of leaves were colonized to a similarly great extent, but the rates of colonization differed. AMF colonized labile leaves rapidly, but colonized recalcitrant leaves slowly. Second, we tested whether or not leaf veins facilitate colonization of dead leaf pieces by AMF. We obstructed leaf veins with nail polish and rubber, and buried obstructed and non-obstructed dead leaf pieces contained in mesh bags for four months. We found significantly less colonization of leaf pieces with obstructed veins. These results suggest that leaf traits may influence the efficiency of nutrient capture from leaf litter by AMF.

11.2 Microscopic analysis of novel, root-associated ascomycetous endophytes from grassland and meadow ecosystems of the Western United States

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We isolated approximately 600 putative endophytes from the roots of dominant plants in long-term ecological research (LTER) sites representing tall grass prairie, short grass prairie, arid grassland, oak savanna, and high montane meadow ecosystems in the western United States. Conspecific isolates were identified by PCR-RFLP analysis of the ITS region and two isolates of the fungi, which occurred at least twice (86 isolates), were selected for resynthesis assays with *Allium porrum*. Inoculated plants were grown for six weeks, then harvested and analyzed microscopically for intracellular hyphae and/or microsclerotia. Thus far, four (*Neophaeosphaeria* cf. *barri*, *Lophiostoma* sp., *Ophiosphaerella herpotricha*, and *Leptosphaeria* sp.) *Allium*-colonizing, novel endophytes have been identified from the short grass prairie ecosystem and three (*Periconia macrospinosa*, *Periconia* sp., and *Microdochium* sp.) have been identified from the tall grass prairie ecosystem. Morphological variation in endophyte root colonization as well as host responses to inoculation will be presented. Additional results from the ongoing analyses of the remaining grassland sites will also be incorporated. Assuming a similar frequency of endophytes for the remaining samples, we expect 10-15 additional taxa capable of producing indicative endophyte structures in host roots. Our studies suggest endophytes are abundant and that different ecosystems host distinct endophyte species.

11.3 Ectomycorrhizal fungi, plant-plant interactions and the dynamics of woodland ecosystems

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Woodlands have begun to replace grasslands in many areas of the world due to changing climate and anthropogenic forces. Mycorrhizal fungi may contribute to grassland-woodland transitions because mycorrhizal abundance and community composition influence woody plant establishment and performance. This talk focuses on the dynamics of ectomycorrhizal fungi (EMF) in woodlands with an emphasis on semi-arid ecosystems of the western US. Data from our studies and others reveal that both facilitative and competitive interactions among established vegetation affect EMF abundance and/or species composition, which then feeds back to affect plant performance. The dynamics of these interactions are complex, as they depend on the developmental stage and physical proximity of the interacting species, as well as climatic conditions. Water addition experiments, long-term comparisons of mesic and dry decades, and analysis of natural moisture gradients demonstrate that variation in precipitation is a key driver of both EMF and tree dynamics. However, these patterns are complicated by the influences of host plant genetics and herbivory. Studies in woodland ecosystems highlight the need for integrative study of biotic and abiotic interactions to improve our understanding of the functional role of mycorrhizal associations in the field.

11.4 Fungal community dynamics and activity in boreal forest soils of interior Alaska: characterizations via rDNA and rRNA in both summer and winter

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We are investigating seasonal dynamics of fungal community composition (using ribosomal DNA genes) and metabolic activity (using ribosomal RNA transcripts). Our site is in mixed spruce (*Picea mariana, P. glauca*) forest near Fairbanks, Alaska, USA (Bonanza Creek LTER, 64.8N 148.0W). We have collected soil samples during October (fall), February (mid-winter), May (spring) and August (mid-summer), 2004-5. We are also investigating the effect of soil temperature manipulation (twenty 1x1 meter snow-exclusion plots) on fungal community composition and activity. In comparison with paired control plots, snowpack elimination has significantly depressed microsite soil temperatures in winter and slowed soil thawing into mid-summer. Here we report on clone-libraries from the resident (DNA) fungal community as well as the active (RNA) community in summer versus winter. Preliminary results on soil collected from under snow during February show the DNA community is dominated by clones with high sequence similarity to ectomycorrhizae (*Amphinema spp., Tuber spp., Tricholoma spp., Wilcoxina spp.*) as well as ericoid mycorrhizae and members of the Helotiales. We also recovered sequences highly similar to known psychrophiles (cold-lovers) *Geomyces pannorum* and *Cryptococcus laurentii*. Preliminary results show the RNA community is also dominated by ectomycorrhizae (*Amphinema spp.*), however, marked differences appear to exist between the views given by DNA and RNA community characterizations.

11.5 Ericoid mycorrhizal colonization increases cranberry nitrate uptake

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There may be a fundamental error in our understanding of nitrate metabolism in cranberry. It has been based primarily on studies of the physiology of nonmycorrhizal plants, despite the common occurrence of ericoid mycorrhizal colonization of cranberry both in their native habitat and in cultivation. Nonmycorrhizal cranberry exhibited linear uptake kinetics for nitrate (Uninduced: $^{15}NO_3^-$ influx (µmol g_{root}^{-1} min^{-1})= .011 + 1.32 * [NO_3^-]; Induced: influx = .011 + 1.08 [NO_3^-]), apparently lacking the saturable high-affinity transport system common to non-ericaceous plants. We have developed methods to inoculate surface-sterilized cranberry cuttings with the ericoid mycorrhizal fungus *Hymenoscyphus ericae* in hydroponics, with about 50% colonization (root length basis) 6 weeks after inoculation. In experiments with the fungus alone, we found that *H. ericae* was capable of high rates of nitrate influx, and responded to nitrate exposure with increased rates of nitrate influx. Mycorrhizal cranberry inoculated with *H. ericae* had on average 8-fold greater NO_3^- influx than non-mycorrhizal cranberry at NO_3^- concentrations from 20 µM to 2 mM. By measuring fungal biomass in mycorrhizal roots, it should be possible to determine if a simple mixing model can account for the increase in nitrate uptake seen in mycorrhizal cranberry compared to non-mycorrhizal plants. This finding is significant for cranberry management when irrigation water sources contain nitrate.

11.6 Boron in sporocarps of ectomycorrhizal fungi

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Fungi are assumed not to have a boron (B) requirement. Because of the affinity of B for forming stable complexes with most simple carbohydrates except glucose and sucrose, it has been hypothesized that B is immobilized in fungal mycelia. We determined B concentrations in a number of fungal species, and found some consistent patterns between species. There are species that can accumulate up to 200 mg kg^{-1} B, whilst in others, the concentration was consistently low, less than 1 mg kg^{-1}. Hence, B is taken up and translocated from the soil to sporocarps, but some species enrich B compared to tree foliage, and other species appear to filter boron. In conclusion, there is a possibility of B immobilization in the mycorrhizal mycelia of some species but not all. On the other hand, we have previously shown that B is taken up and translocated to the host tree by ectomycorrhizal mycelia. As B deficiencies are wide-spread in many climatic zones in the world, the processes related to B mobility in ectomycorrhizal fungi may determine B availability to host plants and the occurrence of deficiency symptoms.

11.7 Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest soil

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In order to understand how fungi re-circulate nutrients from litter back to living plants, we need to know the contribution of different functional groups of fungi at different stages of the process. Using molecular techniques (ITS-PCR, TRFLP, cloning and sequencing), we analysed the vertical distribution of fungal taxa in a *Pinus sylvestris* forest soil. In boreal forest soils, mixing is limited and organic matter accumulates on the forest floor with the youngest material at the surface and the most degraded material directly on top of the mineral soil. The vertical distribution of fungi therefore reflects their temporal succession during degradation of litter to humus. We found that surface needle litter was colonised by needle endophytes, saprotrophic fungi and ascomycetes of uncertain trophic strategy. After about 4-5 years of decomposition, these fungi were replaced by mycorrhizal fungi and other putative root symbionts. During the saprotrophic decomposition phase, the C:N ratio of the litter decreased with age of the organic matter, indicating loss of carbon and retention of nitrogen in the litter. In contrast, the C:N ratio in organic matter colonised by mycorrhizal fungi increased with depth, indicating removal of nitrogen powered by root-derived carbon. Our results suggest that mycorrhizal fungi control humus degradation in the boreal forest and provide their host plants with nitrogen mobilised from well-degraded organic matter.

11.8 Ectomycorrhizal fungi suppress saprotrophs in a tropical monodominant rain forest

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Most tropical trees form arbuscular mycorrhizal (AM) associations. However, a correlation exists between monodominance, where one tree species comprised >50% of the canopy, and ectomycorrhizal (ECM) associations. We tested the hypothesis that ECM fungi suppress saprotrophic microorganisms since, unlike AM fungi they can directly access organically bound nutrients, but are poorer competitors for these nutrients than saprotrophs. To test this hypothesis, we incorporated field and laboratory studies in both a monodominant forest where the ECM tree *Dicymbe corymbosa* forms >80 % of the canopy, and a diverse, mixed forest where most trees are AM. Using mesh litterbags we assessed decomposition rate differences between forests over two years and also measured bi-weekly litter input. We found that decomposition was significantly lower in the monodominant forest, but that litter input was significantly higher in the mixed forest. In the laboratory we used phospholipid fatty acid analysis and denaturing gradient gel electrophoresis to determine total microbial biomass and compositional differences in the fungal communities. Results indicate that in the mixed forest microbial biomass is higher and the dominant fungal groups are saprotrophic. ECM fungi dominate in the monodominant forest and fewer saprotrophic fungi overall were found there. This suggests that ECM fungi may be suppressing saprotrophs and may be one mechanism by which ECM trees become dominant in tropical rain forests.

11.9 A strategy for restoring endangered/endemic species in a dolomitic shrub-land from Sierra de Baza Natural Park (Granada, Spain) based on using AM

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Recovery of endangered and endemic flora is crucial not only for biodiversity conservation *per se* but also for the restoration of the natural habitats supporting such a singular type of vegetation. The *in situ* and *ex situ* conservation of this flora has a number of eco-physiological constraints which prevent both auto-regeneration of these species in their natural habitats and their rooting and multiplication under nursery conditions. It has been postulated that optimization of the mycorrhizal status of these plants is one of such constraints. An endangered community of "dolomitic endemisms" characteristic of the "Sierra de Baza" Natural Park was selected to investigate the impact of mycorrhizas on its restoration. The target species, which include *Jurinea pinnata*, *Thymus granatensis*, *Centaurea boisieri*, *C. granatensis* and *Pterocefalus spatulatus*, scarcely grow adapted to environmental stresses (low nutrients and organic matter, excess in Ca and Mg, and drought) characteristic of dolomitic soils. It was demonstrated that all of these species form AM and depend on AM to grow in the target soil. The diversity (through morphological and molecular criteria) of AM fungi present in the rhizosphere and roots of the target plants is being analysed. A bank of adapted autochthonous AM fungi is being established for restoration purposes. The tolerance/adaptation eco-physiological mechanisms of plants and their AM to the characteristic environmental stresses are being investigated.

11.10 Growth and root architecture responses of tall grass prairie plants to AMF and soil nutrients

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We tested the effects of arbuscular mycorrhizal fungi on the root architecture and plant biomass on tall grass prairie plant species. Ten plant species, including weeds, early, mid and late successional species, were grown in high and low fertility soils, with and without AMF. Late successional species are generally more responsive to mycorrhizal fungi than early successional species and exotic weeds. Weeds and early successional species generally had finer root systems than later successional species. Fine root architecture shifted in response to soil nutrient and mycorrhizal treatments and this shift also varied with plant species. This study has important implications for plant restoration because difficult to establish plants are more responsive to the AMF community.

11.11 Understanding the causes of rarity in plants: Is there a failure on cooperative strategies played by the mycorrhizal partners?

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The prisoner's dilemma game is used to investigate how cooperation between unrelated individuals can evolve by natural selection. In this game, defectors always out-compete cooperative individuals when encounters are random. However, cooperative strategies can be enhanced if multiple encounters with the same partner are allowed and if current behavior is based on past experience. Cooperative individuals interact with one another more often than they would purely by chance. Although cooperative behavior may benefit the group it is also inherently open to exploitation by cheaters who achieve greater reproductive success than other members of the group by enjoying the benefits of cooperation without paying the associated costs. Based on this theory, the project aims to evaluate the hypothesis that in the case of rare plant species, cooperative behaviors (with mycorrhizal and bacterial partners) capable of buffering environmental shifts at the habitat scale are getting less effective because of cheaters among the community. By favoring the survival of the individual rather than the population, cheaters regulate/decrease the density of those plant populations. Five species from the Portuguese rare and endangered plant list have been chosen. A first evaluation on their microbial partners was made. Next, an experimental approach will be proposed where plants are endowed with either a limited set of microbial counterparts, abiotic edaphic conditions or an interaction of both factors.

11.12 Land use and diversity of arbuscular mycorrhizal fungi in Mexican tropical rain forest

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At Los Tuxtlas, Veracruz, Mexico tropical rain forest has been reduced to 40,000 ha during the last 30 years, which represent 10% of the original forest, and its main cause is the land use changes (agricultural practices and livestock farming). This deforestation modifies environmental conditions and reduces biodiversity; this has been reported for animals and plants. However, the same behavior is expected for Arbuscular Mycorrhizal Fungi (AMF). The aim of this study was to determine how AMF spores diversity is affected by changes in land use. Three field sites were selected at Los Tuxtlas Biosphere Reserve according to their cover vegetation percentage. In each of these, 32 soil samples were collected distributed among the land uses considered (tropical rain forest, agroforestry, pasture and maize culture). AMF spores were isolated, morphologically identified, and counted. More than 50 species were found. There were significant differences for spore abundance between sites and land uses; for species richness; significant differences only were found for sites. The site with the medium cover vegetation percentage and the maize culture had the highest diversity. Cover vegetation percentage determines changes in AMF diversity at regional level, and land uses determine changes in spore's abundance at local level.

SESSION 12

Narrowing the gap between theory and practice in mycorrhizal management

Knowns’ and ‘unknowns’ in mycorrhizal management

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Management involves the manipulation of one or more factors for the purpose of acquiring a particular outcome. Increasingly, managers involved with restoring ecosystems or in agricultural production are looking for ways to manipulate mycorrhizal symbioses within their management programs. Unfortunately, the promise of mycorrhizal benefits can be unmet because of insufficient understanding of the circumstances and mechanisms that control mycorrhizal function in managed and natural ecosystems. The purpose of this talk is (i) to examine the factors that need to be considered when managers select management programs for manipulation of mycorrhizas, (ii) to explore what is currently ‘known’ and ‘unknown’ about mechanisms of mycorrhizal function under field conditions, and (iii) to identify practical solutions to overcoming gaps in knowledge for enhancing mycorrhizal benefits at a field-scale.

Using models grounded in stoichiometry to predict plant-mycorrhizal ecology across scales

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Mycorrhizal function and the impact of plants and mycorrhizal fungi on each other vary widely over space and time, and we have no general predictive model for this variation. A variety of models exist, but they tend to predict mycorrhizal dynamics at different scales. Effectively linking models at multiple scales would allow more effective prediction of mycorrhizal dynamics and their consequences. Stoichiometric variables have been demonstrated to have predictive power in other systems, and may also be incorporated into modeling approaches at multiple scales. For example, comparative advantage biological market models utilize stoichiometric ratios to predict the degree of beneficial resource exchange between different pairs of plants and mycorrhizal fungi. Agent-based models can also integrate stoichiometric relationships to explore complex interactions between plants and mycorrhizal fungi predicting patterns of nonlinear dynamics in mycorrhizal communities. Though we have knowledge of stoichiometric resource ratios in some mycorrhizal fungi and their host plants, predictive modeling will be facilitated by datasets in which stoichiometry is measured simultaneously with numerous other traits of multiple species in diverse communities. Only with a thorough understanding of tradeoffs among traits across species, and predictive modeling approaches that incorporate these traits, can we bridge the gap between theory and practice in our understanding of mycorrhizal dynamics.

Arbuscular Mycorrhizal Fungi as a key factor for restoration model of humid forest

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Using ecological restoration is possible to diminish deforestation effects if we focus on ecological processes; just a few studies have considered this point of view. This approximation allows the return to natural plant community and avoiding land deterioration. After 15 years of studies on Arbuscular Mycorrhizal Fungi (AMF) ecology in tropical humid forest in Mexico, a model is proposed with the general principles for restoration of the tropical rain forest using AMF. The model is based on different species responses to native AMF, on microenvironmental conditions (biotic and abiotic), and on plant life history traits. For ecological restoration, we suggest using: 1) plant species according their role in succession (pioneer and late species), 2) specific site conditions that limit species growth, 3) mycorrhizal dependence and, 4) the type of AMF inocula. To analyze plant growth, and survival, ecophysiological variables were considered such as Relative Growth Rate (RGR), Net Assimilation Rate (NAR), and also stem diameter, height and leaves number. AMF use clearly increases growth, and survival of pioneer species, whereas effects late species depend on each species. Results show that accurate restoration strategy of tropical humid forest needs to consider AMF.

Function of arbuscular mycorrhizas in revegetation process in volcanic deposit-affected lands

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Newly deposited volcanic materials are very low in nutrients available for plants and very susceptible to erosion. The revegetation process is very slow, so the acceleration of revegetation is required. Therefore, AM fungi are expected to be beneficial in the establishment of vegetation in such degraded lands. First, we examined the status of naturally occurring revegetation in the lahar (mud flow of volcanic deposit) area of Mt. Pinatubo, Philippines (Soil Sci. Plant Nutr. 50:1195-1203, 2004). Field observations and laboratory inoculation experiments suggest that a key of revegetation is proliferation of AM fungi, which is supported by the pioneer grass species that are not so AM-dependent. Second case is a revegetation project using AM inocula. In Mt. Unzen-Fugendake, Japan, large pyroclstic flows destroyed the previous vegetation on the mountain slope. The revegetataion materials containing plant seeds, AM fungal inocula and some carriers were applied from the air by a helicopter. The applied grass plants were highly colonized with AM fungi and contributed to rapid recovery of vegetation (Plant Soil 244:273-279, 2002). Furthermore, a strain specific molecular probe showed that the inoculated fungus was proliferating at the site, suggesting the effectiveness of AM inocula (Soil Sci. Plant Nutr. 51:125-128, 2005). These observations suggest the significance of AM fungi and their practical importance for revegetation in degraded lands.

Ecological risks *versus* benefits in AMF inoculum production for tropical agriculture

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Tropical soils are mostly acidic and low in phosphorus. These characteristics produce high responses to AMF inoculation in tropical soils. The growing demand of food in tropical countries and the increasing conscience about the negative environmental impact caused by the use of chemical fertilizers and agrochemicals are changing the public mentality in favor of sustainable agriculture. The aim of this work is to present the results obtained in the field with the use of commercial inoculants of AMF produced by substitution of the soil for light materials of local origin. Inocula of *Glomus manihotis*, *Scutellospora fulgida*, *S. heterogama*, *Acaulospora lacunosa* and *Entrophospora colombiana* are being produced, and the results of the field inoculation of lettuce and cassava are presented. An increased of 272 % in the fresh weight of lettuce was obtained when it was inoculated in an acidic soil and 50 % of chemical fertilizer was save when cassava was inoculated with *S. fulgida* in the east plains of Venezuela. However, not all the AMF are equivalent they differ in functional compatibility with both plant and soil, and depending of the ecological condition they can be parasitic and cause a growth depression of the host. These effect need to be anticipated and avoided when inocula are sold. A protocol to predict these effects is proposed and the necessity of adding ethical values to manage the inoculum production as a profitable business in the third world is discussed.

How to narrow gaps between scientific research and commercial application of mycorrhizal fungi products – a plea for support!

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Recently there has been a surge in interest in using mycorrhizal fungi (MF) products. The inoculum producers are usually those who are testing MF in the real world to show the benefits. So companies end up relying on their own studies and customer reports to support product sales and develop the market and hence widespread interest in the MF. The majority of scientists frequently claim that MF are useful and can be applied in plant production to achieve better plant growth, fitness and health in their papers or grant proposals yet rarely take an approach which advances their exploitation. This is often the result of pressures to publish and not necessarily the desire of the scientists. There have been excellent studies on various nutritional and non-nutritional effects of MF on plants, plant communities and the soil environment but rarely do they build what the MF businesses need i.e. to develop strong and defendable USPs (Unique Selling Points). The companies undertaking the production and selling of MF inocula would benefit if the scientific research base could help them explain to their customers the mechanisms behind the often observed MF effects. This is a never ending debate and the load is currently undertaken by businesses themselves. However do customers really care if there are not methods available to trace inoculated fungi in the roots of treated plants? No they don't! What they want to see is the product doing what it says on the packet via the MF placed within.

12.1 Effects of mycorrhizal fungi on mobility of P under leaching of repacked columns of a loamy sand soil in saline conditions

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Two experiments with repacked columns of a loamy sand soil were carried out to investigate the effects of arbuscular mycorrhizal (AM) fungal symbiosis on mobility of phosphorus (P) during leaching in saline conditions. *Trifolium subterraneum* plants inoculated with an AM fungus or not inoculated were grown in cores with low or high P concentrations at two salinity levels for 8 weeks in the glasshouse. Cores were then irrigated with 2500 mL water and the leachate collected. Plant growth and the amounts of P removed by plants, remaining in soil as available P and removed in leachate were measured. AM fungal colonization and development of external hyphae were also determined. Increased salinity decreased plant growth, external hyphae and increased the volume of leachate and dissolved P in leachate. AM fungal colonization significantly increased plant growth and P uptake and decreased soil available P and total dissolved P in leachates in saline conditions. Lower P leaching from cores with AM plants under saline conditions was related to enhancement of plant growth and to scavenging and removal of P from the soil by roots and/or external hyphae. When P was applied AM effects were not observed and available P remaining in the soil after leaching was much higher, regardless of AM fungal colonization.

12.2 Morphological and physiological reaction of micropropagated blueberry cv. 'Bluecrop' to fungi isolated from natural sites in Poland

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Blueberry are propagated by *in vitro* technique or traditionally by cuttings in soilless substrate. In both cases the plants are free from any microorganisms. Thus, introducing of symbiotic fungi can be beneficial for plants cultivated in sustainable system. The objective of this study was to evaluate reaction of micropropagated blueberry cv. 'Bluecrop' to fungi isolated from roots of plants belonging to Ericaceae and growing in natural ecosystems. Response of plants to particular inoculum was assessed by morphological characterisation of canopy and measurements of photosynthetic activity using chlorophyll a fluorescence method. Other measurements as biomass production, chlorophyll and water content and leaf area were done. The data were collected during first two years after removing the plants from in vitro conditions. Among investigated inocula, two of them significantly stimulated and one moderately inhibited plant development. The inoculum which inhibited growth and another one which stimulated development of plants, increased photosynthetic activity but in different "places" of light phase of photosynthetic process. We speculate that inoculum inhibiting vegetative growth could be used for micropropagated plants which are growing vigorously and therefore their yielding is delayed. The inocula which stimulate the shoot growth could be suitable for plants propagated traditionally by cuttings.

12.3 The presence of arbuscular mycorrhiza (*Glomus intraradices*) in strawberry plants modifies the spectral response

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Strawberry is one of the most profitable agricultural crops in Michoacán (Mexico). To achieve the maximum profit per hectare, excessive nitrogen and water are used. In this context, inoculation of the strawberry plants with arbuscular mycorrhizal fungi would allow reducing these inputs. Mycorrhiza induces changes in plants metabolism and development which modify plant photosynthesis and leaf properties. The hypothesis is that these modifications may be detected through the leaves spectral response. In order to test this idea, several vegetation indices were calculated from the leaf spectrum of plants cultivated with three levels of nitrogen fertilization (0.03, 3 and 18 mM), at four sampling dates. The analysis of variance shows a significant effect of nitrogen supply on leaf nitrogen content, chlorophyll content, plant biomass and leaf area, in all sampling dates. Inoculation with arbuscular mycorrhizal fungi produced a significant effect on chlorophyll b content and plant biomass. Most of the calculated vegetation indices were sensitive to nitrogen supply, but only the green reflectance, MTCI (MERIS Terrestrial Chlorophyll Index), REP (Red Edge Position), (Chl)RIgreen and (Chl)RIREP presented a significant variation associated to inoculation, mainly in the first sampling date. These indices are calculated using the wavelengths in the green (550 nm) and in the transition from red to near-infrared (700-720 nm) bands, and are very sensitive to the chlorophyll concentration.

12.4 Application of mycorrhiza in plant production systems – a book project

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Mycorrhiza application is often not considered due to the fact that customers have limited knowledge about the beneficial impact the symbioses can have on plant growth, health and quality. To narrow the gap between science and application a book project covering all aspects necessary for a broader dissemination of knowledge about mycorrhiza is planned. An overview about different mycorrhiza symbioses, such as endo- and ecto-, ericoid and orchid mycorrhiza is given. The main part will contain a detailed presentation of the numerous fields of application as well as possibilities and limitations. As each field has its different needs of mycorrhizal fungi, carrier materials, product design etc. it is important to show the achieved progress of the last years. In general and in detail practical application examples in agriculture, garden- and landscaping, plant production, urban horticulture, nursery, afforestation, reclamation, roof greening and hobby gardening are shown. The editor of the book, Dr. Carolin Grotkass, produces commercial inoculum with her company INOQ GmbH. Carolin Grotkass is coordinator of the German Committee for Application of Mycorrhiza and member of the Federation of European Mycorrhiza Fungi Inoculum Producers (www.femfip.com). The author, Dr. Thomas Fester, is scientist at the Leibniz Institut for Plant Biochemistry. Besides his scientific work he publishes popular scientific media concerning also mycorrhiza (CD "Mycorrhiza", www.ipb-halle.de/myk).

12.5 Morphological and photosynthetic reaction of micropropagated cherry rootstock 'Gisela 5' to mycorrhization with AMF

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Cherry rootstock 'Gisela 5" is widely used in Europe. This rootstock is propagated by *in vitro* technique. However, obtained plants show high variability in their growth and it is very difficult to overcome. The objective of this study was to introduce mycorrhization to protocol of micropropagation 'Gisela 5' to enhance quality of plants and to improve their acclimatisation to *ex vitro* conditions. The initiation and proliferation of cultures were conducted according to the method used in Laboratory of Tissue Culture in Brzezna. Rhizogenesis was carried out in vitro on MS medium containing IBA or without any growth regulators. At the end of rooting stage, both rooted plantlets and unrooted shoots were planted into multiplates filled with peat-light sand and perlite. Half of plantlets received different AMF inocula. After acclimatisation, the plantlets were transferred to the pots filled with peat and perlite and they were placed in greenhouse. The part of plantlets, not mycorrhized before acclimatisation, received AMF. Reaction of plants to different AMF inocula was determined morphologically and by assessment of photosynthetic activity using chlorophyll fluorescence method. Chlorophyll fluorescence parameters showed that photochemical activity of photosynthetic process was the highest for the plantlets inoculated at later stage of their development (after acclimatisation). Also morphologically this group of plants was higher evaluated.

12.6 Response of a local tomato cultivar from the Canary Islands to mycorrhizal colonization under different levels of phosphorus fertilization

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The effect of two AM fungi on plant growth and nutrition under three different levels of phosphorus fertilization was studied, using a local tomato cultivar, Canary Islands ('Manzano negro') under greenhouse conditions. Tomato seeds were established in 1L pots filled with a water steam sterilised substrate mixture of soil, volcanic ash and peat (2:2:1 v/v). *Glomus mosseae* or *G. intraradices* inocula (5 mL/pot) were applied in the moment of seeds establishment in pots. Three weeks after inoculation, a fertilization programme based on Hewitt nutritive solution (Hewitt, 1952) was started. Three different phosphorus (PO_4H_2K) doses were applied: $P_0 = 0.2$mM, $P_1 = 2$mM and $P_2 = 20$mM. Harvests were performed 6, 8 and 11 weeks from the beginning of the experiment and 10 plants per treatment were analysed at each date. In general, both AMF were able to increase plant growth at all P doses and harvests. At the end of the experiment these plants were even less developed than non-inoculated control plants. Highest mycorrhizal dependency values were registered in plants inoculated with *G. mosseae* and fertilized with a medium phosphorus level. Mycorrhizal colonization was higher in plants inoculated with *G. intraradices*. This fungal isolate seems to be less sensitive to different phosphorus doses. Expression of mycorrhiza-specific phosphate transporter- and invertase-encoding genes was assessed to determine functionality of the symbiosis in the plants of the different treatments.

12.7 Establishment of new productive areas of *Terfezia* species in midland of Portugal

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In "Beira Interior Sul" region of Portugal one of the must collected hypogeous fungi is *Terfezia arenaria* (Moris) Trappe occurring in mycorrhizal association with an annual plant *Xolantha guttata* (L.) Raf.. Macroscopic and microscopic characterization of *X. guttata* roots collected from natural productive and non productive spots showed mycorrhizal association. In productive areas the majority of plants presented typical ectomycorrhiza, although some ectomycorrhiza without sheath were detected. On the contrary all roots observed from non productive areas showed ectomycorrhiza without sheath. In order to create the basis for further establishment of new productive areas, experimental plots were set in non productive areas. Mature *Terfezia* spp. ascocarps were collected, cleaned, dried and triturated for inoculum production. At the end of vegetative season *X. guttata* seeds were also collected and sowed on two different areas with and without mixing with dried ascocarps. Six months after plants germination ectomycorrhiza were detected in both areas. During second year, natural regeneration of *X. guttata* was allowed. Although no ascocarps production was observed the presence of ectomycorrhiza was confirmed. Attending to knowledge of local people more than two years will be needed to restore *Terfezia* spp. production after plants destruction. This work was supported by programme AGRO 8.1, project 449 "Establishment of mushroom and toadstool new areas in midland of Portugal".

12.8 Clay impact on arbuscular mycorrhizae and plant growth

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Data are few to relate arbuscular mycorrhizae to soil texture. However, quantity and type of clay both affect nutrient availability, and so they should modify the function of the symbiosis. A field study and a greenhouse experiment were conducted to evaluate the impact of clay quantity in soil on plant growth and mycorrhizal colonization. Clay contents ranged from 3-28%. Clay mineralogy was consistent throughout. Naturally occurring *Aster laevis* had reduced growth in field soils of elevated clay, yet the end-of-season colonization was similar among plants: overall means +/- s.d. were 9 +/- 6% and 58 +/- 12% for the percentages of root length colonized by arbuscules and hyphae, respectively. Soil clay content in the field was correlated significantly with soil pH, organic C, and available K. Mixtures of the same clay material with sand in various ratios were inoculated with arbuscular mycorrhizal fungi under greenhouse conditions using transplants of *Plantago major* from a field loam. *Sorghum* raised from seed following removal of *Plantago* had significantly different root growth but similar shoot growth among soil mixes. Arbuscular colonization for *Sorghum* was also similar among treatments: overall mean +/- s.d. = 25 +/- 15%. We conclude that development of arbuscular mycorrhizae was similar across the range of soil clay contents tested even though plant growth was modified by soil variables associated with differences in texture.

12.9 Molecular identification of the edible ectomycorrhizal fungus *Lactarius deliciosus* in the symbiotic and extraradical mycelium stages

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Specific rDNA ITS amplifications, microsatellite-primed PCR and ITS-SSCP analysis were applied to identify and characterize pre-selected isolates of the edible ectomycorrhizal fungus *Lactarius deliciosus* in different stages of the life cycle. Sampling was performed from pure cultures, mycorrhizas and soil from experimental plots established with inoculated pine seedlings. A newly-designed reverse primer (LDITS2R) combined with the universal forward ITS1 allowed to perform specific amplifications of *L. deliciosus* from all the samples. Microsatellite-primed PCR using the $(GTG)_5$ oligonucleotide as a primer showed clear polymorphisms among the different *L. deliciosus* isolates. The patterns of mycorrhiza samples showed additional bands corresponding to the plant DNA. Single strand conformation polymorphism (SSCP) analysis of the specific rDNA ITS fragment amplified from 18 *L. deliciosus* isolates showed nine clearly different patterns. Mycorrhiza and soil samples showed coincident patterns with their respective fungal isolates. Specific rDNA ITS amplifications had not been previously used for SSCP analysis of ectomycorrhizas and extraradical mycelium. This relatively simple and inexpensive technique allows tracking *L. deliciosus* isolates in different stages of the fungus development. Specific ITS-SSCP analysis is promising in studies of the persistence of inoculated *L. deliciosus* isolates and their competitiveness with native ectomycorrhizal fungi.

12.10 Arbuscular mycorrhiza and nitrogen speciation influence the phenolic content, antioxidative activity, and organosulfur compounds in *Allium* species

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Phenolic and organosulfur compounds in onions are beneficial for human health. Therefore the aim of our study was to investigate whether arbuscular mycorrhizal [AM] colonisation or nitrogen speciation affect phenolic content, antioxidative activity, and organosulfur compounds of *Allium* species. *Allium cepa* 'Centurio', *A. fistulosum* 'Kaigaro', and *A. tuberosum* were grown on perlite and supplied with nutrient solution. The plants were either inoculated with AM inoculum (TerraVital Hortimix, Plantworks Ltd., UK) or remained mycorrhizal free. They received different ratios of nitrate (NO_3^-) and ammonium (NH_4^+) (95:5, 50:50 or 25:75). The mature plants were harvested for determination of dry weight, organosulfur compounds (measured as pyruvic acid), polyphenol content (Folin-Ciocalteau assay), and antioxidative activity, using electron spin resonance spectrometry and trolox equivalent antioxidant capacity assay. Mycorrhization significantly increased antioxidative activity and thus the ability of scavenging radicals, in tissue of *A. cepa* at dominant NO_3^- supply. Moreover, dominant NO_3^- supply increased the antioxidative activity in *A. cepa* compared to dominant NH_4^+ supply. In *A. fistulosum*, mycorrhization had no influence on the antioxidative activity. In this species, the polyphenol content was increased by mycorrhization at dominant NO_3^- supply. Yield (content) of organosulfur compounds was increased in mycorrhizal *A. tuberosum* due to higher shoot dry weight upon mycorrhization.

12.11 Finding your niche in mycorrhizal research

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Collaborative research between disciplines has become increasingly important in mycorrhizal research. This emphasis can be intimidating to new scientists since they have to develop a broad understanding of their own discipline as well as recognize opportunities outside of that discipline. To aid in this, our goal is to objectively characterize current disciplines within mycorrhizal research and to identify linkages among disciplines where collaboration is lacking. We analyzed patterns of keyword usage in the mycorrhizal literature using publicly available software designed for analyzing social networks. Analysis of articles published in 2005 revealed several clusters based on the frequency with which keywords co-occur within records, including those based on soil factors ("aggregate stability", "glomalin", "soil carbon"), plant physiology ("drought", "photosynthesis", "water use efficiency"), and ecology ("competition", "facilitation", "common mycorrhizal networks"). One prediction was that the presence of redundant keywords would hinder this process (e.g., we identified more than ten synonyms for "arbuscular mycorrhizal fungi"); however, preliminary analyses suggested that this method is robust. New scientists could use this technique, in conjunction with advice from mentors, to identify areas for collaboration outside of their discipline and to choose combinations of keywords that allow them to fully explore the literature.

12.12 Implementation of arbuscular mycorrhizas in conventional production of greenhouse grown vegetables

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Arbuscular mycorrhizas (AM) have been shown to increase plant health with respect to growth, nutritional status and tolerance to pathogens. Plants in horticultural greenhouse production systems are often grown in growth medium, where AM fungi are absent. In this project, the objective was to study if implementation of AM in tomato and cucumber grown in rock wool with circulating nutrient solutions is relevant and possible. Initially, commercial AM products were tested for plant root colonisation of different cultivars of cucumber and tomato grown at different levels of P in the nutrient solution. In general, the results showed that growth of cucumber was unaffected by a 50% reduction of P in the recommended nutrient solution (to 20 ppm), and that plants were 10-15% colonised with AM after four weeks, which is the approximate time for transplanting. On the other hand, tomato plants was only colonised at very low P levels, where the plant had unacceptable growth for commercial production. Experiments in three commercial cucumber nurseries were conducted to evaluate the effect of AM fungi on plant growth and yield of cucumber under commercial conditions in two production cycles. AM increased yield in the up to 6.8 % in some experiments and had no influence of yields in others. So far, it seems relevant and possible to implement AM in production of greenhouse grown cucumber, whereas it does not seem to be relevant in tomato in these production systems.

12.13 Long-term effects of arbuscular mycorrhizal inoculation at the nursery stage on field growth of camellias

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Many studies have shown that AM fungal inoculation benefits growth of different cultivated plants in the short term, but few have dealt with the continuity of this effect in the long term. The aim of this study was to evaluate the effects of nursery inoculation with a selected AM fungus on field performance of three cultivars of *Camellia japonica* (`Lady Clare`, `Paolina Guichardini`, and `Compacta Alba`) and one hybrid of *C. japonica x saluenensis* (`E.G. Waterhouse`) during three years. Cuttings of all four *Camellia* material were rooted in nursery beds either with or without *Glomus aggregatum*, then transplanted to pots filled with peat:perlite and kept for one year in the greenhouse. In the spring of 2002, healthy well-rooted plants were transplanted to the field under two fertilization treatments: an unfertilized control and application of an slow-release N-P-K + Mg fertilizer. Roots of non-inoculated *Camellia* plants were extensively colonised by the indigenous AM fungal population. Nursery AM inoculation resulted in improved height, stem diameter, and number of shoots and leaves especially of `Paolina Guichardini` and `Compacta Alba` cultivars both in the unfertilized and fertilized plots, these effects persisting after three years of growth. No differences were observed for AM-inoculated and noninoculated `Lady Clare` plants.

12.14 Responses of mycorrhized strawberry plants to *Verticillium dahliae*

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Verticillium spp. cause wilt of many plant species. This pathogen invades the plant vascular system, blocking transport of water and nutrients. Far-reaching plane of our study is to investigate the mechanisms of mycorrhizal fungi (endo-AMF) activity in enhancing strawberry resistance/tolerance against *Verticillium dahliae*. In the present study, we focused on water status and photosynthetic activity of mycorrhized plants infected with *Verticillium dahliae*. Experiments were performed on cvs 'Elsanta' and 'Senga Sengana', differing in their susceptibility to *Verticillium dahliae*. At time of intensive growth, the plants were mycorrhized and one week later infected with *Verticillium dahliae*. Symptoms of disease were diminished by AMF. Infection with *Verticillium dahliae* decreased water potential for 'Elsanta' in higher degree than 'Senga Sengana'. Mycorrhization counteracts pathogen's activity, increasing water potential. Stomatal resistance decreased and as a direct result transpiration increased in presence of fungi, both pathogenic and beneficial. Photochemical activity of 'Senga Sengana' was little influenced by both types of fungi. Whereas *Verticillium dahliae* decreased drastically and AMF increased photochemical activity of 'Elsanta'. Quenching analysis showed high sensitivity of 'Elsanta' to infection and key role of AMF in recovery of proper ratio among particular forms of quenching.

12.15 Practical application of VA-Mycorrhiza for recultivation and reforestation

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Revegetation of eroded and/or heavy metal polluted soils are thought to be promising areas where AMF are likely to be used in practice. We have used the AMF-inoculation technique very successfully at three field sites: • Waste dump of a copper-mine in Poland • Steep hillside in Hungary • Waste dump extremely heavy metal contaminated (Germany) The used method is a combination from AMF inoculum, a special mixture of grasses, mechanical fixation (net, adhesive, wood fibers), and organic slow-release fertilizer. At the reforestation of problem-locations young plants of Acer platanoides, A. pseudoplatanus, Carpinus betulus, Quercus robur, Q. petraea were inoculated with an inoculum of Glomus intraradices (fixed on expanded clay or by dipping in suspension). We have found an increase growth in the first two years, a diameter increment already in the 3rd year and a mortality rate les as 5% in despite of strong drought stress. From the third year it seems that the effectiveness of ectomycorrhizal fungi surpassed that of AMF.

12.16 Competitive arbuscular mycorrhizal fungi for inoculation of papaya (*Carica papaya*, L.)

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In search of arbuscular mycorrhizal fungi (AMF) that may be used in inoculation programs it must be efficient in conditions of competition with indigenous fungi. Two experiments with papaya, Tainung nº1 variety were conducted in greenhouse conditions at National Center of Research of Cassava and Tropical Fruits-Embrapa, Brazil, aiming to evaluate competitive capacity of selected fungi, using simultaneous inoculation with augmented native populations. It was used a Yellow Oxisol fertilized with 30 mg dm^{-3} P. In both experiments, different ratios of spores of selected fungi and native populations were used: 0/0%, 0/100%, 25/75%, 50/50%, 75/25% and 100/0%, in percentage of a total of 200 spores per plant. Inoculum was composed by spores in the first experiment, and soil, in the second. Plants were cultured for 75 days after transplanting, when shoot dry weigth, nutrient contents and total and qualitative colonization were evaluated. *Gigaspora margarita* was more competitive and efficient than population nº 35, which contained *Acaulospora mellea* as predominant species. But this changed when *G. margarita* was put together with populations dominated by spores of *G. etunicatum*. A native isolate of *Gigaspora*, obtained from papaya plantings was more competitive than the exotic *G. margarita*, resulting in a higher efficiency. In conclusion, fungi previously selected in fumigated soil were efficient in conditions of competition, but this depended on the competitive population.

12.17 Mycorrhiza in The Netherlands: A changing environment

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Horticulture is traditionally an important sector of the Dutch economy. A high degree of intensification en optimisation makes Dutch horticulture very competitive. So far research and technical support have focused on high-input production systems and there has been little strategic research on the application of mycorrhizas. A few attempts to use mycorrhizas in such systems were doomed to fail. Under the growing pressure to develop more sustainable, alternative plant production systems, mycorrhizas are gaining importance as part of an integrated culture system with lower input of fertilizers, pesticides and irrigation and more organic soil amendments and bioagents. Also in the landscaping sector there is growing interest and awareness to develop sustainable methods. However, in spite of this changing environment the potential benefits of mycorrhizas are still largely unknown in The Netherlands or the application of mycorrhiza is approached with scepticism. Servaplant B.V., a new Dutch company, is aiming at bridging the gap between theory and practice in mycorrhiza management by providing a range of good quality European mycorrhiza inoculants with adapted strains for specific applications combined with competent technical support for growers and landscapers. We contribute to strategic research on practical implementation under Dutch conditions and support the distribution of knowledge under (potential) users of mycorrhizas.

12.18 Altering C:N:P ratios to assess mycorrhizal function

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Arbuscular mycorrhizae are ubiquitous symbioses between plant roots and specialized fungi. Previous research indicates that resource availability (specifically soil P, soil N, and light) are important controllers of the mutualistic function of arbuscular mycorrhizal (AM) symbioses. Global changes such as increases in atmospheric CO2 and anthropogenic N deposition, pollution, land use change, introduction of exotic species are known to alter the relative abundances of C:N:P; however the ramifications of these impacts on AM symbiosis are unknown. In this study, the native tallgrass prairie grass, big bluestem, was grown under varying C:N:P ratios, with and without mycorrhizal symbiosis. Carbon was altered by growing the plants under ambient light, or 30% and 60% shading. Soil N and P ratios were altered by using soils from three different sites that naturally varied in N:P ratios. Seedlings planted in low P soil failed to grown without the symbiosis, regardless of C or N. Moderate levels of soil P allowed for growth of nonmycorrhizal seedlings, but these were smaller than their mycorrhizal counterparts. Photosynthetic rates of mycorrhizal plants were higher than nonmycorrhizal counterparts, however, shading reduced both photosynthetic rate and growth of mycorrhizal plants at all N:P ratios. In this study, mycorrhizal symbiosis was beneficial to big bluestem seedlings regardless of C:N:P ratios.

SESSION 13

Mycorrhizal networking

Vegetative compatibility, self recognition and nonself incompatibility in arbuscular mycorrhizal networks

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Arbuscular mycorrhizal (AM) fungi have a wide host range, which allows them to colonize and interconnect plants of different species, genera and families, by means of hyphae extending from one root system to another. Such AM networks, first visualized and quantified by means of two-dimensional experimental systems, are able to spread from colonized roots into the surrounding environment at growth rates ranging from 738 to 1067 mm per day, depending on the host plant, and reach hyphal lengths of 10-40 mm per mm of root length. AM networks may be highly interconnected by hyphal fusions (anastomoses), whose formation depends on a highly regulated mechanism of self recognition between compatible mycelia (1). By contrast, AM mycelia of individuals belonging to different genera and species, and to geographically different isolates of the same species, are unable to fuse, and show rejection responses, such as cytoplasm withdrawl, septa formation, vacuolization, lysis or cell wall depositions (2). Here, recent results which evidenced AM fungal ability to discriminate self from non-self and opened the way to genetic tests on vegetative compatibility/incompatibility in AM fungi will be discussed. (1) Giovannetti M. et al. (2004). Patterns of belowground plant interconnections established by means of arbuscular mycorrhizal networks. New Phytologist 164:175-181. (2) Giovannetti M. et al. (2006). At the root of the wood wide web. Plant Signaling and Behavior 1: 1-5.

Extraradical arbuscular mycorrhizal fungi development in different soil environments

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Arbuscular mycorrhizal fungi (AMF) are soil organisms that may have requirements of their own. The influence of the soil environment on extraradical AMF (AMF$_{\text{ext}}$) development, as indicated by the abundance of the PLFA 16:1ˆ5 extracted from soil, was sought within 83 asparagus-planted soils of 4 Québec (Canada) regions located along a climatic gradient, and in a long term experiment in which different N, P, and C input treatments had been applied to wheat for 37 years in southwestern Saskatchewan (Canada). Discriminant analyses based on soil chemical and physical descriptors revealed asparagus plantation soils differing with regions and distinct wheat-growing soils that received different N, P, and C inputs. Soil microbial community structure varied with regions in the asparagus experiment and with N, P, and C input treatments in the wheat experiment. PLFA 16:1ˆ5 had a large weight in microbial community discrimination in both experiments. PLFA 16:1ˆ5 was rare in northern asparagus fields, fair in the intermediate region and similarly abundant in both southern regions, and increased with increasing soil P. Under wheat, low PLFA 16:1ˆ5 and high NLFA 16:1ˆ5 abundance under conditions of low available N suggested accumulation of reserve C and limited extraradical development under condition of N scarcity. AMF$_{\text{ext}}$ development was unrelated to root colonization. We conclude that AMF$_{\text{ext}}$development is influenced by soil conditions and can be enhanced by nutrient availability.

Spatial pattern and control of arbuscular mycorrhizal fungal community diversity in an old-field meadow

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Experimental manipulations of the diversity of arbuscular mycorrhizal (AM) fungi have shown that changes in AM fungal diversity can influence plant diversity and productivity. Variability in the composition of these belowground communities over space could have important implications for the structure and function of aboveground communities. However, spatially explicit variation in AM fungal community composition has rarely been measured. Also, we know little about the factors that control local AM fungal diversity. We examined spatial heterogeneity of AM fungi in an old-field plant community. At a large-scale across a 50m X 50m grid, AM fungal spore species diversity over space ranged from 0-10 species. Distinct spatial patterns for 15 different AM fungal species were apparent. There was little change in spatial pattern for most species over a two-year interval. Although there were significant associations observed between specific AM fungal and plant species, there was no significant relationship between AM fungal diversity and plant diversity over space. At the level of the individual plant we found that AM fungal community assembly was largely constrained by their phylogeny. Maximum species richness (and maximum productivity) was achieved when AM fungal communities were phylogenetically overdispersed. These data show that AM fungi have spatially explicit patterns in the field, and that their life-history characteristics can influence local community composition.

Partial mycoheterotrophy - a novel nutritional mode of forest ground vegetation

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In the mycorrhizal symbiosis plants usually exchange photosynthates for mineral nutrients aquired by fungi from the soil. This mutualistic arrangement has been subverted by a few hundreds of mycoheterotrophic plant species that lack the ability to photosynthesize. The most numerous examples of this behaviour are found in the largest plant family, the Orchidaceae. The non-photosynthetic orchid species are known to be highly specialized exploiters of the ectomycorrhizal symbiosis of trees and have been shown to be distinguished in their C and N stable isotope natural abundance from co-occurring autotrophic vegetation in a similar way as ectomycorrhizal fungi are distinguished from co-occurring plants by their isotope signature. Despite of the occurrence of mycoheterotrophy among orchids, green representatives of the orchid family have been thought to be fully autotrophic. Recent stable C and N stable isotope abundance data, however, provide evidence that some of the putatively autotrophic orchids live partially at the cost of the fungal partners. This partial mycoheterotrophy is associated with a change of the fungal partner and explains how orchids can thrive into the deepest shade of forests. Furthermore, stable isotope abundance data provide evidence that the partially mycoheterotrophic nutrient gain among forest ground vegetation is not limited to representatives of the orchid family.

First evidence of mutualism in orchid mycorrhiza: Net carbon and nutrient fluxes between an orchid and its mycorrhizal mycelial network

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Orchids were amongst the first plants in which mycorrhizal associations were recognized to be beneficial, but the extent to which this symbiosis benefits the fungal partners is uncertain. A defining feature of orchid mycorrhiza is that during symbiotic germination and establishment of the tiny dust seeds of orchids there is net flux of both carbon (C) and nutrients from fungus-to-plant so the relationship appears not to be mutualistic. Most adult orchids develop green leaves and thus have potential to supply photosynthate in exchange for fungal-acquired mineral nutrients in a fully mutualistic relationship as seen in arbuscular, ecto and ericoid mycorrhiza. However, such mutualism has never been established for any orchid. Here we demonstrate in the green forest orchid *Goodyera repens*, using double labelled [^{13}C-^{15}N]glycine supplied to external mycelium and $^{14}CO_2$ supplied to the plant shoots, that a net flow of photosynthate-derived carbon from plant-to-fungus can occur in orchid mycorrhiza. In return for this carbon the fungus supplies nutrients taken up through the mycelial network. This study demonstrates for the first time (1) mutualism in orchid mycorrhiza, (2) bi-directional transfer of C between a green orchid and its fungal symbiont, and (3) a fungus-dependent pathway for organic N acquisition by an orchid. These findings suggest that the investment of fungal C in seedling establishment may be repaid with interest by adult orchid photosynthate.

The role of ectomycorrhizal fungal communities in the success or failure of tree establishment

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Seedlings of many trees require ectomycorrhiza to obtain nutrients and grow, but the fungi cannot grow without the presence of trees as a source of carbon. This presents a classic chicken and egg paradox. Since each partner requires the other, how do populations get established and spread? The key to resolving this paradox may lie in differences between the fungal communities of plants that are limited by a lack of mycorrhizal fungi with those that are not. I summarize the case of *Quercus* (oaks) in North America, where ectomycorrhizal fungi are a major factor limiting invasion into old fields. This contrasts with work from other groups showing *Pinus* (pines) can invade similar sites. I believe the difference lies in fungal communities of seedlings growing distant from trees. While *Quercus* have very low infection distant from trees, pines are able to become infected with pine-specific ECM fungi that are much less dispersal limited. I then move to New Zealand, where *Nothofagus* (southern-beech) is severely limited by a lack of mycorrhizal infection distant from trees, yet *Pinus* has become a successful invader in the same habitats. Again, the success of *Pinus* may be related to its mycorrhizal associates: belowground analysis shows little overlap between the mycorrhizal communities of *Nothofagus* and *Pinus*. Differences in fungal communities between these tree species may be key to understanding how some ectomycorrhizal trees are able establish as ecosystem pioneers.

13.1 Do common mycorrhizal networks facilitate conifer regeneration under environmental stress?

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Theory predicts plant-to-plant facilitation to be most important under high environmental stress. To date, however, research has focused on abiotic factors underlying mechanisms for this facilitation. In this study, we hypothesize that C and water transfer through common mycorrhizal networks from mature trees facilitates establishment of ectomycorrhizal conifer seedlings of the same subspecies under water-limited conditions. This will be tested along a climatic gradient in the field, where we will manipulate the presence of common mycorrhizal networks and distance from mature trees. Different pathways for water and C transfer will be controlled with different pore-sized mesh barriers in the field and in growth chambers. Temperature, moisture, and CO_2 concentration will be regulated in growth chambers, where labeling with $^{13}CO_2$ and D_2O will be used to detect C and water transfer. The effects of these various factors on growth and survival of Douglas-fir seedlings can be modeled for predicting climate change effects on regeneration of Douglas-fir in the drier portions of its range in western North America.

13.2 A new example of myco-heterotrophy within the genus *Pyrola*

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During the evolution of myco-heterotrophy plants have lost the ability to photosynthesize, reversed the normal flow of carbon in mycorrhizal interactions, and become specialized on relatively narrow lineages of fungal associates. However, it is not know in what sequence these events take place in independently evolved myco-heterotrophs. We are investigating two sister taxa *Pyrola picta* and *P. aphylla* (Ericaceae), which are photosynthetic and non-photosynthetic, to determine whether partial myco-heterotrophy and fungal specificity precede the loss of photosynthesis. From stable isotope analysis of ^{15}N and ^{13}C we have found that *P. picta* has access to the same nitrogen source as *P. aphylla*, which is distinct from other understory vegetation and most similar to that of fungi. Also, *P. aphylla* is enriched in ^{13}C compared to other understory plants, and has $\delta^{13}C$ values most similar to those of ectomycorrhizal fungi and other obligate myco-heterotrophs. Using DNA sequencing we have found that single *P. picta* plants associate with a broad range of ectomycorrhizal fungi including species of *Piloderma*, *Russula* and *Rhizopogon*. From these results we have found no fungal specificity in the closest living relative to *P. aphylla*. Our ongoing studies will determine whether *P. aphylla* associates with a narrow range of fungi and if under light limited conditions heterotrophic gain of both C and N increases in *P. picta*.

13.3 Inoculation with native AM fungi modifies intraclonal integration between ramet pairs of *Potentilla reptans* and *Fragaria vesca*

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Intraclonal physiological integration has been repeatedly demonstrated for stoloniferous or rhizomatous plants, which means that ramets subjected to advantageous conditions support the growth of ramets facing a more adverse environment. Surprisingly, the effect of arbuscular mycorrhizal (AM) fungi with their wide-spreading extraradical mycelium on the growth of clonal plants and intraclonal integration has received little attention. As model plants for the present study, two stoloniferous species coexisting at the same field site were selected, *Fragaria vesca* and *Potentilla reptans*. Pairs of their ramets were grown in compartmented rhizoboxes, each ramet rooted separately on one side. In half of the rhizoboxes, stolons connecting the ramets were left intact, whereas they were severed in the second half of the rhizoboxes. Different inoculation treatments were established and either parent or daughter ramet or alternatively both of them were inoculated with native AM fungi. A negative growth response of the plants to AM inoculation was observed and fewer stolons and ramets as well as lower shoot biomass were recorded in inoculated plants. The inoculation, particularly in the treatment where both rooting compartments were connected by the extraradical mycelium, significantly influenced intraclonal integration between parent and daughter ramets in favour of the latter ones. The study was financially supported by the Grant Agency of the Czech Republic, project No. 526/05/P063.

13.4 *Glomeraceae* and *Gigasporaceae* differ in belowground mycelia interconnections

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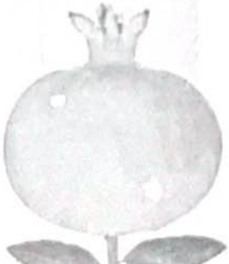
Arbuscular mycorrhizal (AM) fungi can interconnect plants by means of a common mycelial network. For *Glomus* strains anastomoses play a crucial role in the interconnection of extraradical mycelia and therefore on the formation of large networks. The question whether this mechanism also exists in *Gigasporaceae* strains remains unresolved and may have major implications to understand how AM fungi exploit new environments and substrates. An *in vitro* tri-compartmental culture system was developed to study the anastomosis formation in and between networks of the same AM fungal isolate of *Glomeraceae* and *Gigasporaceae* strains. In *Glomeraceae*, 14-21 % of the anastomoses were formed between hyphae of different networks. Anastomoses were mainly formed between different hyphae of the same network (78-87 %), rarely within the same hypha. In *Gigasporaceae*, no anastomoses were observed between hyphae belonging to different networks. Anastomoses were mainly formed in the same hypha (95-97 %) while 3-5 % were observed between different hyphae of the same network. *Glomeraceae* and *Gigasporaceae* drastically differ in their capacity to interconnect mycelial networks, suggesting divergent strategies for the fungal colonies to explore and exploit new environments and to create large networks interconnecting different plants in a community.

SESSION 14

Mycorrhizosphere interactions/biology



Effects of mycelial exuduates from an arbuscular mycorrhizal fungus on soil bacterial community composition *in vitro*

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Mycelial exudates may influence the activity and composition of soil microbial communities. We studied the influence of mycelial exudates of the arbuscular mycorrhizal (AM) fungus *Glomus* sp. MUCL 43205 on the composition of a soil bacterial community *in vitro*. Two-compartment systems of mycorrhizal and non-mycorrhizal root organ cultures were used to create compartments with or without the influence of extraradical mycelium of the AM fungus. Liquid medium from the hyphal compartments was incubated with a bacterial community. The chemical composition of exudates was analysed by HNMR. Initial and post-exposure bacterial communities were characterised at time intervals by TRFLP in combination with cloning and 16S rDNA sequencing of individual taxa. Bacterial growth and vitality was monitored using a vitality stain. Glucose, formiate and acetate were the main carbohydrates exuded by the mycelium. Exudates stimulated bacterial growth and changed the bacterial community composition. Two taxa related to *Legionella wadsworthii* and *Flavobacterium* sp., respectively, were detected only in samples with mycelial exudates. A taxon related to *Buttiauxella gaviniae*, significantly increased in response to mycelial exudates. In addition, a *Flavobacterium* sp. and an unknown proteobacterium were only detected in the absence of exudates. The relevance of extraradical mycelial exudates in relation to changes in soil bacterial communities is discussed.

Influence of *A. niger* fermented sugar beet waste on AM hyphal growth and P uptake, and on microbial populations in soil

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Sugar beet (SB) waste, a lignocellulosic residue, can be used as a soil amendment after static fermentation with *Aspergillus niger* in a rock phosphate (RP) supplied medium. The application of *A. niger* treated SB waste, in a soil-plant system, increased plant growth and nutrition, especially in mycorrhizal plants, as well as soil enzymatic activities. In order to assess the influence of SB waste on the growth and P uptake of AM fungi, plants were grown in compartmentalised growth units. Each unit consisted of a root compartment (RC) and two lateral root-free compartments (RFC). The RFCs were amended or not with sugar beet waste, either untreated (SB) or treated with A. niger (ASB). The soil in each pair of RFC was labelled with ^{33}P and ^{32}P and soil microorganisms were measured using biomarker fatty acids. Whilst ASB increased growth and activity of AM mycelium, there was no AM fungal growth in RFC amended with SB. Modification of soil microbial communities as a consequence of the addition of the amendments, and the different composition of ASB compared to SB (nutrient content and *A. niger* exudates) could possibly explain these results. Moreover, higher shoot P content was obtained in mycorrhizal plants amended with ASB in the RFC, as a result of the solubilization of rock phosphate by *A. niger* during the fermentation. In conclusion, SB waste fermented by *A. niger* facilitates AM hyphal growth and P uptake, however the underlying mechanism needs to be further examined.

Contribution of arbuscular mycorrhizal fungi to the bioremediation of PAH in the mycorrhizosphere

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AM fungi generally improve plant survival and growth in polluted soils, including plant tolerance towards heavy metals. For soils polluted with organic pollutants such as polycyclic aromatic hydrocarbons (PAH), interactions between the plant and its microbial communities in the rhizosphere control microbial degradation processes. AM fungi are not known to have direct biodegrading activities. However, in pot experiments with industrial soils and in compartment devices with spiked sand, the concentration of PAH increased with the distance to roots and was lower in the mycorrhizosphere than in the rhizosphere of nonmycorrhizal plants. *G. mosseae* increased PAH degrading bacterial populations and the expression of the naphthalene dioxygenase gene in the mycorrhizosphere of ryegrass. Therefore there is a potential for including AM fungi in bioremediation schemes. AMF may also improve plant establishment and growth in polluted soils after remediation treatment. However, in other studies, mycorrhizal fungi on the contrary had no effect on PAH degradation. Competition for water and mineral nutrients between roots and soil microorganisms, or modification of root exudation by mycorrhizal roots, may be limiting factors to biodegradation. Further studies should focus on effects of environmental parameters controlling PAH bioavailability and biodegradation (nutrient levels, root exudation or bacterial communities) and understanding the biodegradation processes in the mycorrhizosphere.

Mineral weathering - how important are ectomycorrhizal fungi?

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In boreal forests mineral weathering provides the main long-term buffer against soil acidification and serves as an important nutrient supply for the forest ecosystem. Ectomycorrhizal (EcM) fungi have been shown to accelerate mineral weathering, but it has proved difficult to quantify these effects. Here we describe three approaches we have used to quantify components of ectomycorrhiza weathering of minerals. In a dune soil chronosequence (0- 5000 years) we studied the contribution of fungal tunnelling through feldspar grains to feldspar weathering. Assuming that the tunnels were created entirely by EcM fungi, this component of mycorrhiza-weathering accounts for less than 1% of total feldspar weathering. Next, we modelled the effect of EcM oxalate exudation based on fungal distribution over the main minerals in the soil chronosequence. Oxalate exudation was estimated to contribute 2% to total K-feldspar weathering and 4% to Na/Ca-feldspar weathering. Proton release associated with organic acids will have negligible additional effects on weathering. In the final study, we supplied either muscovite or hornblende to a pot system with EcM *Pinus sylvestris*. The *Paxillus involutus* mycorrhizas nearly doubled K-release from muscovite, compared to *Suillus bovinus, Piloderma croceum* and non mycorrhizal controls, but there were no effects on hornblende. Our results suggest that the importance of EcM in weathering of minerals is species, mineral and context-dependent.

Mechanisms behind mycorrhiza helper effect exerted by *Streptomyces laceyi* AcH 505

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The mycorrhiza helper bacterium *Streptomyces laceyi* AcH 505 promotes extension of *Amanita muscaria* hyphae and mycorrhiza establishment, but suppresses the growth of *Hebeloma cylindrosporum*. Using co-culture techniques, microscopy, gene expression analyses (suppressive subtractive hybridisation), and HPLC separation of bacterial exudates, we could show that AcH 505 accelerated fungal growth by interacting with the actin cytoskeleton and by modulating fungal gene expression (signalling pathways, metabolism, cell structure, cell growth). These effects could in part be related to bacterial substances. The fungal growth promoting substance, auxofuran, caused increased lipid-metabolism-related gene expression in *A. muscaria*. Co-cultivation of strain AcH 505 with *A. muscaria* stimulated the auxofuran production. The growth-inhibiting effect with regard to *H. cylindrosporum* could be related to the antibiotics WS-5995 B and C, substances that caused cell stress-related gene expression in *A. muscaria*. We have recently observed that AcH 505 also improves Norway spruce colonisation by the plant pathogenic fungus *Heterobasidion abietinum* 331, a fungal isolate whose growth is not affected by AcH 505. We will present evidence that the streptomycete AcH 505 assists spruce colonisation by *H. abietinum* by suppressing plant resistance. This indicates that both improved hyphal extension and assisted colonisation result in the mycorrhiza helper effect.

Diversity and expression of nitrogenase genes (*nifH*) from ectomycorrhizas of *Corsican pine* (*Pinus nigra*)

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The diversity of bacterial nitrogenase genes (*nifH*) and their mRNA transcription in ectomycorrhizas of Corsican pine (*Pinus nigra*) were examined. DNA and RNA were extracted from surface-sterilised and non-sterilised Corsican pine roots colonised by the ectomycorrhizal (ECM) fungi, *Suillus variegatus* and *Tomentellopsis submollis*. DNA-derived *nifH* PCR products were obtained from all samples, but only a few RT-PCRs for *nifH* mRNA were successful, suggesting that nitrogenase genes were not always transcribed. Several different *nifH* sequences were detected and the bacteria actively transcribing *nifH* were different from those whose genes were detected through DNA-based PCR. Putative nitrogenase amino acid sequences revealed that more than half of the *nifH* products were derived from methylotrophic bacteria, such as *Methylocella* spp. The next most frequent sequence types were similar to those from *Burkholderia*.

14.1 Mechanisms of P mobilization from rock phosphates by *Glomus intraradices*

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The arbuscular mycorrhizal (AM) symbiosis is known for its capacity to increase the inflow of slowly mobile nutrients, mainly phosphorus (P) to plant roots. Although P taken up by AM fungi is considered to be from the same labile pool used by plant roots, the growth of mycorrhizal plants has been shown to respond to the application of insoluble forms of inorganic P such as rock phosphate (RP). Nevertheless, the mechanisms underlying this fact are not fully understood and there is a lack of experimental data supporting the production of chelating agents by these fungi. The objective of this work was to determine if AM fungi facilitate the use of RP by plants and, if so, which mechanisms regulate the process. We hypothesize that: 1) *Glomus intraradices* can solubilize and transfer P from RP to plant roots; 2) susceptibility to RP solubilization by the AM fungus is positively correlated with the mineral's reactivity; 3) the AM fungus has access to RP through localized alterations of pH and the production of organic anions as chelating agents. We grew carrot root-organ-cultures in presence or absence of *G. intraradices* for 8 weeks under different P treatments (control, igneous RP, sedimentary RP and tricalcium phosphate). Surprisingly, after 8 weeks, we did not observe a change in pH of the bulk medium. We are currently analysing localized variations in pH, P root content and organic acids which will help to further determine how AM fungi may access insoluble sources of P.

14.2 Effectiveness of autochthonous bacteria from semiarid mediterranean ecosystem in increasing *Glomus intraradices* development in dixenic culture

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This study investigates the interaction between drought adapted beneficial microorganisms such as autochthonous moderately halophyte bacterial strains (*Pseudomonas putida* and *Bacillus megaterium*) and the mycorrhizal fungus *Glomus intraradices*, both isolated from semiarid mediterranean ecosystem. All the *G. intraradices* life cycle steps (spores, hyphae and branched absorbing structures (BAS) production) were analysed along 12 weeks, in dixenic culture, under two levels of NaCl concentrations (0 and 35 mM), in presence or absence of bacteria. Without salt, bacterial treatments increased spore number by 50% (*P. putida*) or by 123% (*B. megaterium*) and BAS by 28% (*P. putida*) and by 91% (*B. megaterium*). Under saline conditions, *G. intraradices* did not lose its ability to grow, but hyphae and BAS produced were highly reduced compared to those formed under non-saline conditions. However, under salinity, both bacteria increased spores production and hyphal growth by *G intraradices*. Under such stress conditions, BAS formation was particularly enhanced as affected by the bacterial treatments being increased by 306% (*P. putida*) or by 477% (*B. megaterium*). The direct effect of autochthonous salt-adapted bacteria, particularly *B. megaterium*, produced greater growth, propagules formation and saline stress resistance/tolerance of *G. intraradices* under dixenic culture. The stimulating metabolic compounds produced by these bacteria, under such saline conditions, are now under evaluation.

14.3 Use of a novel root window technique to visualize spatial variation in soil phosphatase activity *in-situ* across a forest chronosequence

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Ectomycorrhizal fungi increase soil nutrient availability by excreting hydrolytic enzymes. We installed plexiglass root windows across a chronosequence of mixed Douglas-fir and paper birch stands in the Interior Cedar-Hemlock biogeoclimatic zone in B.C., Canada to characterize *in-situ* soil phosphatase activity. In addition, soil samples from active and non-active areas of enzyme activity were taken and the ectomycorrhizal fungal community profiled. Filter paper impregnated with a colorimetric substrate (p-nitrophenyl phosphate) was applied to intact soil horizons exposed through these windows to detect *in-situ* phosphatase activity. Total *in-situ* phosphatase activity and intensity increased with stand age, reaching a maximum at stem exclusion (60-70 years). Areas of *in-situ* phosphatase activity also became larger and more intense at stem exclusion. A substantial increase in areas of less intense activity was also seen at stem exclusion, while older sites had a more even distribution in the intensity of phosphatase activity. Small soil samples (0.25 g) were taken directly from areas in organic and mineral soil layers revealing phosphatase activity in *in-situ* assays. Additional samples were taken from the same layers in locations lacking phosphatase activity. The fungal community profile in these samples is being analyzed by Denaturing Gradient Gel Electrophoreses (DGGE). Preliminary results suggest that some fungi are unique to soil microsites with high phosphatase activity.

14.4 Arbuscular mycorrhizal fungi do not compete for intra- or extraradical resources, but show distinct substrate colonization strategies

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Competition for intra- and extraradical resources, and hyphal interactions / substrate colonization strategies of the arbuscular mycorrhizal fungi (AMF) *Glomus intraradices*, *Glomus proliferum* and *Gigaspora margarita* were investigated under *in vitro* conditions. The AMF were polyxenically-cultured with a Ri T-DNA transformed carrot root organ culture (ROC) in either big Petri plates containing three separate culture compartments and a common hyphal compartment (i.e. an independent host root for each AMF); or two by two in the culture compartment of regular bi-compartmented Petri dishes (i.e. a common host root and a common hyphal compartment). Maps of the extraradical mycelial development of the three AMF were obtained. Two distinct substrate colonization strategies (Glomus-type and Gigaspora-type) were identified, reflecting intrinsic differences between AMF genera/families. Our data reveal a general lack of antagonism between the different isolates tested when extraradical hyphae explore and exploit the substrate out of the root influence zone; however certain growth restrictions were imposed by *Gi. margarita* extraradical mycelium when developing in the proximity of the host root, and by *G. proliferum* intraradical hyphae. This work highlights once more the appropriateness of AM *in vitro* culture systems to perform *in vivo* studies on the biology of this symbiosis, and opens new avenues to the formulation of *in vitro*, AMF inoculants.

14.5 Interaction effect between *Glomus mosseae* and plant growth promoting fungi on anthracnose suppression and their root colonization in cucumber plants

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Cucumber plants were inoculated with arbuscular mycorrhizal fungus, *Glomus mosseae*, and plant growth promoting fungi (PGPF), *Phoma* sp. and *Penicillium simplicissimum* isolates, in order to elucidate their interaction effect on disease suppression and root colonization. Induction of systemic resistance in cucumber by inoculation of *G. mosseae* and PGPF isolates against the anthracnose disease caused by *Colletotrichum orbiculare* was evaluated. Root colonization of each fungal species which could be influenced by their interaction was also evaluated. Plants were grown in potting soil inoculated with *G. mosseae* and each PGPF isolate and grown for 4 weeks. The leaves were then challenge inoculated with pathogen, *C. orbiculare*. Plants induced by each PGPF isolate showed considerable protection for the disease however significant protection was not observed in plants inoculated with *G. mosseae*. Furthermore, combined inoculation of *G. mosseae* with *Phoma* isolate reduced the level of disease protection induced by single inoculation of *Phoma* isolate. In contrast, high level of protection induced by *P. simplicissimum* was not altered by the combined inoculation with *G. mosseae*. Root colonization of *Phoma* isolate was adversely affected by co-inoculation of *G. mosseae*, while root colonization of *P. simplicissimum* was not. The percent root length colonized by *G. mosseae* was not antagonistically affected by both PGPF isolates tested.

14.6 Xyloglucanases in pea root colonized by *Fusarium oxysporum-126*, *Trichoderma harzianum*, *Penicillium chrysogenum* and *Glomus mosseae*

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We studied the production of xyloglucanase enzymes of pea roots inoculated or not inoculated with the arbuscular-mycorrhizal (AM) fungi *Glomus mosseae* in presence or in absence of the saprobe fungi *F. oxysporum-126*, *Trichoderma harzianum* and *Penicillium chrysogenum*. *G. mosseae* increased the dry weight of pea. All the saprobe fungi increased the mycorrhization of pea, but only *P. chrysogenum* inoculated together with *G. mosseae* increased the dry weight of pea. The AM and saprobe fungi not only were able to produce xyloglucanases but they increased the production of these enzymes by plant roots. The level of xyloglucanases activities and the number of xyloglucanolytic isozymes in plants inoculated with *G. mosseae* and most of the saprobe fungi tested was higher than the other treatments in which both microorganisms were inoculated separately. The possible relationship between these activities and the capacity of the inoculated microorganisms to improve plant dry weight and AM colonization of plant root was discussed.

14.7 Mycorrhiza-mediated weathering of minerals: from nanometre scale to environmental systems. The WUN Weathering Science Consortium

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The boreal forest is the world's largest vegetation system and is dominated by ectomycorrhizal trees that have recolonised vast areas of bare rock and raw mineral substrates exposed at the end of the Devensian glaciation circa 10,000 years ago. Ectomycorrhizal trees are strongly implicated in weathering rock-forming minerals and in the formation of intensely weathered spodosols characteristic of boreal regions. Our interdisciplinary consortium funded by the Natural Environment Research Council (NERC) and by the World Universisites Network (WUN) brings together mycorrhiza researchers, geologists, biogeochemists, nanotechnologists, and environmental modellers to study mycorrhiza weathering at scales ranging from nanometers to whole soil profiles. The central hypothesis we are testing is that rates of biotic weathering are proportional to the biotic energy fluxes directed at the scale of reacting grains. Mycorrhizal fungi typically produce 200-600 km of mycelia per kg of boreal forest soil. These fungi receive 20-30% of net photosynthate, and contribute 50% of the soil dissolved organic C. To test our hypothesis the carbon energy fluxes in the mycorrhizospheres of pine and birch trees are being quantified and rates of weathering determined by advanced physical, chemical and imaging technologies. The importance of exudates versus surface-contact interactions is a special focus of the work which aims to create a step-change in our understanding of mycorrhiza-weathering.

14.8 Evaluation of the microbial populations in the mycorrhizosphere of *Vaccinium* plants

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The presence of microorganisms associated to the mycorrhizosphere of *Vaccinium* plants (Ericaceae) in two Colombian soils differing in their organic matter (OM) content was investigated. Both soils were added or not (controls) with two types of organic matter in two proportions (3:1 and 1:3). The microbiological analysis carried out after six months of plant growth included: counting of CFU, phosphate-solubilizing bacteria, actinomycetes and fungi. Bacterial isolated were identified. The development of mycorrhizal Blue Berry plants correlated with the OM content of the substrate. Both the number and diversity of microbial components also correlated with OM content of the substrate. *Xanthomonas, Salmonella, Serratia marcescens, Pantoea agglomerans. Pseudomonas putida, P. fluorescens, Enterobacter cloacae, Acinetobacter iwoffi, Streptomyces, Streptoverticillium, Nocardia, Penicillium, Aspergillus, Trichoderma, Fusarium, Paecilomyces* and *Mucor.* In spite of *Vaccinium* is not an AM-host, the number and type of AM spores was also determined in its rhizosphere. AM spores were present in all the substrates but at a higher concentration (180 spores/g) when the OM content was higher, and lower (5 spores/g) in control soils (OM not added). Mycorrhizal colonization levels ranked from 23% to 57%. Bacteria predominate relatively to fungi when mycorrhizal colonization was higher.

14.9 Methodology of glomalin-related soil protein extraction

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We examined standard methods of soil extraction for glomalin, a glycoprotein complex produced by arbuscular mycorrhizal fungi. We added 5 mg/g bovine serum albumin or a glycoprotein, bovine mucin, to five different field-collected soils to see if the standard extraction procedure for total glomalin would destroy these other proteins. We found that one hour of autoclaving clayey soils appeared to eliminate the added proteins, but failed to do so for sandy soils. After a second one-hour extraction cycle, however, the added proteins were no longer detectable by Bradford protein assay. We also have found that following a single half-hour autoclave extraction: 1) short term (up to 3 h) room-temperature incubation before centrifugation diminishes Bradford protein, but 2) incubation for several days at room temperature increases the amount of Bradford protein extracted. Also, increasing the ratio of volume of extracting solution to weight of soil removes more Bradford protein than the standard ratio (8 ml to 1 g). Similarly, if 1 g soil samples are successively extracted for both easily-extractable glomalin and the remaining extractable glomalin, the sum of these extracts' Bradford protein contents exceeds Bradford protein extracted solely by the total glomalin extraction procedure.

14.10 Xyloglucanases in the interaction between *Sinorhizobium meliloti*, *Rhizobium leguminosarum* bv. *viceae* and *Glomus mosseae*

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We studied the production of xyloglucanase enzymes of alfalfa and pea roots inoculated with the arbuscular-mycorrhizal (AM) fungi *Glomus mosseae* and the rhizobia *Sinorhizobium meliloti*, *Rhizobium leguminosarum* bv. *viceae*. The inoculation of the plants with the rhizobia inoculated either alone or together with *G. mosseae* increase their shoot dry weight. The shoot dry weight of both plants inoculated with its rhizobium and *G. mosseae* was significatively higher than with the inoculation of both microorganism individually. The rhizobia increased the mycorrhization of plants but only *S. meliloti* inoculated together with *G. mosseae* increased the nodule number of pea. Similar level of xyloglucanase activity of alfalfa inoculated with *S. meliloti* than the noninoculated controls was observed. However, the level of xyloglucanase activity of pea inoculated with *R. leguminosarum* bv. *viciae* was higher than the no inoculated controls. The xyloglucanase activity of plants inoculated with *G. mosseae* alone or together with its specific *Rhizobium* were higher than the noninoculated controls, being this activity higher in alfalfa when both symbionts were inoculate together. The number of xyloglucanolytic isozymes in plants inoculated with AM fungi and the rhizobia tested was higher than when both microorganisms were inoculated separately. Our results indicate that xyloglucanases can be involved in the interaction between both microorganisms and in their action on the plant physiology.

14.11 Interactions of ericoid mycorrhizal fungi and root pathogens in *Rhododendron* plantlets grown in a sterile culture system

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We are testing the hypothesis of a biocontrol potential of ericoid mycorrhiza towards root pathogens. Ericoid mycorrhizal fungi (ERMF) with distinct growth inhibiting activity against Oomycetes in dual agar plate tests were studied in a sterile *Rhododendron* test system favorable for continuous observations of the infection processes. Micropropagated rooted *Rhododendron* plantlets were transferred into a mineral solution culture system with *Oidiodendron maius* and two isolates from heather (E3-8; E3-24a). After 4 weeks of mycorrhizal establishment in the hair roots, inoculations with root pathogens (*Pythium* spec., *Phytophthora cinnamomi*) were set up in a renewed solution. Microscopic assessment covered mycorrhizal development as well as pathogen infection sites, infection progress and fungal development in mycorrhizal roots and non-mycorrhizal control plants. A ranking of the three ERMF in the root colonizing intensity was made up (*O. maius* > E3-8 > E3-24a). Independent of their colonization capacity, all ERMF reduced the frequency of pathogen infection sites. Microscopic analyses revealed undisturbed pathogen infections in mycorrhiza-free tissue zones, sparse infections in non-mycorrhizal cells adjacent to mycorrhizal cells, and seldom dual infections of single cells. The tested ERMF seem to prevent roots from pathogen attack by pre-infectional, antibiotic mechanisms.

14.12 The growth of arbuscular mycorrhizal fungi is stimulated by decomposing host tissues in the soil

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Arbuscular mycorrhizal (AM) fungi are considered to be obligate biotrophic organisms depending on organic nutrition from the host plant. Consequently, their interactions with soil organic matter seem to be less relevant than the relationship with living hosts. However, AM fungi are in contact with soil organic matter, including dead plant tissue releasing bioactive compounds during its decomposition. This lead us to study this interaction, which may be important for understanding the biology of AM fungi. Effects of plant tissue decomposition on the AM fungi *Glomus claroideum* BEG23 and *Glomus intraradices* PH5 were investigated in vitro and in a hydroponic cultivation system. Alfalfa roots and shoots as well as pure cellulose were left to decompose in nonsterile soil at two mineral supply levels. Water extracts of such soil samples stimulated axenic growth of hyphae of *G. intraradices* in vitro. Similarly, stimulation of root colonization by *G. claroideum* in the presence of soil with decomposing plant tissues was observed. Chemical analysis of the soil by pyrolysis-GC-MS revealed that many organic soil compounds were associated with increased AM root colonization, hyphal growth and increased levels of some plant growth characteristics. The results indicate that decomposed plant tissues have a strong impact on the development of AM fungi under field conditions where plant residuals occur.

14.13 Can photosynthesis rates of legumes be stimulated by sink strength of rhizobial and mycorrhizal associations?

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Legume growth is enhanced through association with rhizobia and mycorrhizal fungi but such associations involve carbon costs. We study how legumes balance the costs and benefits of the rhizobial and mycorrhizal symbioses. We hypothesise that plants increase the photosynthesis rates to compensate for the carbon costs related to microbial symbioses. Triose phosphates, the first molecules of photosynthesis, can be processed into starch or sucrose. The synthesis of sucrose and its export via phloem lead to release of inorganic P (Pi) to the chloroplasts: if sink strength is increased, Pi recycle is faster and photosynthesis is stimulated. Rhizobia and mycorrhiza change the sink strength by affecting the sucrose export and activity of developing tissues. The magnitude of effects might differ because both symbionts compete for C but only rhizobia demand P. Photosynthesis is simulated assuming the sink stimulation by microbial symbioses. Sink stimulation will probably not result in increased plant biomass, but it shows the plant flexibility in response to symbiotic associations. Parameters for the model are being gathered from literature or obtained experimentally. The effects of symbiotic sink stimulation will be assessed by comparing costs with nutrient-fed plants that have the same shoot nutritional content. We will measure the symbiosis effects on photosynthesis of soybean by performing factorial designed experiments (mycorrhiza, rhizobia, P and N) under glasshouse conditions.

14.14 Enhancement of the mycorrhizal status of an established ginger crop through mycorrhization of a companion onion crop

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Arbuscular mycorrhizal and mycorrhiza-free seedlings of onion (*Allium cepa* L.) were raised in the greenhouse and transplanted in the field along rows of previously established plants of ginger (*Zingiber officinalis* Roscoe) in order to evaluate the possibility of enhancing the arbuscular mycorrhizal status of ginger via a companion crop. Arbuscular mycorrhizal (AM) fungal colonization of ginger and onion roots determined 136 days after transplanting of onion showed that roots of all plants were colonized by the fungi. However, the level of AM fungal colonization of onion roots was significantly higher if onion seedlings were mycorrhizal prior to transplanting than if they were not. Dry matter yield of onion was nearly tripled by mycorrhizal inoculation. The level of AM fungal colonization of ginger roots was significantly enhanced if the companion onion was mycorrhizal than if it was not. However, the yield of ginger was unaffected by the presence of onion irrespective of the latter's mycorrhizal status. Our data suggest that the mycorrhizal status of an established plant may be enhanced through the introduction of a compatible companion crop colonized by AM fungus.

14.15 Gene expression in white clover with and without colonisation by arbuscular mycorrhizal fungi

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White clover (*Trifolium repens*) is an important component of low input pasture systems and can form effective associations with both N-fixing and arbuscular mycorrhizal fungi (AMF). We have exploited near-isogenic lines of white clover to identify genes involved in legume-symbiont interactions with a view to assigning functions to genes involved in these interactions and generating molecular markers for improved symbiosis in new varieties. Differential display methods were used to identify changes in gene expression in both leaves and roots from plants following colonisation with *G. mosseae* and *G. intraradices* and rapid amplification of cDNA ends (RACE) is currently being applied to confirm and extend the obtained sequences to further elucidate the genetic factors that control AMF symbiosis in white clover. The results obtained indicate clear differences in gene expression in white clover plants colonised by AMF. Future work will focus on how AMF modify the growth and senescence of roots and will involve comparing gene expression patterns in individual root tips from plants with and without colonisation by *G. intraradices* using cDNA AFLP methods. We will use microcosms which enable us to follow the growth of individual roots and allow us to observe individual root tips throughout their life cycle and extract RNA at different stages of development.

14.16 Take-all disease caused by *Gaeumannomyces graminis* var. *tritici* is locally and systemically reduced in roots of mycorrhizal barley plants

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The effect of mycorrhization by *Glomus mosseae* on take-all disease caused by *Gaeumannomyces graminis* var.*tritici* (Ggt) was studied in barley. Take-all disease was reduced when barley plants showed high levels of AM root colonization, whereas a low AM root colonization exhibited no effect on Ggt infection. A similar pattern was observed in split-root systems of barley with one side mycorrhizal and the other side inoculated with Ggt. High AM root colonization on one side of the split-root system clearly reduced take-all disease on the other side. These results show a local and systemic bioprotectional effect of mycorrhization against Ggt. The bioprotectional effect seems to depend on the on the degree of root colonization by the AM fungus.

14.17 Characterization of soil fungal communities in *Castanea sativa* forests with special emphasis on mycelia of the *Boletus edulis s.l.* species complex

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The aim of this study was to characterise the soil fungal community of *Castanea sativa* forests in the area of Borgo Val di Taro (Italy). Due to the fact that the *Boletus edulis* complex is a main source of income in this valley the emphasis was put on this type of fungus. The main focus was given to the differences in the species components belonging to natural and cleaned forests. We examined two natural areas and two forests which were cleaned within the last 6 or 12 months. Altogether 16 soil samples were taken directly under the basiodomes of *Boletus edulis* s.L.. The fungal were assayed by cloning techniques. The results revealed that there are distinctive differences between natural and cleaned forests. In comparison to cleaned forests saprobial fungal dominate in the natural areas; whereas the treated forests showed a richness in ectomycorhizal species belonging to the Agaricales, Russulales and Boletales. Furthermore, a greater amount of ericoid mycorrhiza was discovered in the cleaned areas. Although the basiodomes of the *Boletus edulis* s.L. complex dominated on the ground, only few mycelia could be detected in the soil. Finally, it is to mention that the above and below ground fungal community rarely overlap.

14.18 Interaction effect between *Glomus mosseae* and *Phoma* sp. on suppression of cucumber damping-off and microbial populations in the rhizosphere soil

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Glomus mosseae and plant growth promoting fungus, *Phoma* sp., were co-inoculated to cucumber in order to evaluate disease suppression effect. After growing cucumber seedlings in potting soil inoculated with *G. mosseae* and *Phoma* sp. for 10 days, the seedlings were transferred to *Rhizoctonia solani* infested soil. Disease protection (%) was evaluated 7 days after the transplanting. Although disease protection effects were high in plants induced by single inoculation of *G. mosseae* and *Phoma* (52 and 46 %, respectively), the effects were even higher (74%) in the plants induced by co-inoculation. Reduction of root colonization by *Phoma* sp. was observed in co-inoculated plants with *G. mosseae* while no adverse effect was observed in *G. mosseae* colonization by co-inoculation of *Phoma* sp. Qualitative and quantitative changes of fungal and bacterial populations by inoculation of *G. mosseae* and *Phoma* sp. were further evaluated using soil dilution plating and PCR-DGGE methods. Both fungal and bacterial populations were reduced in the rhizosphere by co- and single-inoculation. Increase of both fungal and bacterial species in the rhizosphere by single-inoculation was observed by PCR-DGGE. Further more, common fungal and bacterial species were observed in the rhizosphere by single inoculation of *G. mosseae* and *Phoma* sp. and they were more strongly detected by co-inoculation of *G. mosseae* and *Phoma* sp. The role of these specific microorganisms on disease suppression was discussed.

14.19 Plant growth promoting bacteria from the genus *Paenibacillus* suppress the arbuscular mycorrhizal fungus *Glomus intraradices*

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Interactions between *Glomus intraradices* and bacteria from the genus *Paenibacillus* (*P. macerans* and *P. polymyxa*) were examined with and without organic matter. Both *Paenibacillus* species suppressed mycorrhiza formation, but *P. macerans* only in combination with organic matter. Combining mycorrhiza and *P. polymyxa* caused severe plant growth depressions. Inoculation with *G. intraradices* increased dehydrogenase activity, which correlated positively with the concentration of phosphorous in the growth substrate, indicating that mycorrhiza inoculation accelerated the decomposition of organic matter. Background microbial communities as measured with fatty acid biomarkers were increased by mycorrhiza in the absence of organic matter, but unaffected in the presence of organic matter, except for other soil fungi (18:2w6,9) and protozoa (20:4), which were increased by mycorrhiza, irrespective of the presence of organic matter. Inoculation with *Paenibacillus* increased all measured microbial groups, except for the AM fungus biomarker 16:1w5, which tended to be suppressed by both isolates of *Paenibacillus*. On the other hand, mycorrhiza reduced the amount of a biomarker for Gram-positive bacteria (15:0a), which is present in *Paenibacillus* in high amounts indicating that both isolates of *Paenibacillus* were inhibited by mycorrhiza. In conclusion, isolates of *Paenibacillus* may not only have mycorrhiza helper features but also suppressive effects on AM fungi and cause plant growth depressions.

14.20 Arbuscular mycorrhizal fungus-bacteria interactions: implications for adaptation via bacterial associations

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In nature, symbiosis is the rule rather than the exception. Increasing evidence suggests that the arbuscular mycorrhizal (AM) symbiosis is often a tripartite relationship between not only plants and AM fungi, but also many bacteria. AM fungi associate with bacteria ranging from free-living to obligately endosymbiotic. Evidence from numerous studies suggests that bacteria could play a major role in the ecology of AM fungi. Specifically, I propose that AM fungi can incur increased fitness by selecting beneficial bacteria. Two ways AM fungi can select for particular bacteria are via dispersal of bacteria on and within spores and releasing exudates (i.e. organic acids, specific carbohydrates, and enzymes). An extensive search of published, peer-reviewed studies of AM fungus-bacteria interactions revealed: 1) six instances of AM fungi altering bacterial community composition (e.g. cultures of six isolates of three *Glomus* spp. possessed mutually different bacterial communities), 2) eight instances of AM fungus-bacteria specificity (e.g. *Gigaspora margarita* and *Candidatus* Glomeribacter gigasporarum), and 3) six instances of positive effects of bacteria on AM fungi (e.g. *Paenibacillus validus* supported sporulation of *Glomus intraradices* in plant-free culture). This potentially widespread mechanism of AM fungal adaptation has ramifications for studies of AM fungal function and diversity, as well as implications for AM fungal culture and use in agriculture and restoration.

14.21 Diversity of *Glomus mosseae* spore-associated bacteria and their antagonism on soilborne plant pathogens *in vitro*

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The arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe was shown to reduce diseases provoked by different root pathogens. As this obligatory biotrophic species cannot be routinely cultured in monoxenic conditions, on hairy roots, it is difficult to produce an inoculum absent of associated bacteria and to distinguish between the contribution of AMF itself from the one of these bacteria on root disease alleviation. In order to investigate this issue, *G. mosseae* spores were collected from leek pot cultures, surface sterilized and inoculated on Tryp-Soy Agar. The total genomic DNA of the 19 bacterial isolates obtained was extracted, the bacterial 16S rDNA gene was amplified using PCR and sequenced. By similarity search in GenBank, the isolates were shown to belong to three genera: *Methylobacillus*, *Bacillus* and *Paenibacillus*. Antagonism over *Phytophthora nicotianae* Breda de Haan, *Fusarium oxysporum* Schltdl. and *F. solani* (Mart.) Sacc. on V8-agar was recorded for isolates among these three genera. The results support the hypothesis that the biocontrol induced by *G. mosseae* is due, at least in part, to its associated bacteria. Study of the effect of these bacteria on other fungal plant pathogens is currently ongoing.

14.22 Yeheb (*Cordeauxia edulis* Hemsl.) associated micro-organisms and their effects on its growth and pathogens

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Yeheb (*Cordeauxia edulis* Hemsl.) is an endangered shrub because of overexploitation. For its cultivation outside its native habitat we studied the microbes, living with yeheb, particularly arbuscular mycorrhiza, beneficial bacteria and their role for its growth. AMF spore density and root colonization were estimated in field rhizosphere soils and roots respectively. Yeheb was evaluated 11 months later in a greenhouse experiment after inoculation of 8 months old plants with its native AMF, a commercial inoculum,Vaminoc and *in vitro* cultured *G.intraradices*. Isolation of bacteria from pre-germinated yeheb seeds and soils was conducted and screened for *in vitro* inhibition of seed borne pathogens: *Aspergillus niger, A. biciliate and A. versicolor* and their ability to improve seed emergence. Selected bacteria were tested in the greenhouse. Effects of AMF and bacteria were measured in terms of plant growth and nutrients contents of the shoot. AMF spores (ranges from 2-18 spores/g dry soil) and colonized roots (>10-60%) were observed in field samples. Inoculation of AMF resulted in root colonization of yeheb and was higher in Vaminoc and *G. intraradices* . Shoot dry weight and nutrient contents increased, except for N and K and were insignificant. Some bacterial strains showed pathogen inhibitory activity and seemed to increase plant height ($P=0.052$) and magnesium content ($P=0.042$) in shoot.These bacteria were identified and belong to *Pseudomonas, Klebsiella* and *Pantoea*.

14.23 Variable effect of *Trichoderma* bio-inoculants on ectomycorrhizal colonisation of *Pinus radiata* seedlings

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Trichoderma bio-inoculants, applied whilst *Pinus radiata* seedlings are in the nursery, provide protection from infection by *Armillaria* spp. during their establishment in forestry plantations. However, the colonisation of *P. radiata* seedlings by ectomycorrhiza (ECM) could be compromised if the *Trichoderma*spp. have inhibitory, non-target effects. *Trichoderma* spp. suppress plant pathogens by mechanisms such as mycoparasitism, antibiosis and highly competitive saprophytic ability. In a pot experiment to investigate the effect of *Trichoderma* inoculation on ECM colonisation of *P. radiata* seedlings a *Trichoderma* isolate specific effect on the overall percentage ECM colonisation was observed. One *Trichoderma* isolate increased the percentage ECM colonisation while the other isolates, including a mix of all isolates, had no effect. In dual plate bioassays, conducted to assess the individual interactions between five ECM species (*Suillus granulatus*, *S. luteus*, *Rhizopogon* spp., *R. parksii*, and *R. luteolus*) known to be early colonisers of *P. radiata*, and six *Trichoderma* isolates, isolate specific interactions were seen. *S. granulatus*was not mycoparasitised or out-competed by any of the six *Trichoderma* isolates. While *R. parksii* was out-competed by all *Trichoderma* isolates and, although not mycoparasitised, was extensively overgrown by *Trichoderma*hyphae. The effect of *Trichoderma* application on colonisation of *P. radiata* seedlings by the five ECM species is currently being investigated.

14.24 Transformation of humic acids by forest litter fungi

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Transformation of forest litter results in genesis of humic compounds, whose substantial part is formed by humic acids (HA). Several studies reported the ability of fungi to degrade HA. The aim of the present study was to evaluate the effect of different fungi cohabiting the forest litter on HA degradation. Liquid media amended with HA isolated from the litter were inoculated with saprotrophic, endophytic and mycorrhizal fungal strains. Absorbances of media amended with or without HA were regularly measured and finished by the extraction of dark pigments from mycelia and the cell walls after 40 days. Simultaneously with media decolourisation (attributable to HA degradation and deposition in fungal cell walls), melanins were produced into media by dematiaceous fungi. We hypothesize that melanins contribute to the genesis of HA and vice versa – HA stimulate fungal growth and production of melanins. Next to the liquid media study, spruce needle litter was inoculated by several saprotrophic fungi and their effect on HA isolated from the litter was studied by MALDI-TOF mass spectrometry. It was found that fungi modified both the pattern of detected compounds/fragments and their quantity. We conclude that forest litter fungi "transform" rather than just "degrade" HA as they take part both in humification and HA degradation processes. We report herein that particular fungal strains differ in their ability to transform HA.

14.25 Ectomycorrhizal soil fungal communities in an alpine primary successional habitat

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Ectomycorrhizal (ECM) fungi are essential for establishment and maintenance of ECM plant communities. However, establishment and developmental dynamics of mycorrhizal associations are still poorly understood, especially in primary successional habitats. We evaluate soil fungal communities (SFC) using direct isolation of internal transcribed spacer (ITS) rDNA genes by PCR and sequencing of cloned fragments. Fungal biomass is indirectly investigated by ergosterol contents. Investigations were carried out of the Rotmoosferner glacier forefront (2350 m Ötz valley, Austria) in August 2005. Three ECM host plants co-occur at the moraine ridge of 1920 in a patchy distribution: the herbaceous plant *Polygonum viviparum* (Polygonaceae), the Cyperaceae *Kobresia myosuroides* and the dwarf shrub *Salix herbacea* (Salicaceae). Bare soil was used as a control. The main aim of the present study was to assess the interaction of ectomycorrhizal soil fungi with different host plants. We were especially interested if plant cover influences the soil fungal community in primary successional soils. Our results clearly show that soil ECM fungal communities and biomass change significantly due to the influence of plant cover. Typical ectomycorrhizal fungi are missing in bare soil, and comparatively rare in *Kobresia* soil. In contrast, soils with *Salix* and *Polygonum* show high relative abundances of ectomycorrhizal Basidiomycota.

14.26 Effects of inoculation with *Gigaspora rosea* and *Frankia* on the development of root system architecture and root hairs in *Alnus glutinosa*

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Glasshouse experiments were conducted to analyse how interactions between an arbuscular mycorrhizal fungus (AMF) and *Frankia* affect the development of roots and root hairs on inoculated seedlings of *Alnus glutinosa* roots. Seedlings were inoculated with the AMF at planting. Inoculation with *Frankia* occurred either simultaneously or was delayed for up to 30 days. Data showed that nodulation by *Frankia* was increased when plants were colonized by AMF and colonization was greater in dual inoculated plants, when inoculation with *Frankia* was delayed until 30 days following planting. Root system architecture was altered significantly by inoculation, and seedlings inoculated with AMF had substantially more third and fourth order lateral roots. An important and novel discovery is that development of root hairs was also affected by AMF, with numbers of root hairs reduced significantly in plants colonized. Practically these data suggest that growth of dual inoculated, young alders can be strongly affected by the timing of inoculation. There is also a suggestion that some of the negative growth effects previously reported could be explained by changes in the root system of the host.

14.27 *Lavandula* spp and *Thymus* spp act as 'fertility Islands' in *Cupressus atlantica* G. revegetation strategies

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The mycorrhizal status of several representative shrub species (*Lavandula* spp. and *Thymus satureioides*) in Moroccan ecosystems, was evaluated as well as their contribution to the mycorrhizal potential of the soil. Furthermore, the rhizosphere soils collected under these target species have been tested for their influence on the growth of *Cupressus atlantica*. Soil samples were collected from the rhizosphere of *L. stoechas*, *L. dentata* and of *C. atlantica* existing in the experimental area. Control samples were randomly collected from bare soil sites, away from plant influence. All the target species formed AM symbiosis and the extent of AM fungal colonization was not significantly different between plant species. No significant difference was detected between the total number of AM fungal spores of the bare soil and those recorded in the root zones of target species and *C. atlantica*. The number of mycorrhizal propagules in soil originating from around the four target plant species was significantly higher than the one in the bare soil. In the glasshouse experiment, the growth of *C. atlantica* seedlings was significantly higher in the *C. atlantica* and in the shrub species soils than in the bare soil. Key words: Arbuscular mycorrhiza, *Cupressus atlantica* G., degraded forest ecosystems, Nurse plant, fertility Islands, Revegetation strategies.

14.28 Arbuscular mycorrhizal colonization and microbial activities in the rhizosphere of gypsophytes

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Damage to soil and plant in arid and semiarid areas is not easily repaired because these are fragile and ecologically sensitive ecosystems. Hence, our main aim is to improve the revegetation efficiency, using mycorrhization as a key-instrument to restore a rubbish dump located in a semiarid soil landscape of Villena (Alicante). In this preliminary study five randomly points have been selected to evaluate the microbial activity under four endemic species, exclusively grow on gypsisols (Gypsophytes): *Teucrium libanitis* (TL) (Lamiaceae), *Ononis tridentata* (OT) (Fabaceae), *Gypsophila struthium* (GS) (Caryophyllaceae), *Helianthemun squamatum* (HS) (Cistaceae). Rhizosphere and no rhizosphere soil were sampled to analyze: Enzyme activities: Dehydrogenase, protease-BAA, acid phosphatase and ,-glucosidase , organic matter, AM fungi propagules number and aggregate stability. First results from this study show in general, significantly higher enzyme activities on rhizhosphere soil compared to no rhizospheric soil, where *Ononis tridentata* species stands out above the rest. It is also noticed a higher organic mater content, next to infective arbuscular mycorrhizal potential and aggregate stability in rhizhospheric soil. There are statistical differences between phosphatase and ,-glucosidase according to the species. Results show similar behavior between TL, GS and HS species in acid phosphatase activity, and different on OT. Also, these results have been reported to ,-glucosidase activity.

14.29 Ectomycorrhizal carbon and its role in denitrification in boreal forests

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Denitrification occurs when soil nitrate is reduced to N_2O and N_2. N_2O significantly contributes to global warming, with atmospheric loading increasing by 0.3% per year. Although many studies have focused on N_2O emissions from agriculture, it is important to develop an understanding of emissions from different land-uses, microbial communities and processes, in order to enable efficient mitigation. Denitrifying bacteria require carbon for the respiration of nitrate to N_2O under oxygen-limiting conditions: where carbon is readily available, N_2O is further reduced to the environmentally benign N_2. Low molecular weight (LMW) carbon is readily metabolised by soil bacteria, and LMW exudates from ectomycorrhizal roots may provide an important carbon source in boreal forest systems. The effect of this carbon on N_2O and N_2 production during denitrification can be quantified using stable isotopes techniques. It is unknown whether it is carbon quality or quantity that drives the final reduction step in denitrification. To address this, we examined the effect of different carbon substrates, predominantly exuded by ectomycorrhiza (glucose, mannitol and oxalic acid), on denitrification in forest soils. Future investigations of the role of ectomycorrhiza in soil denitrification will include measurement of N_2O and N_2 from mesh-cores inserted into pots containing ectomycorrhizal trees, as well as measurements from the common ectomycorrhizal fungus *Tylospora fibrillosa* grown in liquid culture.

14.30 The effects of ectomycorrhiza on growth of *Picea glehnii* seedlings in immature volcanic soil condition

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Picea glehnii, is light demanding conifer and is one of the dominant species in the mesic region of northern Japan, and can grow in infertile soil. We examined whether ectomycorrhizal infection have the positive effect on growing of *Picea glehnii* in immature volcanic soil or not. The nutrient contents (e.g. P, K, Ca, mg and Na) in volcanic ash soil (VAS) were significantly lower compared to in brown forest soil (BFS). However, the percentage of ectomycorrhizal infection for fine roots (< 5mm) of seedlings was significantly higher ($P < 0.05$) in VAS than in BFS. Therefore, it may result in the phosphate contents of different age needles (2, 4, 6 and 10 yr) were markedly larger in VAS than in BFS. Although the survival of needles was reduced by ageing in both two types of soil, it was higher in VAS than in BFS. The photosynthetic rate of seedlings grown in VAS was also higher than that of seedlings in BFS. Nevertheless, the nitrogen content and total chlorophyll content of 2-yr needles were lower in VAS than in BFS. We conclude that the significant ectomycorrhizal infection has an important role for *Picea glehnii* seedlings' establishment in infertile soil, such as immature volcanic soil, by uptake and translate phosphate to needles to maintain a higher photosynthetic rate.

14.31 Mycorrhizal fungal inoculum potential and diversity of amending materials used for revegetation of the Canadian tar sands tailings

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In northern Alberta, surface mining of tar sand produces massive areas of disturbed lands with composite tailings and tailing sands as by-products. These materials are considered challenging substrate for reclamation and revegetation, because of high alkalinity and salinity, low in organic matter, poor nutrition, and lack of mycorrhizal inoculum. Several amending materials have been used to amend these disturbed lands. Mycorrhizal fungi can play a critical role in ecosystem dynamics and productivity; however, the mycorrhizal status of these materials needs to be evaluated. This study used a bait plants system to investigate the natural mycorrhizal inoculum potential and diversity of amending materials used for reclamation of Canadian tar sands tailings originating from the oil sand industry in Alberta. To evaluate the fungal inoculum potential and diversity, one-year-old jack pine and white spruce seedlings were transplanted onto two sites composed of tailings covered with amending materials: LFH, fresh peat, and stockpiled peat. After two growing seasons, seedlings were sampled for analyses. Besides using morphological typing, this study also used molecular typing to identify the ECM species present. Results demonstrated some level of inoculum potential and diversity in all amending materials. Results suggest that the indigenous inoculum levels of these materials are low and there is a need for pre-inoculation of nursery seedlings for revegetation of Canadian oil sand lands.

14.32 Impact of AMF on the community structure of bacterial diazotrophs and denitrifiers in the mycorrhizosphere

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Rhizodeposition, the output of organic C from roots to surrounding soil, is a key driver of microbial community structure and function in the rhizosphere. Ability to utilise specific rhizodeposit signals selects for a sub-set of microbial genotypes from bulk soil that play a critical role in processing rhizodeposit C and mediate interacting biogeochemical functions, for example, diazotrophy and denitrification. However, most studies regarding rhizosphere functional diversity have ignored the mycorrhizal status of the plant, despite the ubiquity and importance of the AM symbiosis. It is known that AMF colonization impacts (mycor)rhizodeposition through modification of root system architecture, alteration of exudation, and as a result of the activity and senescence of extraradical hyphae. Therefore, our hypothesis is that colonization by AMF (*Glomus mosseae* BEG 12) will impact the diversity of diazotrophs and denitrifiers in the (mycor)rhizosphere of *Plantago lanceolata*, a species important ecologically in the UK. Initial experiments characterized a soil which had been sterilized and re-inoculated with a filtered (<2.5 μm) suspension of indigenous bacteria, necessary to achieve soil free of indigenous AMF and to impose the *G. mosseae* treatment. Experiments using PCR-DGGE with primers targeting *nif*H (encodes nitrogenase reductase; N fixation) and *nir* (encodes nitrite reductase; denitrification) genes to dissect AMF impacts on functional diversity, will be discussed.

14.33 Interaction between ericoid mycorrhizal fungi and testate amoebae (Rhizopoda, Testaceae)

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Interactions between mycorrhizal fungi and soil protozoans and possible effects of such interactions on plant fitness have been so far studied only to a limited extent. This contrasts with worldwide distribution of both mycorrhizal fungi and soil protozoans, and their proposed key role in nutrient cycling in the rhizosphere, hence their importance for plant nutrition. Here we report our observations on the presence of testate amoebae (Rhizopoda, Testaceae) in the rhizosphere of ericoid mycorrhizal plants and their association with fungal mycelium emerging from those roots. We show that testate amoebae occur frequently in the rhizosphere of ericoid mycorrhizal plants, and that mycorrhizal fungi have the potential to utilize testae of dead amoebas as a source of nutrients.

14.34 Stimulated growth of EM mycelia in response to apatite amendment diminish after P fertilization when studied under field conditions

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Certain ectomycorrhizal (EM) fungi can stimulate mineral weathering to obtain nutrients from soil minerals. The contribution of EM fungi to total weathering is not known but it is probably influenced by the nutrient status of the trees. Forest trees exposed to P deficiency allocate more C to roots and to their EM symbionts, which will increase their potential to stimulate weathering of P containing soil minerals such as apatite. Most work to study the influence of EM fungi on mineral weathering have been performed under laboratory conditions but we have developed methods to study these processes under field conditions using in-growth mesh bags. In a study of 4 Norway spruce forests in south-western Sweden, with documented low P status, we found that apatite amendment stimulated the production of EM mycelia in mesh bags by approximately 60%. This enhancement of EM growth diminished when subplots of the forests were fertilized with P indicating that EM fungi only responded to apatite when the host trees where exposed to low P availability. This result emphasises the importance of controlling the nutrient status of the host tree when studying growth responses by their associated EM fungi. Rare earth elements originating from the apatite accumulated in EM root tips outside the mesh bags suggesting an EM effect on the dissolution of apatite. The composition of the EM community in mesh bags from this experiment is reported by Erland et al. at this conference.

14.35 The role of arbuscular mycorrhizal fungi in aggregate stability and soil carbon and nitrogen storage in tallgrass prairie

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Arbuscular mycorrhizal fungi (AMF) may be lost from soil through various agricultural practices such as conventional tillage, slash-and-burn, and excessive fertilization, or from natural ecosystems due to introduction of invasive species, increases in anthropogenic nitrification, and alterations in land use. These effects may influence processes at the ecosystem scale by altering the ability of AMF to aggregate soils and store carbon. Here we use a continuum of AM hyphal abundance to examine the role of AM fungi in soil aggregate stability, and carbon and nitrogen storage in grassland soils. Increases in AM hyphae (through N fertilization) were strongly correlated to increases in water-stable soil macroaggregates and C sequestered into the soil. Loss of hyphal networks (via fungicide applications) resulted in a loss of water-stable macroaggregates and a subsequent reduction of the C storage in the soil. While several previous studies have reported correlations between the production of macroaggregates and abundance of EMH, no study to date has observed a degradation of macroaggregates in response to elimination (or suppression) of EMH. Our study provides strong evidence that soil structure and stability, as well as enhancement of soil sequestration of C and organic N, are largely defined by the abundance of AM hyphae.

SESSION 15

Mycorrhiza in alternative production systems

Diversity of ectomycorrhizal fungi in wooded banks of different age in The Netherlands

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Wooded banks are characteristic for the agricultural landscape in The Netherlands and have a high ecological value. However, the economic value is often considered as low and a considerable number of wooded banks have been removed. From 2002-2006, the ecological and economic contribution of ectomycorrhizal fungi to wooded banks was studied. The economic contribution could be that edible mycorrhizal fungi can be sold by land owners as a delicacy for human consumption. This study focused on monitoring ectomycorrhizal fungi in two wooded banks of different age (3 and 60 years old) in The Netherlands. *Quercus robur* occurred in both wooded banks. This study investigated whether topsoil removal and addition of rain water could raise the above- and below-ground diversity of ectomycorrhizal fungi, including edible fungi. Fruitbodies were counted and molecular (PCR-based) analysis was used to describe below-ground ectomycorrhizal communities. Chemical composition of the soil was determined. Highest above- and below-ground ectomycorrhizal diversity was observed in the old wooded bank and was related to the low nutrient concentrations in the soil. The old wooded bank contained numerous ectomycorrhizal species, including edible species. Addition of rain water raised the number of ectomycorrhizal fruitbodies in the old wooded bank, especially in dry periods of season. Removal of topsoil was not effective within the first three years after application, but longer term effects are expected.

Biotechnology and cultivation of desert truffles: an agricultural tool for degraded semiarid lands

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Although *Tuber magnatum* and *T. nigrum* are the most studied and appreciated truffles, there is increasing interest in the commonly called desert truffles. Their geographical distribution is limited to arid and semiarid lands, particularly in the Middle East and Mediterranean-bordering countries. Numerous biotechnological procedures have been used to obtain elite host plants from the genus *Helianthemum* (*in vitro* micropropagation protocols, seed germination improvement and nursery cultivation conditions), a good fungal inoculum from *Terfezia claveryi, T. olbiensis* and *Picoa lefebvrei* (mycelium growth in bioreactor and good spore germination) as well as to obtain selected mycorrhizal plants. This type of mycorrhiza has been morphologically and molecularly characterized. Eight different plantations were established in the southeast of Spain from 1999 until 2004, using *H. almeriense* inoculated with *T. claveryi*. During the last five years, the carphophores have fructified yearly and production has been increased by adequate irrigation when necessary. *T. claveryi* reduced the negative effect of drought stress on *H. almeriense*, suggesting that this mycorrhizal symbiosis aids adaptation to semi-arid climates. This crop will improve, not only the quality of the land, avoiding soil erosion, but also the economy of social and economically depressed areas, thus increasing the value of these territories. This work was supported by Project REN2003-08241/GLO, MEC, Spain.

Practical application of endomycorrhizal fungi in China: retrospect and prospect

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Application of endomycorhizal fungi as 'biofertilizers' has been widely investigated in China, mostly on arbuscular mycorrhizal fungi (AMF) and orchidaceous fungi. Growth promoting effects of AMF inoculation have been found in various crops. For example, sweet potato yield increased by up to 10% following inoculation with AM fungi under field conditions. A mixed inoculum of *Glomus mosseae* and *Glomus versiforme* increased the yield of micropropagated potatoes by 21%. Similar effects have been demonstrated in watermelon. AMF can also influence plant quality, for example the minerals, sugar and carotene contents of sweet potato tubers. AMF inoculation has increased disease resistance in plants, for example Fusarium wilt disease of watermelon and cucumber. Preliminary studies have begun on the role of AMF in degraded ecosystems. Plant cover and plant density and biomass were much higher after inoculation with a mixed inoculum of *Glomus mosseae* and *Glomus etunicatum* in the Gurbantunggut desert, northwest China. Endophytic fungi enhanced he growth and medically important components of Chinese medicinal plants. Total alkaloids and polysaccharides in *Dendrobium nobile* were increased by endophytic fungi. Growth promoting effects on *Dendrobium candidum* and *Anoectochilus roxburghii* were related to the metabolites of the fungal hyphae. Seed germination rate and yield of *Gastrodia elata* were simulated by a strain of *Mycena dendrobii*. Future work on these topics will be discussed.

Alteration of the production of phytochemicals in sweet basil under the influence of the arbuscular mycorrhizal symbiosis

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Interest in herbal remedies and natural products has grown rapidly in recent years as a result of a large number of people seeking natural medicines and healthier food products. Phytochemicals found in comestible plants (herbs, vegetables, etc.) are a good source of natural antioxidants that are increasingly sought after in the natural product industry. As there is a growing interest to develop plant varieties with higher concentrations of phytochemicals, we aimed to assess the potential of arbuscular mycorrhizal fungi (AMF) to enhance the production of antioxidants in sweet basil (rosmarinic and caffeic acids, RA & CA) and tomato (lycopene and ,-carotene). A first experiment revealed that *Glomus intraradices* increased the biomass and concentrations of RA and CA in sweet basil after six weeks of growth. However, AM and non-AM plants had different phosphorus concentrations, which might lead to an indirect effect on phytochemicals production. After adjusting P nutrition so that P concentrations and yield were matched in AM and non-AM plants, a new experiment showed that *G. caledonium* increased RA and CA production in the shoots of basil. *Glomus mosseae* also increased shoot CA concentration in basil under similar conditions, but *G. intraradices* yielded similar RA and CA concentrations to non-AM plants. Further work is underway to screen for the production of antioxidants in a wild-type tomato (76R) and its mutant (*rmc*, which is AM defective) under different AM treatments.

Farming systems and carbon sequestration

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We discuss new understanding of traditional management of crops, using data from research of cotton production. Wet/dry cycles and fallow in cropping reduce the population of AM fungi in the lab. Colonisation of roots from extremely low densities of AM fungi is remarkably rapid in cotton. In the field, continuous drought for three years did not significantly reduce colonisation of roots at 6 weeks. Abundance of AM fungi is sustained for seedling growth and production of cotton. Molecular techniques indicate that diversity of AM fungi in cropping soils is similar in rotations and fallow for up to 7 years. AM fungi produce a recalcitrant putative protein called glomalin, measured as Bradford Reactive Material (BRM). BRM is reduced in cropping soils, but can it be increased by managing AMF?

Mycorrhizae inoculated seedling production systems in organic farming under greenhouse and field conditions

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Since the Mediterranean soils are commonly poor in availability of macro and micronutrients because of their high clay and lime contents, mycorrhizal inoculation seem to be a good strategy for sustainable plant growth. Under semi arid conditions, most of indigenous, field and horticultural plants are strongly mycorrhizal dependent. Since it is still very difficult to produce large quantity of mycorrhizal inoculum for arable land, it is sound to produce mycorrhizal inoculated seedlings. The experiments revealed that under field conditions, mycorrhizae spores effectively infected horticultural plants and mycorrhizae successfully resulted in better plant growth. Tree plants such as citrus and pistacia are highly mycorrhizal dependent and it is sound to produce mycorrhizal inoculated seedlings. The results showed that the mycorrhizal inoculated seedling significantly increased dry matter production, seedling quality, root infection and nutrient uptake. Mycorrhizal species were also different in term of their effect on seedling growth. It seems that for horticultural plant mycorrhizal seedling production is the most reliable method, but for field crops it is better to manage the soil and crop for sustainable agriculture.

15.1 Green manure leguminous and sunflower arbuscular mycorrhizal influence on sugar cane crop system in Piracicaba, Brazil

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Leguminous (Fabaceae) plants can be used for the supply, substitution and complementation of mineral nutrition, and soil fertility restoration. Besides the known symbiosis between those plants and the nitrogen fixing bacteria, their can do an association with arbuscular mycorrhizal fungi (AMF) to make up a triple symbiosis. This research aimed to evaluate the natural occurrence of AMF and it influence on green manure leguminous and sunflower. After the cultivation of those crops, sugar cane variety IAC 87-3396 (*Saccharum* spp.) was planted in the area in a randomized block design with eight treatments and five replications. Sugar cane production was measured during three consecutive cuttings. Greater AMF colonization was observed in peanut cv. IAC-Caiapo; sunflower cv. IAC-Uruguai; soybean cv. IAC-17; and velvet bean. It was observed a higher production of sugar cane in the fields previously cultivated with leguminous in comparison to the control. It was observed a higher production of sugar after cultivated area with peanuts, mung bean and valvet bean. There was a highly positive correlation between the greater AMF colonization and the height sugar cane plant in the first cutting, (P=0,0105 and R=0.52). These results suggest a strong positive relationships between microbiological occurrence and sugar cane growth.

15.2 Effect of *Azospirillum*, mycorrhiza and *Streptomyces* with manure on yield and yield components of wheat (*Triticum aestivum* L.)

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In order to study the effects of some biofertilizers with manure utilization,a research has confirmed by factorial experiment in completely randomized block design with 4 replications and 4 factors, *Azospirillum*, mycorrhiza, *Streptomyces* and manure(dung) was used in which 2 levels of each one (one with and one without) were applied. Results showed that *Azospirillum* and mycorrhiza, individually, caused significant increase in spike/m^2, grain/spike and grain yield. Also mycorrhiza increased 1000 GW significantly. Otherwise manure increased most of characters specially whean it used with *Azospirillum* and mycorrhiza. Results of threefold and fourfold interactions of experimental factors showed that use of *Azospirillum*, mycorrhiza and manure increased most of characters but there were a negative or antagonistic interactions between mycorrhiza and *Streptomyces* and there were not any antagonistic interactions between *Azospirillum* and *Streptomyces*.

15.3 Effect of a natural AM inoculum obtained by pre-cropping with either AM-host or non-host plants on soybean growth and P acquisition in natural soils

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A mesocosm experiment was carried under open greenhouse conditions to investigate the effect of a nuturally-generated AM inoculum, in interaction with soluble P, on biomass and P accumulation and AM formation by soybean plants grown in soils from three sites, i. e. Caazapá, San Pedro and Itapúa (Paraguay). A previous experiment was developed to induce stimulation of the AM fungal population naturally present in the test soils. For that, either a non-host plant (radish) or an AM-host (sunflower) was pre-cropped. The core experiment used pots containing 3.5 kg of soil in which radish was pre-cropped, either alone or added ("inoculated") with a 7 % soil in which sunflower previously grows. These AM-"inoculation" treatments were combined with the application of Triple Super Phosphate (TSP) at doses of 0, 1 and 3 g/pot. The benefits of AM "inoculation" on the accumulation of biomass and P by soybean plant and on spore production in soil (available for further cropping) were higher when soluble P was not added, but it was still effective in combination with the lowest P doses (1 g TSP/pot). For two of the three locations, adding 3 g TSP/pot (which also lowered the AM formation level) did not improve the growth benefit of the lowest dose (1 g TSP/pot). The results of this experiment therefore show that the use a nuturally-generated AM inoculum and a low level of P can support sustainability of agricultural systems.

15.4 Influence of soil physico-chemical characteristics on the capacity of a mycotrophic plant to weather rock phosphate in semi-arid soils

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Phosphorus is generally considered as one of the major abiotic factors limiting the agricultural production in West Africa. Large amounts of rock phosphates are frequently recorded in West African soils but they are generally slightly altered in neutral soils. Since it has been shown that some soil microorganisms (i.e. mycorrhizal fungi) could solubilize P from natural rock phosphate, management by mycotrophic plants of the Mycorrhizal Soil Infectivity (MSI) could combine a positive effect on soil microflora and, consequently, on its fertility and its relatively weak cost. In the present study, the impact of a mycotrophic plant (Medicago sativa L.) on the solubilization of rock phosphate from Khouribga (Morocco) was determined in controlled conditions with 4 different soils collected from Morocco. Results were compared with those obtained with a non-mycotrophic plant Brassica oleracea. The leguminous plant significantly improved available P content in some soils whereas in others, the effects of both plant species were comparable. The role of mycorhizal symbiosis and its associated microflora (mycorrhizosphere and hyphosphere microflora) in the biological processes involved in rock phosphate weathering is strongly dependent to the soil characteristics.

15.5 Bacteria associated with arbuscular mycorrhizal spores and their influence on growth of mycorrhizal and non-mycorrhizal plants

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Arbuscular mycorrhizal fungi (AMF) and their bacterial associates (AMB) are living components of the soil microbiota. AMB can be important for the mycorrhizal development, plant growth and disease protection. In our earlier study, we compared the cultivable populations of AMB from two types of AMF spores originating from rhizosphere soils of two plant species grown in field as monocultures. AMB were functionally characterized for their antimicrobial activity against potato pathogens. In the current study, AMB showing strong *in vitro* inhibition of *Rhizoctonia solani*, *Phytophthora infestans* and/or *Erwinia carotovora* were investigated for their impact on potato and rapeseed respectively in greenhouse. The purpose was to examine their compatibility and efficiency in plants with and without AMF. AMB belonged to *Stenotrophomonas maltophilia*, *Bacillus subtilis*, *Pseudomonas putida*, and *P. fluorescens* when identified by FAME profiles and molecular methods. The growth of potato and rapeseed was promoted in presence of some AMB and *G. mosseae* combinations. Impact of AMB inoculation on AMF establishment was compared with that of native AMF populations in potato in field. Our results demonstrated that the AMB from spores and antagonistic to pathogens, e.g. *P. putida* seems to be plant growth promotory (PGPR). Results on to what degree PGPR is also mycorrhiza helper bacteria and whether the interaction of PGPR with AMF is dependent on the bacterial isolate involved will be presented.

15.6 Combined vegetative micropropagation and mycorrhizal biotechnology to improve wormwood culture in the southern Alps of France

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Artemisia umbelliformis (wormwood) plants collected in natural and cultivated sites in the southern Alps of France showed no or a low level of root colonisation by arbuscular mycorrhizal (AM) fungi (between 0 and 38% and 0 and 12%, respectively for wild and cultivated plants). Under controlled conditions, mycorrhizal colonization of micropropagated wormwood used as trap plants in one soil collected from an alpine cultivated site reached only 20 % while it increased significantly to 60% in co-culture with nurse plants (*Medicago sativa* or *Trifolium pratense*). Therefore a method for the efficient production of alpine wormwood, based on microcuttings, and compatible with mycorrhizal inoculation has been developed in dual cultures with *T. pratense* using an appropriate AM fungus. This method will be presented together with ongoing experiments aimed to evaluate the impact of this technology on acclimatization, survival and growth of micropropagated alpine wormwood in nursery, and their outplanting in alpine agroecosystems.

15.7 Evaluation of plant resistance to white root rot in fruit tree rootstocks: A response dependent on the mycorrhizal status

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Plant breeding programmes for fruit-tree rootstocks include as a main trait the search for plant resistance to pathogens. Experimental results have demonstrated that plant inoculation with selected arbuscular mycorrhizal fungi can increase plant growth, enhance survival in replant situations and induce tolerance against soilborne pathogens. In this work, eight *Prunus* rootstocks were evaluated against *Rosellinia necatrix* Prill, a soilborne fungus causing white root rot. Pathogenic lesions in the root and the stem-end were monitored both in mycorrhizal plants inoculated with *Glomus intraradices* Schenck & Smith BEG 72 and in non mycorrhizal plants. Results showed that mycorrhizal colonization mitigates the severity of pathogenic symptoms and thus modifies the expression of resistance in plant materials against disease.

15.8 Performance of mycorrhizal grapevines in a replant vineyard infested with *Armillaria mellea* (Vahl:Fr.) P. Kumm

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The white root rot fungus *Armillaria mellea* (Vahl:Fr.) P. Kumm has been identified as the most damaging soil pathogen in Mediterranean replanted vineyards. The search for control alternatives to chemical treatments is focused currently on the use of beneficial soil microbiota, including soil antagonistic agents and arbuscular mycorrhizal fungi (AMF). Plants of the vine rootstock 110 Richter (*Vitis berlandieri* Planch x *V. rupestris*) were inoculated with three AMF, two indigenous fungi isolated from the wine growing area in the Northeast of Spain, identified as *Glomus intraradices* Schenck & Smith and *Glomus aggregatum* Schenck & Smith emend. Koske, and with *Glomus intraradices* BEG 72. Plant response was evaluated in a replant vineyard heavily infested with a highly virulent isolate of *A. mellea*. Although the three isolates evaluated significantly increased 110 R plant development under greenhouse conditions, only *G. intraradices* BEG 72 was effective in the infested field. Plant inoculation with this selected fungus increased plant growth and reduced transplant mortality during the first growing season.

15.9 Cryopreservation methods for long term conservation of root organ cultures of arbuscular mycorrhizal fungi

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Whatever the micro-organism considered, "preservation techniques range from continuous growth through methods that reduce rates of metabolism to the ideal situation where metabolism is halted" (Smith and Onion, 1994). Root organ cultures have increased in notoriety in the last decades with numerous species belonging to several genera successfully maintained on excised roots. This has resulted in the creation of GINCO (http://www.mbla.ucl.ac.be/ginco-bel), the first international collection of AM fungi strictly cultured in vitro. At present, root organ cultures are most often maintained through sub-cultivation. Although, this could be time-consuming and does not guarantee stability of the material over successive generations. Half a decade ago, cryopreservation was successfully achieved in GINCO with a strain of Glomus intraradices (MUCL 41835) (Declerck and Angelo-Van Coppenolle, 2000) opening perspectives for long-term storage of high quality material. Here, we review the different preservations techniques used for Germplasm collections of AM fungi. We also report on the extension of the cryopreservation process to various other species and genera of GINCO and proposed some refinements to improve the reliability of this technique.

15.10 Influence of green compost and mycorrhizal inoculum on plant growth, fruit production and quality of tomato (*Lycopersicon esculentum* Mill.)

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Green compost, produced from green wastes, is characterised by high quality standards. The influence of amendment with green compost and with an arbuscular mycorrhizal (AM) fungal-rhizobacterial mixed inoculum on plant growth and on yield and quality of fruits in tomato (*Lycopersicon esculentum* Mill. var. Guadalete) was studied. Inoculated and non-inoculated plants were transplanted in pots with soil added with different compost proportions (0, 25, 50, 75 and 100%) and cultivated for six months in a greenhouse. Tomato fruits were weighed and analysed for glucose, fructose, nitrate, nitrite, malic and citric acid, carotenoids and ascorbic acid content. The addition of compost to substrate increased shoot dry weight and fruit production, with the best results with inoculated plants and 75% compost. In general, fructose prevailed on glucose and citrate on malate. AM tomato plants showed significantly higher concentrations of glucose and malate in fruits. Citrate was higher with 50% and 75% compost, while its lowest concentration was detected in plants without compost. Higher amounts of compost increased ,-carotene, lycopene and lutein content; ascorbic acid levels were significantly higher in control plants. Nitrate content was increased by compost and AM inoculum; nitrite content was decreased. These results show that the use of green compost and AM inoculum can improve fruit quantity and quality, affecting biochemical composition and relative proportion of various compounds.

15.11 Root organ cultures (ROC) of arbuscular mycorrhizal fungi as a tool for Germplasm collections

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Germplasm collections, whichever micro-organism is concerned, are essential to preserve biodiversity. Arbuscular mycorrhizal (AM) fungi are particular soil micro-organisms which can not be cultured without a suitable host. As a result most culture collections are based on pot cultures, which present a number of drawbacks related, for the most important, to the purity and monospecificity of cultures. The emergence of the root organ culture technique and the increased number of strains maintained in such system offer a unique opportunity to enter AM fungi into a new era of quality management. With this objective, GINCO, the Glomeromycota IN vitro COllection (http://www.mbla.ucl.ac.be/ginco-bel), was created as the first international culture collection of AM fungi based exclusively on *in vitro* produced material. This collection contains approximately 30 strains belonging to *Glomus*, *Gigaspora* and *Scutellospora* . Since august 2005, GINCO has accepted to host a unique "sunbag" trap culture collection of more than 200 well described strains of AM fungi including type and ex-type species belonging to nearly all the families of Glomeromycota. Thanks to that richness, the challenge in GINCO is now to improve the classical *in vitro* techniques in order to offer the scientific community *in vitro* cultured type species belonging to different families. Here we present results (phylogeny, growth pattern, …) on these newly produced species and genera in ROC.

15.12 Arbuscular mycorrhizal fungi - weed interactions in different cover crop-based arable aystems.

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Most research on arbuscular mycorrhizal fungi (AMF) is focused on crop – AMF interactions but there are indications that AMF influence weed competitiveness in the field. This suggests that AMF may play a role in the ecological management of weeds in sustainable cropping systems. The aim of this study is to assess the influence of different cover crops on AMF colonization of the following crop in the rotation (maize) and on mycotrophic weeds growing in the field. A long-term field experiment based on a maize – winter wheat rotation started in 1993 at CIRAA 'E Avanzi', University of Pisa, Italy. The experiment is arranged as a split-split plot design with four replications. The main plot factor includes a Low Input System (LIS) and a Conventional System (CS), the sub-plot factor includes four nitrogen rates (0, 100, 200 and 300 kg N ha-1 in the maize crop), and the sub-sub-plot factor includes four soil cover types, namely three cover crops (*Vicia villosa*, *Trifolium squarrosum* and *Brassica juncea*) and a control without cover crops. AMF colonization of the cover crops and two mycotrophic weeds (*Sonchus asper* and *Poa* spp.) has been studied in the 2005/2006 growing season. Data from the LIS N0 and N200 plots show that *B. juncea* was not colonized by AMF and that the legume cover crops were strongly colonized. Preliminary data indicate that weeds in legume cover crop plots show higher AMF colonization than weeds in control or *B. juncea* plots.

15.13 Ecological and economic value of popular forests in The Netherlands: description of above- and below-ground diversity of ectomycorrhizal fungi

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Popular forests are characteristic for the landscape in the southern part of The Netherlands. Although the ecological value is high, the economic value is often considered as low. Therefore, these popular forests are threatened by possible removal for agricultural or urban use of the landscape. In 2005, a project was started to study the ecological and potentential economic value of ectomycorrhizal fungi contributed to popular forests. In the project, the above- and below-ground diversity of ectomycorrhizal fungi in two different popular forests in the southern part of The Netherlands was determined and related to abiotic soil conditions. Furthermore, possibilities were inventoried to raise the number and diversity of ectomycorrhizal fruitbodies, including edible fungi. Both popular forests were dominated by *Populus canadensis*. Observations releaved only a limited number of ectomycorrhizal fruitbodies in both forests. *Laccaria* species occurred to be the most abundant above-ground species. Molecular analysis with fungal specific primers revealed that the diversity of ectomycorrhizal fungi below ground was higher than observed above ground. The low number of fruitbodies observed can be explained by the relatively high temperatures in the autumn of 2005. The observed below-ground diversity suggest that there are some possibilities for increasing the economic value of these forests by the production of edible ectomycorrhizal fungi.

15.14 Effect of mycorrhizal inoculation and P rates on yield and quality of processing tomato

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The application of Arbuscular Mycorrhizae (AM) can be useful in agricultural sustainable systems. It has been demonstrate that AM association enhances phosphorus (P) uptake by plant roots. As a result of the increased P uptake, AM plants frequently yield more than without AM. The aim of this work was to evaluate the influence of P and mycorrhiza application on tomato crop performance. The research work was carried out in the province of Foggia, the most important area for this crop in Italy, where high P rates (200-250 kg ha^{-1}) are often applied to the crop. Three rates of P_2O_5 (0, 60, 120 $kg \cdot ha^{-1}$) were compared with (WM) or without (NM) AM inoculation in the soil with *Glomus intraradices* strains in a split-plot design with 3 replications. The transplanting was done on 3 May 2005 and the harvest was performed 99 days later. During the growing season, leaf area; fresh and dry weight of the leaves, stems and fruits were assessed. At the harvest, plant P-content, total and marketable yield were determined. The greatest fresh biomass (5.7 kg per plant) and total yield (127 $t \cdot ha^{-1}$) were obtained with 60 $kg \cdot ha^{-1}$ of P under mycorrhizzated conditions; the highest number of total and marketable fruits (71 and 61 per plant, respectively) and marketable yield (113 $t \cdot ha^{-1}$) were recorded with the WM treatment. Total P plant content was 2.4 g per plant on average, independently of P2O5 rates; significant differences between WM and NM plant were also recorded (2.7 vs 2.2 g per plant).

15.15 Higher diversity of AMF in Dutch organically managed onion fields than in conventional fields

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Onions (*Allium cepa*) form an important crop in Dutch organic farming. As onions are known for their arbuscular mycorrhizal (AM) fungal dependence, the AM fungal status of soils is considered of importance. Also, it is known that management regimes can influence the AM fungal status of the soil. To obtain a better insight in AM fungal diversity in Dutch arable soils and the factors influencing it, we carried out an AM fungal survey in cultivation areas at five organically and five conventionally managed onion fields. The fields were located at young soils in the Flevopolder and relatively old soils in Zeeland. Per field ten onions were sampled. Mycorrhizal colonization of onion roots was determined with staining techniques and molecular analysis was used to describe diversity of AMF. After DNA extraction, nested PCR reactions were applied with AMF-specific primers (GLOM5.8R, GIGA5.8R, GLOM1310, ACAU1660, ARCH1311 and LETC1670). In total, 15 different AM fungal types were observed and AM fungal colonization of onion roots was 84%. Interestingly, highest numbers of AM fungi were present in organically managed fields at the young soil. This suggests that organic farming can positively affect development of AMF in young soils while conventional farming can have a negative effect on AM fungal communities in these young soils. Furthermore, the data suggest that in relatively old soils, adoption of organic farming does not automatically lead to an increase in AM fungal diversity.

15.16 Arbuscular mycorrhizal colonization plants native of pitahaya (*Hylocereus undatus* Britt & Rose) in the dry tropics

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The objective of this study was to determine % of root colonization and spore populations of fungi AM in pitahaya growing on native tutorales plants and the low-input system called Family Gardens, in dry tropic. The pitahaya is a cactus native of the regions of Mexico and South America. The fungi AM are in most of the cultivated plants and native, their function is important for the water obtaining and mineral nutrients, nevertheless also help in the protection against the drought or attack of pathogens. The sites studied in field, are located in the Mexican state of Colima. The ground samples (0-20 cm) were collected in 24 sites of four diverse geographic zones during February and March of the 2006. The first examined condition was pitahaya in native tutórales plants in border zones to rivers and stream water. A second condition is the low-input system (Family Gardens), where pitahaya with other species of fruit trees coexists and the cultural practices are minimum. Root colonization was much higher and consistent in the low-input system than in native tutorales plants. The colonization levels of root pitahaya were from 62 to 31% in both conditions, nevertheless, a variation in each site was demonstrated. Spore abundance was higher in native tutorales plants (53 and 29 respectively), but showed constant variation. The results suggest a strong effect of the ecosystems on AMF of the plants of pitahaya in conditions of the dry tropic.

15.17 Characterization and histochemical localization of phosphatase activity from ascocarps of *Terfezia claveryi* Chatin

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Phosphatases are a group of enzymes which hydrolyze phosphate esters and their role in mycorrhizal symbiosis has been widely studied. *Terfezia claveryi* is an edible hypogeous mycorrhizal ascomyces called desert truffle. In natural ecosystems, it establishes symbiosis with species of genus *Helianthemun*. Before studying the phosphates in mycorrhizal plants we have characterized this enzyme activity in the fungus, and this is the first report on acid phosphatase activity from *T. claveryi* ascocarps. Both acid (ACP) and alkaline (ALP) phoshatase activities were detected using *p*-nitrophenylphosphate (*p*-NPP) as substrate. ALP activity was partially purified, precipitating with PEG_{8000}. With this method a substantial reduction in the lipid, phenolic compound and protein content of the extract was obtained. These phosphatase activities were kinetically characterized using *p*-NPP. Their optimum pH were pH 10.0 and pH 5.0, their K_m, 9.06 mM and 14.6 mM, and their optimum temperatures were 45°C and 35°C for ALP and ACP, respectively. The effect of different inhibitors on both phosphatase activities was also studied. Moreover, ACP and ALP activities were histochemically localized within *T. claveryi* ascocarps using the azo-dye method and the combination of BCIP and NBT reaction, respectively. This work was supported by Project REN2003-08241/GLO. MPG holds a contract from Programa Ramón y Cajal, MEC and FEDER.

15.18 "Mycorrhizal: the best friends of plants"

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"Mycorrhizal: the best friends of plants", is an educational brochure which explains in a clear and specific way, the features of the natives mycorrhizae (NM) genera found in the region, their isolation, identification and craft spread in plots. This publication is the result of the work developed in the Combeima´s canyon zone by the Tolima University, with participation of students and teachers of the Combeima Environmental Technique Institute in Villa Restrepo and the Mariano Melendro Agricultural Institute of Chapeton. The student community had the really active participation, motivated for their interest in the environmental maintenance and improvement of the natural resources. The results of this study give the opportunity to include the mycorrhizals topic in the scholar environmental projects and provided some laboratory materials and equipment in order to keep developing laboratory and field practices, and bring the possibility to continue with these activities. In this work were isolated NM genus Glomus, Gigaspora, Acaullospora, Entrophospora y Scutellospora in the plants species Cedrela odorata L, Ficus benjamina L, Bambusa arundinacea Retz, Cecropia peltata L, Coffea arabica L, Pisum sativum, Psidium guajava and Helianthus annus L. In this way Tolima University, World Bank and the Ibagué´s Government, contributed to the development of the environmentally sustainable technology and their social fitting in order to improve the life quality in the population of Tolima.

15.19 The effects of rock phosphate, compost and mycorrhizal application on citrus plant growth and nutrient uptake

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The use of organic and inorganic materials instead of chemicals are important in sustainable agriculture. This research tested the use of rock phosphate treated with compost as part of the substrate for production of citrus seedlings enabling the establishment and expression of mycorrhizal symbiosis. The experiment was carried out in a greenhouse at the University of Cukurova, Department of Soil Science. Three levels of compost 0,20,40 g compost/kg soil (4 kg soil each plot) and rock phosphate 0,2 and 4g rock phosphate/kg soil, were tested with different mycorrhizae species (as Control, *G. mossea, G. caledonium, G. etincatunium, G. clarum*, Indigenous mycorhiza and Cocktail) in the experiment. Results obtained after 180 days of culture showed that plants have higher growth in mycorrhizal plant then control plants. Since rock phosphate takes longer time to be dissolved or decomposed, it had no additional effect on plant growth and P and Zn uptake. All levels of compost had no effect on nutrient uptake but the use of 40 g compost had increased root inoculation. Inoculation of *G. mosseae* is the most efficient species for development of plants. According to the results, mycorrhizae were more efficiently compared with rock phosphate and compost on growth and nutrient uptake by the maize plant. A high increase in the uptake of phosphorus and zinc was determined. *G. mossea* was determined as the most efficient among the mycorrhizae species.

15.20 Inoculation of an avocado (*Persea americana* Mill.) clone of commercial interest with *in vitro*-produced, native arbuscular mycorrhizal fungi

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The prospective benefits of application of AMF as biofertilizers and bioprotective agents are enormous, and they perfectly fit within EU directives reinforcing and promoting sustainable agro-ecosystems management. Nevertheless the production of large-scale AM fungal inoculants of certified quality has been hindered up to now by the clear disadvantages presented by open-cultured AMF inoculants: i) it is almost impossible to certify the absence of undesired and potentially harmful microorganisms in them; ii) the microscopic nature of AMF and the non-transparent nature of soil-based substrates largely difficults for non-experts to confirm the product propagule-richness; iii) the absence of strict EU directives for AMF biofertilizer production and commercialization allows fraud to occur; and iv) the lack of technical experts for evaluating AMF commercial inoculants makes it almost impossible to detect such potential frauds. We should add to all this the potential risk of introducing foreign, exotic AMF isolates, often contained in commercial biofertilizers, in distant agricultural/natural soils which may compete with AMF natural populations, resulting detrimental for soil equilibrium. *In vitro* production of AMF may largely overcome these problems. We will present the results of some inoculation tests in which *in vitro*-produced native AMF strains were used on a micropropagated avocado (*Persea americana* Mill.) clone of commercial interest.

15.21 Arbuscular mycorrhizal fungi: the hidden help for sustainable shifting cultivation

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Shifting cultivation, one of the oldest forms of agriculture, remains the only means of livelihoods for many people in the mountainous region of Southeast Asia. Rotational shifting cultivation can be productive and sustainable when fallow regeneration has accumulated sufficient nutrients and effectively suppressed weeds and pests, usually requiring fallow periods of 10 years or more. This paper reports on how arbuscular mycorrhizal (AM) fungi contribute to productivity of a rotational shifting cultivation system on acidic infertile soil in Northern Thailand with the fallow period reduced to 6 years. A local fallow enriching tree, *Macaranga denticulata*, accumulated 8 times as much biomass, and 5-6 times as much nutrient with the AM fungi which occurs naturally, as without in an acidic infertile soil. Upland rice grown after 6 years of Macaranga dominated fallow yielded 2.62 t/h, compared with only1.32 t/ha where the trees were few. This was not a pre-existing difference because when *Macaranga* dominated fallow was slashed, burned and cropped after only 3 years the rice yield was only about 1 t/ha. The benefit of AM fungi on rice yield is likely to be indirect via *Macaranga*. Upland rice has shown no response to AM fungi although 40-60% of its roots were infected by the fungi in the field. Coix, sorghum, maize, sesame and cowpea grown in this system also responded to the AM fungi. The role of AM fungi is sustainable land use should be more closely examined.

15.22 Project AGRO 689: *In vitro* mycorrhization of *Castanea sativa* and *Pinus pinaster* plants

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The present work concerns results from the Project AGRO 689 "Agronomic, economic and environmental interest of macrofungi associated with pinus (*Pinus pinaster*), chestnut (*Castanea sativa*) and oak (*Quercus pyrenaica*), in the Northeast of Portugal: Production of mycorrhizal pinus, chestnut and oak plants". We intend to contribute to the study of the capacity of two ectomycorrhizal fungi, *Pisolithus tinctorius* / *Boletus edulis* and *Pisolithus tinctorius* / *Lactarius deliciosus* to establish symbiosis with, respectively, *Castanea sativa* and *Pinus pinaster* roots, under *in vitro* conditions, either alone or in co-culture. Evaluation of their effects on plant growth is also being performed. Fungal growth and fungal association with the root system (mantle and Hartig net formation) was monitored at regular time periods. Growth rates of mycorrhizal and nonmycorrhizal plants were determined. This *in vitro* system enables us to compare growth capacity of each fungus, alone and in co-culture, in the presence or absence of the plant, as well as the establishment of mycorrhization. Differences in fungi growth were observed when growing alone or in co-culture. We discuss the preliminary results of mycorrhization with one and two fungi, both in terms of structure and growth of the symbionts.

15.23 Is microbial inoculation a feasible biotechnology for revegetation of spoilbanks?

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The aim of our study was to find out the potential of microbial inoculants – arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria (PGPR) – to reduce duration and costs of agricultural reclamation of coal-mine spoilbanks in northwestern Bohemia. Growth effects of mycorrhization were tested for *Medicago sativa* and high biomass crops *Canabis sativa*, *Linum usitatissimum* and *Phalaris arundinacea*. Plants were cultivated in spoilbank substrate with or without organic additives (compost, lignocellulose) commonly used within spoilbank reclamation. High mycorrhizal colonisation potential was determined for spoilbank substrate; however, inoculation significantly increased mycorrhizal colonisation. Addition of compost in commonly used amounts almost eliminated the formation of mycorrhiza, whereas addition of lignocellulose in corresponding dose did not have any inhibitory effect. Therefore, the compost dose was optimised to maintain its nutritional effects on the crop plants without inhibiting the development of AMF. Mycorrhizal growth response was neutral to positive depending on plant species, with the highest benefit for *Linum*. Co-inoculation with PGPR did not influence the effect of mycorrhiza on plant growth, nor changed the level of root colonisation. The results will be tested *in-situ* in a field experiment. The authors are grateful to the Ministry of Education, Youth and Sports of the Czech Republic for financial support within the project IM6798593901.

15.24 The arbuscular mycorrhizal symbiosis as soil quality indicator in forage maize agroecosystems

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Soil quality is one of the main factors affecting agricultural sustainability. Several physical, chemical and biological characteristics of soils have been proposed as environmental indicators of soil quality. Arbuscular mycorrhizal (AM) fungi are usually present in natural soils, but can be negatively affected by agronomic practices when soils are cultivated, thus potentially being key indicators of soil health and agroecosystem sustainability. In this work, forage maize was used as crop plant in three field experiments to study the effect of a conventional mineral fertilization management and the fertilization with dried broiler litter (a by-product of the poultry industry) on the principal physical and chemical properties of the cultivated soils, and on the colonization of maize roots by the indigenous AM fungal population. Soil samples were taken at three soil depths: 0-15 cm, 15-30 cm, and 30-45 cm. Fertilizer treatments mostly affected the upper 30 cm of the soils, resulting in increased levels of P, K, Ca, Mg, and micronutrients respect to the soil layer at >30 cm depth. Maize roots were extensively colonized by AM fungi under both fertilization managements both in the 0-15 cm and the 15-30 cm layers, whereas the percentage of AM colonization in roots taken at >30 cm were significantly lower, suggesting that the AM symbiosis was influenced by factors other than soil nutrient availability.

15.25 The arbuscular mycorrhizal fungi of soils derived from volcanic ashes in Mexico

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In the last 10 years has been carried out the investigation on the symbiosis of arbuscular mycorrhizal fungi in several wild and cultivated species grown in soils derived from volcanic ashes (Andisoles), of the State of Mexico. These soils are acid, fix high concentration of phosphorus, present deficient in nitrogen and rich content of organic matter of low mineralization. The presence of the arbuscular mycorrhizal fungi was analyzed in leguminous wild and cultivated in several towns of the state, as well as the efficiency of the arbuscular mycorrhizal symbiosis to colonize the roots from the cultivations when modifying the characteristics from the soil when applying fertilizing and compost. Experiences are also presented with the inoculation of arbuscular mycorrhizal in cultivations of corn, bean, tomato, beetroot and coriander, in those that it was evaluated the yield and the mycorrhizal colonization. The inoculum of arbuscular mycorrhizal fungi from Andisoles was efficient, since it increased plant growth and yield in the above mentioned crops. The inoculated was constituted of genus *Glomus, Acaulospora and Scutellospora*. +Universidad Nacional Autónoma de México. Facultad de Estudios Superiores Zaragoza. °Centro de Edafología, Colegio de Postgraduados, Montecillos Edo. de México.

15.26 Study of environmental risk of phosphate rock application in relation to toxic element uptake and AMF colonization of red clover host

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Phosphate rocks (PRs) could be economic P sources in moderately or strongly acid soils. However, PRs application may cause soil contamination due to their heavy metal and other potentially toxic pollutant content. Arbuscular mycorrhizal fungi (AMF) have an important role in improving plant nutrition, vitality and fitness. However high concentration of available phosphate restricts the AM formation, but it is the symbiosis that could inhibit the metal accumulation in host plants. Therefore the analysis of PRs effect is necessary from the agronomic efficiency and the environmental protection points of view, too. The effect of Senegal and Morocco PRs was investigated on root colonization of indigenous AMF, biomass production and metal uptake of red clover in a pot experiment on an acidic sandy soil at 0, 100, 400, and 1600 P_2O_5 mg kg^{-1} dry soil. The shoot yields gave a better response to the PR from Morocco than to the Senegal PR, which had a low specific surface, P solubility and high metal content. The effect of various PR levels on arbuscular richness was more pronounced than on the frequency of infection. Compared to controls, significantly higher arbuscular richness was found at Senegal PR rates of 100 and 400 mg total P_2O_5 kg^{-1} soil. The AMF colonization decreased Cd, Ni, Pb and Cr contents of the shoots, as compared to non-infected plants at both PR treatments. Only Cd concentration of shoots increased significantly in proportion to levels of Senegal PR.

15.27 Community composition of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilisation trial in Sweden

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We investigated the impact of organic and inorganic fertilisers on the AM fungal and bacterial community in the mycorrhizosphere of maize. Roots and root-associated aggregates were sampled four times during the growing season, in unfertilised plots and in plots amended with $Ca(NO_3)_2$, $(NH_4)_2SO_4$, green manure, farmyard manure, or sewage sludge. Community composition of AMF in roots and bacteria in soil aggregates were characterised using cloning and sequencing of fungal 18S rDNA and PCR-TRFLP of bacterial 16S rDNA, respectively. Both richness and the occurrence of specific AMF and bacterial taxa were influenced by the treatments. Fewer AMF and bacterial taxa were detected in plots amended with $(NH_4)_2SO_4$. There was a significant reduction in the occurrence of *Glomus intraradices* and six different bacterial taxa in plots fertilised with $(NH_4)_2SO_4$. In contrast, amendment with $Ca(NO_3)_2$ had a positive effect on *G. intraradices*, and $Ca(NO_3)_2$ and sewage sludge had a positive effect on bacterial taxon richness. Ordination analyses revealed that bacterial community profiles of the $(NH_4)_2SO_4$ and sewage sludge, were markedly different from other treatments, and that these two treatments were in addition different from each other. Although pH explained most of the changes in the bacterial community composition, other environmental variables were also correlated with the species composition. Time of sampling and harvest influenced the bacterial community composition, but not the richness.

15.28 Mycorrhizal colonisation of willows in plantations and adjacent natural stands

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Fast-growing species of willow (*Salix* spp) are increasingly used in coppice plantations for the production of biofuel. Willow species can host both ecto- and arbuscular mycorrhizal fungi. The degree of colonisation and types of mycorrhizal fungi on willow roots may have an impact on pest- and disease resistance as well as on productivity, and it is therefore of interest to study whether the degree and diversity of mycorrhizal colonisation of willows in plantations differs from that of naturally occurring willow species in the same area. Four willow plantations in the vicinity of Uppsala, Sweden were sampled for the study of ecto- and arbuscular mycorrhizal colonisation. Three natural willow stands adjacent to the plantations were sampled in the same way. Preliminary results show that both the degree of mycorrhizal colonisation and the diversity of ectomycorrhizal fungi are, in general, lower in the plantations compared to adjacent natural stands of willow. Species specific differences in the willows as well as stand age may be of importance for explaining these differences, but these may also be a result of management regime and clone selection in the plantations. Willow clones for biofuel production are mainly selected for their above-ground biomass production and this, in combination with a regular cutting and removal of above-ground plant parts, may negatively influence the belowground carbon allocation and thus the microbial community.

15.29 AMF inoculum cultured on-farm from soil gathered under local relict vegetation can enhance crop and tree growth in low-input semi-arid agriculture

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In semi-arid areas, where soil is degraded through loss of vegetation cover and erosion, AMF populations can become impoverished to the extent that the soil's infection potential falls below the threshold level necessary for optimum colonisation of host plants. This deficiency in AMF is a key factor in a vicious circle which can thwart attempts at revegetation and low-input crop production in semi-arid climates. In these circumstances reintroduction of the native soil biota, particularly AMF, can be beneficial. We are using a simple pot culture technique (the "Munro method") to multiply mixed, locally adapted indigenous AMF populations collected from nearby forest soil or pockets of relict vegetation. The inoculum thus produced is used in local reforestation and crop production projects. We currently have trials in Almería province, SE Spain, Tanzania and Burkina Faso. Our Spanish project involves both crop and revegetation trials, whereas in Tanzania and Burkina Faso the technique is primarily used to enhance the establishment and growth of trees of ecological and economic value in agroforestry contexts. Results so far show a consistent advantage from inoculation. The technique is recommended for village tree nurseries or small farmers. Keywords: indigenous, pot cultures, semi-arid, low input, crops, agroforestry, revegetation

15.30 Mycorrhization of *Pinus halepensis* Miller using different substrate mixtures of treated sewage sludges and paper waste in the nursery

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The agronomic use of treated sewage sludge has increased lately because it represents an excellent source of organic matter and nutrients for plants. However, the variety of its origins means that the sludge composition also varies, and government regulation is required for its use as a substrate. The paper industry produce a huge quantity of waste material containing cellulose, calcic carbonate, caolin and other residues is produced. In the present present work, we evaluate sludges from two treatment plants, as well as an industrial waste from the paper industry for use as a forest substrate in conjunction with ectomycorhizal fungi. *Pinus halepensis* was seeded in the sludges and the paper waste (except the controls) at three different concentrations (10%, 25% and 50% v/v) mixed with peat and pine bark. The efficiency of two fungi, *Pisolithus tinctorius* (Mich.: Pers.) Coker & Couch, and *Lactarius deliciosus* (Linnaeus ex Fries) S.F. Gray, was evaluated for their contribution to pine development in all the treatments. The shoots of the seedlings from each different treatment were oven dried and the nutrient content was studied. In general, the pines did not respond positively to mycorrhization and only the treatments containing *P. tinctorius* showed mycorrhizae. Contaminations appeared for both, fungi and controls. Further research is needed to elucidate the factors affecting inoculations using these substrates to obtain good infection rates.

15.31 Necessity of AMF for the transfer of phosphorous from animal-bone-charcoal to plants

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Only a limited percentage of livestock biomass can be consumed by humans directly. In the EU during slaughtering and food processing from e.g. cattle about 1 million tons/year bone by-products have to be handled. Bone meal can be processed at high temperatures under exclusion of oxygen to animal bone charcoal (ABC). This sterile material contents high amounts of calcium and phosphorous and only low contaminations with heavy metals. The P-fraction in the ABC is not easy available to plant roots. This makes ABC interesting as a P-fertilizer for organic and low input farming vegetable production. Greenhouse experiments with different mycorrhizal and non-mycorrhizal plants are shown regarding growth and P-uptake in quartz-sand with ABC as the only P-source or in soil. Dependence on AMF as a P-bridge from ABC varied between host plants. Non-mycorrhizal plants being able to exude high amounts of organic acids into the rhizosphere obviously are much more able to take up P from ABC than others. However, in the latter plants growth and P-uptake can be increased dramatically in the presence of AM.

15.32 *Helianthemum violaceum* Pers., a new host plant for mycorrhizal desert truffle plant production

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Terfezia claveryi establishes mycorrhizal symbiosis with species of the genus *Helianthemum*. The species *H. almeriense* has already been micropropagated and successfully *in vitro* and *in vivo* inoculated with desert truffle mycelia for mycorrhizal plant production by our group. *H. violaceum* was selected as a new host plant because it colonizes different territories to *H. almeriense* and both could be used in desert truffle plantation programmes in Spain. Moreover, truffle formation is related to plant flowering and *H. violaceum* is characterized by its high spontaneous *in vitro* flowering. After micropropagation, the rooted explants were *in vitro* inoculated with *T. claveryi* mycelium and *in vivo* inoculated with spore suspensions. The *in vitro* mycorrhization percentage was measured after two and three months (47 % and 51 % respectively), while the *in vivo* mycorrhization percentage was 62 % after five months. The differences observed were probably related to the phenolic compounds measured in the root cells in *in vitro* conditions. Moreover, the type of cell colonization depended on the mycorrhizal culture conditions, and was more intracellular in *in vitro* than in *in vivo* conditions. This work was supported by Project REN2003-08241/GLO from MEC, Spain.

15.33 A study on effects of two AM fungi on growth of cassava (*Manihot esculenta*) in cassava fields in South China

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The colonizations of two mycorrhizal fungi, *Glomus intraradices* (Gli) and *G. manihotis* (Glm), to the roots of Cassava (*Manihot esculenta* Crantz) and their mycorrhizal effects were studied by field trial in two experimental sites in South China between 2002-2004. The result showed that the treatments with inocula and the control could form arbiscular mycorrhizae (AM) and the mycorrhizal infection rates were high. It was indicated that the indigenous AM fungi were actively involved in the formation of cassava mycorrhizal associations. The effects of the two AM associations varied with site condition. In the successive planting site, the treatment with Glm and mixed inoculum had higher growth than the control. The total biomasses of these treatments were 11.4-27.0% higher than the control. By contrast, the treatment with Gli had poorer growth than the control. In the new planting site with poor soil fertility, the treatments with the inocula had higher tuber yields than the control. That with Gli showed the best growth and tuber yield, which were 61.8% and 22.3% higher than the control. Those with Glm inoculum were 36.3% and 20.6% higher than the control. The tuber yield and total biomass of the mixed inoculum were similar to the control. Further study of the ecological mechanism of these mycorrhizal effects is required.

SESSION 16

Improved inoculum technology and implementation

In vitro mass production technology for arbuscular mycorrhizal fungi: Scientific and industrial aspects

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The axenic cultivation system has become a valuable tool to produce contaminant-free arbuscular mycorrhizal (AM) fungi, allowing the realization of large-scale production under strictly controlled conditions. The *in vitro* mass production technology developed by Centre for Mycorrhizal Research (CMR), has established its importance in Indian and Middle East region. The technology has been transferred to five Indian industries. The industrial production has increased from 70 to 2500 tonnes of mycorrhizal biofertilizer annually during past years. One large-scale use of the mycorrhizal product developed through this technology is in south of India, where 17,000 sugarcane farmers are using this product. TERI has made significant progresses in mass production of AM fungi as advanced versions on a semi-synthetic medium under sterile environment. One technological breakthrough is the co-cultivation of multiple species (three or more) on the same host and another forthcoming advancement of AM fungal species is developed through hyphal fusions, which are capable of performing diverse functions.

Different arbuscular mycorrhizal inoculant technologies used under tropical and subtropical conditions. A study case on a Cuban experience

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The benefits of arbuscular mycorrhizal (AM) association are widely known, mainly the great impact that it has on the ecosystem productivity. The present status of AM application is not far from the practical technology used in the field today. The optimization of appropriate inoculation technologies and the use of effective AM strains that favors an adequate symbiosis relationship is a possible approach for its exploitation. It has been the main objective of our work team, who for more than 20 years has developed mycorrhizal inoculants to be applied in different agricultural models. Such contribution is about the results of the program of using and developing different types of Cuban mycorrhizal inoculants: MicoFert® and EcoMic®, a solid formulation containing specific AM fungal-colonized soil and colonized root fragments. Rates from 5 to 10 g.plant^{-1} are used to inoculate seeds, micro-propagated plants transplanted in multi-pots, polyethylene bags or pots; in the case of EcoMic®, it was specifically designed to seed dressing with a little amount of inoculant, using about 6-10% of the total seed weight used per hectare. More recently, LicoMic®, a liquid AM spore suspension was developed, that is mainly applied through irrigation systems in protected crops. Their essential production process should also be considered and the principal results obtained with their use under different types of soils and application technology both in Cuba and Latin-American countries.

Do commercial mycorrhizal inocula need to be tuned for target environments?

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There are differences in specificity of various mycorrhiza types to plant species, however, there is also a strong fungal strain specificity to environment. Geographical isolates of mycorrhizal fungi are either adapted or at least tolerant to particular soils and climate they originated from. There is certainly a large plasticity and ability to adapt to new conditions, however, if mycorrhizal technology (i.e. introduction of inocula) is being used in highly specific conditions such as degraded ecosystems, artificial cultivation media (e.g. soil-less substrates), the tolerance of fungi to soil features should be taken into account. That implies that the efficient and successful use of generic commercial inoculum products or even single isolate products for all type of soils and environments has its limitations. Several applied studies showed either failure to implement generic product containing non-adapted fungi or successful application of those adapted ones in more extreme environment. Protocols for commercial inoculum production of the EcM fungi, ericoid mycorrhiza and AM fungi are discussed in respect to production process and expected outcome of inoculum implementation in particular in degraded ecosystems. Examples of laboratory tests as well as large-scale applications will be presented to assess potentials of mycorrhizal technology in plant production and planting schemes. Support of Ministry of Education, Czech Republic, grant No. 1M6798593901 is acknowledged.

Challenges for development of mycorrhizal inoculants adapted for specific markets

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Mycorrhizal fungi provide numerous advantages to different categories of plants allowing a large range of applications to different market sectors. For example, mycorrhizal inoculants are currently developed for agriculture, professional horticulture, forestry, restoration and home gardening. Since each market has its own specifications in term of cultural practices, equipment and application methods, it is necessary to develop different products specifically adapted to users' needs. In order to ensure that mycorrhizal inoculation will become a common practice when growing plants in future years, technical challenges have to be addressed by the scientific community as well as the industry. A better understanding of the impact of different agronomic parameters on the performance of mycorrhizal inoculants in the field is required. Also, it is important to improve our knowledge of factors that can influence survival, conservation, germination and resistance of mycorrhizal propagules in order to improve product formulation. Finally, the development of methods for evaluating inoculant quantity and quality is needed to establish standards in this growing industry.

Improved method for inoculation of *Populus tremula* with ectomycorrhizal fungi

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European aspen (*Populus tremula*) is one of the most widespread tree species in the world. It is a fast growing pioneer tree and grows on diverse soils ranging from shallow soils with poor nutrient status to fertile mineral soils. It forms mycorrhizal associations with various ectomycorrhizal fungi. These fungi are known to affect the bioavailability and uptake of both essential and non-essential elements. Some aspen clones have been found to accumulate heavy metal elements beyond the hyper accumulation threshold. Thus European aspen is a promising candidate for use in phytoremediation. *Paxillus involutus* is one of the most studied ectomycorrhizal fungi. It has a wide host range and forms functional mycorrhizas with various gymnosperm and angiosperm tree species. It has also been found to form mycorrhizas with several *Populus* species and hybrids, but mycorrhiza formation in association with *P. tremula* under in vitro conditions has so far not been reported. Initial experiments using numerous published methods for mycorrhizal synthesis resulted in no functional mycorrhizas. A series of further experiments was therefore set up to develop a successful inoculation procedure, particularly concentrating on adaptations of plant propagation and the composition of the synthesis medium. Successful mycorrhization was evaluated under a dissecting microscope. A detailed analysis of semi thin sections confirmed characteristic structures of mycorrhizal associations.

Field inoculation with AMF in agriculture and rehabilitation. Integration of technologies

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Sustainable land use requires that soil management practices be adopted to conserve and increase soil resources. The effects of AMF in agriculture, horticulture and land-reclamation are almost exclusively beneficial. AMF also play a key role in ecosystem sustainability. AMF inoculation can enhance crop productivity in the field and help crops respond to soil borne pathogens and fertilisation regimes. Field inoculation with AMF has for a long time been limited to small-scale high value crops, however because of the advances in AMF inoculum production the application of larger quantities of inocula is now possible. Successful field inoculation in agriculture and rehabilitation of degraded areas has to consider intrinsic characteristics of the plant and target soils besides other issues that involve the integration of inoculation with already established agronomic practices. The effect of applying AMF inoculum at different stages of the development of a crop has been evaluated under field conditions for several Mediterranean woody crops. Hydroseeding is a technique increasingly used to introduce vegetation on large degraded areas, such as large-scale road construction sites and quarries. Native grasses and legume species are used as a first step in the recovery of such sites, prior to the establishment of native forbs and shrubs that occurs at a slower pace. The effect of adding AM inoculum to a hydroseeding mixture was investigated both under greenhouse and field conditions.

16.1 Reclamation of industrial wasteland and development of green cover using mycorrhizae: a demonstration at two industrial sites in India

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Wasteland reclamation was carried out at two industries, a cane-molasses based distillery and a small-scale agro-residue based paper mill using *in vitro* mycorrhizal technology for the establishment of selected plant species. Commercially viable economic plantation like bamboo, *Gliricidia*, *Acacia*, *Saccharum*, and biofuel plantations of *Jatropha curcas* were planted and established on these sites using mycorrhizal technology. After four years of technology implementation at distillery and eighteen months later at paper mill, an evaluation was made of soil physico-chemical parameters, plant and microbial diversity and mycorrhizal infection of plant community developed. The vegetation was healthy and regenerating by vegetative means. On the treated site, plant species richness was much higher and several perennial herbs and shrubs had grown. Most of these were mycorrhizal thus establishing the presence of a mycorrhizal network in the system, which helps in developing plant cover on a completely barren and vegetation-devoid terrain. Another advantage was prevention of groundwater contamination due to leaching. Presently both the sites have been turned into commercially viable area with restored green cover with enhanced loading of effluent in unit land area, lower level of groundwater contamination, and reduced land pollution of adjoining agricultural lands.

16.2 Protective effect of the AMF in the "Grand Nain" banana clone

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In the production of "Grand nain" banana clone, the nematodes are one of the main problems. Likewise, it has been demonstrated that the employment of AMF and benefical bacteria (PGPB) besides improving the aspect of the plant, improve their sanity and increase their tolerance to different stress types. Using plants of "Grand nain" banana clone propagated *in vitro* and inoculated with *Glomus intrarradices* and/or the PGPB 11B, we studied if this benefical microorganisms (BM): a) contribute to diminish the susceptibility to the nematodes, b) if the time of colonization has some influence, and c) if the activity of the peroxidase was modifies. As expected, the population of BM was increased with the time, reaching values, for the AMF, among 12.4 to 46% (depending on the treatment) and, for the PGPB,> 107 CFU/g of root. Also, we find a marked reduction in the population of nematodes in the plants inoculated with the BM (between 26.7 and 77.1%, depending on the treatment), influenced primarily by the AMF. Likewise, we found that the times of colonization of the BM and the time of maturity of the roots, were important factors to decrease the susceptibility to the nematodes. This way, we found 1.38 and 1.91 times more resistance in more mature plants and for a bigger time of colonization of the BM, respectively. On the other hand, the peroxidase activity was increased in the plants inoculated with the BM (among 1.44 to 2.49 times depending on the treatment).

16.3 Persistence of *Lactarius deliciosus* mycorrhizas in the roots of nursery-inoculated *Pinus pinaster* seedlings planted in two locations in Soria (Spain)

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Two field plantations of *Pinus pinaster* Ait. seedlings, mycorrhizal with *Lactarius deliciosus* Fr., were established in the spring of 2003 in two burnt areas of the municipalities of Tardelcuende and Matamala (Soria, Spain). In both plantations, seedlings were divided in two lots, A and B, according to the nursery grown conditions, the seed origin and the inoculated fungal strain. Lot A was inoculated and grown in IRTA and lot B in MICOFORA nursery (MICOFORA S.L., Girona). At plantation, the percentages of *L. deliciosus* mycorrhizas in inoculated plants of lots A and B ranged 51-57% respectively. Both plantations were established following a randomized block design with 5 blocks and 4 treatments, inoculated and non-inoculated seedlings of lots A and B. In spring and autumn of 2004, 4 pines of each treatment were examined to determine the persistence of *L. deliciosus* mycorrhizas, and the presence of other ectomycorrhizal fungi in the roots of inoculated and control plants of both lots. In the two locations *L. deliciosus* ectomycorrhizas had high persistence rate in pine roots. In the Spring of 2004, seedlings of lot A had in average a 80% of mycorrhizas of *L. deliciosus* , while seedlings lot B had 40%. In Autumn of 2004, all seedlings maintained percentages of *L. deliciosus* mycorrhizas arround 45%, without significant differences between lot origin or field location. *Cenococcum geophylum* and *Suillus variegatus* were the most abundant ectomycorrhizal competitors in both locations.

16.4 Physiology of ectomycorrhizal conifer seedlings in saline and sodic habitats

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The physiological response of ectomycorrhizal (ECM) white spruce (*Picea glauca* (Moench) Voss) and jack pine (*Pinus banksiana* Lamb.) seedlings to sodic stress was evaluated to determine the benefits of inoculation for growth and survival. These experiments aimed to improve nursery seedling production for revegetation of reconstructed soils from tailing sands originating from the oil sand mining in Northeastern Alberta (Canada). Initially, we showed that the inoculum potential of reconstructed soils of different ages and materials used in soil reconstruction was very low or null. Secondly, the resistance and physiological response of five ecto- and ectendomycorrhizal fungal species (*Hymenoscyphus* sp., *Phialocephala* sp., *Suillus tomentosus* (Kauff.) Sing., Snell and Dick, *Laccaria bicolor* (Maire) Orton, *Hebeloma crustuliniforme* (Bull) Quel.) from different sources were assessed in vitro. Finally, white spruce and jack pine seedlings inoculated in greenhouse with the three ECM fungi evaluated in vitro were exposed to different concentrations of NaCl. Using several physiological indicators, mycobionts were shown to influence host response to a given NaCl concentration. Under sodic conditions, inoculation with an isolate of *S. tomentosus* from a saline and sodic site increased seedling biomass production and inoculation with an isolate of *H. crustuliniforme* increased seedling osmotic adjustment capabilities. The latter two fungi are potential candidates for tree nursery inoculation.

16.5 Evaluation *in vivo* of light emitting diode (LED) effects on AMF development in the rhizosphere of bahiagrass (*Paspalum notatum* Flügge.)

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Light could induce hyphal growth and branching of AMF (Nagahashi and Douds, 2004; Yachi et al., 2001). This experiment was done to evaluate the best combination of red and blue LED to stimulate AMF propagules formation in the rhizosphere of bahiagrass, following previous results found *in vitro* by Ishii et al. (2003). This experiment was carried in a greenhouse with special acryl pots separated in 5 sections, where in the extreme ones was planted bahiagrass and in the middle about 60 spores of *Gigaspora margarita* were inoculated. In the two sections between the middle and the extremes were added glass beads (1mm diameter). In these glass bead sections there were two kinds of LED: red (R) and blue (B), and control pot was prepared by keeping these sections in the dark (D). Another pot was prepared with this treatment: R for 80 days, R and B for 15 days and then only B for 15 days (R-B). The whole experiment was conducted for 110 days, and the spore formation in the glass bead sections, AMF colonization in bahiagrass roots and the hyphae density were evaluated. The new spores formed had a diameter of around 150Ìm. The R-B treatment promoted a higher spore formation in both sections with glass beads and soils with bahiagrass. However, the highest hyphae density in the bahiagrass sections and AMF colonization had their values decreased by the blue light as compared to the red, whereas the D and R-B pots has no significant difference.

16.6 Management of the arbuscular mycorrhizal liquid inoculant, LicoMic under different crop systems

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The arbuscular mycorrhizal inoculant applied by liquid way is a challenge, due to the low osmotic protection of the fungal spores under an adverse media, as it's a case of water carrier. The study has the goal to the managing the arbuscular mycorrhizal liquid inoculant LicoMic (*Glomus* sp4), under different crop systems. In the study, four crop systems were selected: tomato and lettuce under localized water supply, tomato under field conditions, rice under flooding conditions and direct sowing of sorghum and maize crops. In all cases, a control was used to compared with LicoMic. The inoculant was applied at 20 spores. plant^{-1}. Several variables, such as growing dynamics, mineral contents, polyamines, yield and fungical were measured. Results showed a good functioning of symbiosis with this kind of inoculant. Significant crop yield increases were obtained at the inoculated treatments, from 20 up to 45 %, even in lettuce and rice under strong soil salinity conditions. A consistent response to mycorrhization was recorded, with high colonization (43-80%), plant mineral contents and polyamines concentration, mainly in the rice flooding system, where a low colonization rate was expected and surprising high values of colonization closely to 45% were obtained. Furthermore, it was really important to comment only the presence of arbuscular fungal phase with the total absent of vesicle formation in rootles during the colonization time, not even in the later stage of symbiosis.

16.7 Effect of the ectomycorrhizal fungi *Amanita rubescens* on *Quercus spp.* seeds and seedlings inoculated with *Fusarium moniliforme* and *F. oxysporum*

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The efficacy of the ectomycorrhizal (ECM) fungi *Amanita rubescens* as a biological control agent against pre-emergence, post-emergence and late damping-off caused by *Fusarium moniliforme* and *F. oxysporum* on *Quercus pyrenaica* and *Q. ilex* seeds and seedlings was studied in greenhouse experiments. *F. oxysporum* and *F. moniliforme* failed to produce a decrease in germination of seeds or mortality of seedlings. However, a visible reduction in the *Quercus* spp. first-step seedling growth was observed in plants inoculated with *F. moniliforme*. *F. oxysporum* affected older seedlings more intensively in *Q. ilex*. *A. rubescens* inoculation improves germination of *Q. ilex* seeds compared to treatments without the mycorrhizal fungus. The ECM fungus did not affect the germination of *Q. pyrenaica* seeds. The growth of *Quercus* spp. seedlings increased in the in presence of the ECM fungus, in particular in the root tissue. Monopodial-pinnate ectomycorrhizae appeared in all the *A. rubescens* inoculated treatments. These ectomycorrhizae were classified as *Amanita* type. Acknowledgements: Financial support was received from the Ministerio de Ciencia y Tecnología and from Fondos Europeos de Desarrollo Regional (FEDER) within the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica 2000-2003 (Project AGL2001-1771), and from the Consejería de Educación y Cultura, Junta de Castilla y León (Project VA031/01).

16.8 Inoculation of pine seedlings with *Suillus luteus* decreases damage caused by *Fusarium* damping-off in absence of mycorrhization

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The role of the ectomycorrhizal (ECM) fungus *Suillus luteus* as a biological control agent against the damping-off (DO) caused by *Fusarium moniliforme* (Fo) and *F. oxysporum* (Fm) on Scots and Stone pine was studied in greenhouse experiments. The vegetative mycelium of *S. luteus* in a vermiculite/peat carrier was added to a potting substrate before inoculation with Fo or Fm spores. Seedlings were also inoculated only with the ECM, Fo, Fm or water for treatment comparison. Scots pine seedlings inoculated with *F. moniliforme* and *F. oxysporum* reduced the Seedling Disease Index (SDI) when co-cultured with *S. luteus*. Stone pine seedlings inoculated with *F. oxysporum* significantly reduced damage in presence of the ECM fungus, but no reduction of disease symptoms was observed in the seedlings co-cultured with *F. moniliforme*. The protective effect exerted by *S. luteus* against damping-off caused by *Fusarium* spp. was not related to the number of mycorrhizal apexes in the roots of Stone and Scots pine seedlings. Thus, mycorrhizal formation in co-cultures with *F. moniliforme* was low and nonexistent in co-cultures with *F. oxysporum*, despite the fact that *S. luteus* inoculation induced a greater antagonism against this pathogen.

16.9 Development of the novel ecologically safe technology for growing of plants on the basis of application of complex biopreparations

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The objective of our work is to substantiate a choice of complex of biopreparations and to develop a method of their application (insecticides, fungicides, growth and development promoters and immunomodulators from mycorrhiza endophytic fungi-simbiont) in order to obtain high-quality grain. In course of grain growth and development, the sowings are treated with the following three types of biopreparations: bioinsecticide Bikol, which is effective against more than 80 species insects , fungicide Lutan, and plant growth and development promoter Nikfan. It effectively suppresses the development of the fungi genuses: Fusarium, Alternaria, Rhoma, Ascochuta, Verticillium, Rhizocini, Scleratinia, Botrytis, Septoria. The dose of Nikfan preparation comprises from 1 to 3 ml per 1 ha. The results: - increase in biomass of plants by 13.5%; - increase in number of barley-corns in an ear by 7.6%; - increase in mass of 1000 barley-corns by 4 %; - increase in corn mass by -22 %; - increase in root formation at a tillering stage by 16.7%, at a full booting stage by 18.8%, at a milky-waxy stage of ripeness by 9.6%; - increase in plant height by 18 % on average; - decrease in infectiousness of plants with phytopathogens by 81.5 %. The suggested technology can be transformed to be applicable to the growing of the other crops such as wheat, sunflower, sugar beet, soybean, which makes it more favorable in the view of commercialization.

16.10 Effects of mycorrhiza and soil bacteria on acclimatization and development of micropropagated Lucumo (*Pouteria lucuma* R. and Pav.)

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Plantlets of Lucumo (*Pouteria lucuma* R. & Pav.) var. La Molina, after multiplication and rooting in vitro were transplanted to a substrate previously enriched with mycorrhizal inoculum (*Glomus intraradices* Schenck & Smith) or beneficial soil bacteria associated with AM fungi, in order to determine the effects of this inocula on acclimatization and development of micropropagated lucumo. Standard survival rates for lucumo microplantlets after greenhouse acclimatization were low (20-25%) Inoculation of AM fungi improved significantly survival (70%), growth and development of lucumo plants and induced changes to the root morphology. Best results in acclimatization of the lucumo microplantlets were obtained when substrates were inoculated with beneficial soil bacteria, with eighty percent of survival rate. Bacterization do not induced changes to the root architecture. This communication describes, for the first time, arbuscular mycorrhizal (AM) colonization of this species. Lucumo mycorrhizal colonization was low, about 20% of roots were colonized after 4 months of acclimatization.

16.11 Mycorrhization of *Pinus halepensis* with selected fungi improves seedling establishment one-year after panting in a degraded gypsum soil

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Mycorrhizal *P. halepensis* was obtained by inoculation with vegetative inoculum of *Amanita ovoidea* (isolate ccma-44), *Suillus collinitus* (isolates ccma-1, ccma-5, ccma-24) and spores of *S. collinitus*. Inoculation with all fungi, except ccma-1, significantly improved seedlings growth during the greenhouse breeding period. This significant growth stimulus was maintained one-year after planting. In general, seedling survival was also performed by inoculation. One-year after planting, seedlings previously inoculated with the different fungi showed higher percentages of ectomycorrhizas than controls although in general low fungal diversity was found. Five mycorrhizal morphotypes were described, and molecular analysis revealed two as ascomycetes (probable *Geopora* and *Wilcoxinia*), one as *Suillus* and two as unidentified fungi. Summarily, inoculation of *P. halepensis* with selected fungi aided seedlings to overcome post-transplanting stress, even if the permanence of introduced fungi was not confirmed in all treatments. Spore inoculum of *S. collinitus* was the most effective inoculation treatment significantly promoting seedlings survival and growth. Our results indicate that inoculation of seedling stock with selected ectomycorrhizal fungi is a useful technique to be used in afforestation programmes of degraded soils.

16.12 *In vitro* based mass production technology development for *Glomus* sp. using monosporal cultures as starter germplasm source

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The standardization of large-scale production technology and distribution of viable inoculum at the field level is a must in the technological transfer of knowledge to the grass root's levels. Production of AM inoculum using Ri T-DNA transformed roots is very promising and one already proved in Indian context. In this technique dual culture of Ri T-DNA-transformed roots and AM fungi is established under axenic conditions. This system allows extensive internal colonization and enormous spore production in a small space and over a short time with no contamination. The work was undertaken to culture one *Glomus* sp. (currently unidentified) under *in vitro* condition using transformed roots of carrot. This isolate was selected as the functionally efficient isolate for wheat based cropping system through field and greenhouse research. To mass multiply this isolate, the monosporal cultures initiated from trap cultures were used as a starter material for attempting in-vitro based spore germination and mass production of this species. The spore sterilization protocol was optimized to reduce the contamination from spore for the in vitro raising of arbuscular mycorrhiza using Ri T-DNA transformed host root. The successful spore germination was observed and the *in vitro* AM culture, presently being unidentified is successfully grown and mass multiplied and is available in plenty to apply in fields.

16.14 Determination of mycorrhizal potential from natural *Cedrus libani* stands in the Mediterranean Region of Turkey and applying to seedlings

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Aim of this study, identification and determination of natural mycorrhizas of *Cedrus libani* that is widespread forest trees in Eastern Mediterranean Region, determination of their potential and for cultivation and used in nursery. Mycorrhizal fruit bodies were collected from cedar forests. Surface of fruit bodies were cleaned. Three mycorrhizal fruit bodies were transferred to MMN medium (Modified Melin Norkans) with agar. The medium were incubated at 24 0C until grown their mycelium. After obtaining pure cultures, their sub cultures were produced. Completely random block design was with the replicate. In our study have three replicates and three different growth medium (a-1/3 forest soil+1/3 compost+1/3 sand. b- 2/3 compost+1/3 volcanic (tuff) stone, c-1/3 compost+2/3 andesitic sand) and three different species mycorrhizal fruit bodies. Half of these of mediums will be sterilized. Two inoculation methods are to be used. The first method the seeds were dipped into the mycelium and the other is germinated seedlings roots dip into the mycelium. Seedlings were measured at the end of the one year of age. Variance analysis was done collar-diameter and height. The first inoculation method was better than the second in diameter and height. Inoculation of mycelium treatments was better than the control in diameter and height.

16.13 Molecular characterization and diagnostics of edible ectomycorrhizal fungi by analysis of rDNA-ITS region

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Morphological characterization of ectomycorrhizae is a time consuming procedure, and sometimes it is difficult and even inaccurate. Molecular characterization was made by PCR amplification and analysis of the internal .transcribed sequence (ITS) of ribosomal DNA. Ten ectomycorrhizal strains belonging to 8 different species, and 4 plant species were used. The size of DNA amplified fragments were evaluated by agarose gel electrophoresis and by capillary electrophoresis (ABI PRISM® 310) and it ranged from 695-699 bp in *Amanita rubescens, Laccaria laccata* and *Suillus luteus*; from 732 to 737 bp in *Lactarius* and *Rhizopogon roseolus* and 806 bp in *Boletus fragans*. In plant species DNA fragment sizes found were 556bp in *Pinus nigra*, and about 677 bp in *Pinus pinaster, Pinus sylvestris* and *Quercus pyrenaica*. In order to discriminate ectomycorrhizal species, several restriction endonucleases were used, and the RFLP patterns allowed to separate the fungal genera. Mycorrhized plants were tested, and the amplified fragments corresponded only to fungal DNA showing that ITS1-ITS4 primed reaction favoured the amplification of fungal ITS region against plant ITS region. Fungal diagnostics were positive in *Lactarius* inoculated plants, and negative in *Rhizopogon roseolus* inoculated plant in which a 719bp fragment was amplified. Neither fungal species nor plant species used on this study produced this fragment size, so it could be a foreign fungus colonizing the roots.

Workshops

Workshops

Effect of activated carbon fiber on growth of arbuscular mycorrhizal fungi

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It is well known that the application of charcoal to the soil enhances the growth of plants and arbuscular mycorrhizal (AM) fungi (Ishii and Kadoya, 1994; Ogawa, 1987). In order to improve the axenic cultural methods for AM fungi (Ishii et al., 1995; 1998; 2003), we examined effects of activated carbon fiber (ACF) as a unique material for microorganisms on the fungal growth. Furthermore, we have already reported that red light ranged from 612 nm to 730 nm remarkably stimulate the growth of *Gigaspora margarita* and blue light shows inhibitory effects (Yachi et al., 2001). Thus, the interaction of ACF and light condition *in vitro* was also investigated. *G. margarita* spores sterilized were put on 1% gelrite media containing 0.1% of ACF, activated charcoal (AC) and carbon fiber (CF). The spores were incubated at 27?C in the dark, red LED (light emitting diode), blue LED and red LED + blue LED. Ten days after the incubation, the hyphal length and the number of auxiliary cells were measured. The growth of hyphae and the formation of auxiliary cells were stimulated by the addition of AC and ACF. In particular, this stimulation was strengthened by the lighting of red LED or red LED + blue LED. CF had no effect on hyphal length and auxiliary cell formation. Interestingly, ACF in red LED + blue LED induced hyphal branching and the hyphal re-growth from auxiliary cells. These results suggest that ACF is greatly valuable for proliferation of AM fungi *in vitro*.

A H^+ ion flux signature for the hyphal asymbiotic development of the mycorrhizal fungus *Gigaspora margarita*

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Various evidences have shown that environmental and developmental signals can elicit a differential activation of membrane H^+ fluxes as one of the primary responses of plant and fungal cells. Here, the net flux of H^+ around hyphae of the arbuscular mycorrhizal fungus *Gigaspora margarita* during its asymbiotic growth was determined using a H^+-specific vibrating probe. A pattern was observed in which specific membrane domains of H^+ flux activation that seems to play an organizational role in the hyphal emergence, branching and apical cell growth. Further the activation of the H^+ effluxes and hyphal morphogenesis were sensitive to phosphate (P), sucrose (Suc), glucose (Glu) and vitamins (Vit) exogenously supplied. The exclusion of P from the medium promoted the highest H^+ effluxes located mainly at the hyphal subapical region. Likewise, in a medium witht Suc, an enhanced H^+ efflux was also observed when compared with hyphal grown in complete nutrient medium. Excluding Vit from the medium, converting the H^+ efflux into an influx and affecting the hyphal growth in a similar manner to Suc. No Glu effects were observed in the hyphal growth and H^+ flux. Pharmacological analyses on hyphal H^+ fluxes suggest a possible asymmetric distribution of different H^+-pump isoforms. Altogether, these data depicts spatial and temporal alterations of the hyphal H^+ fluxes, which might define a pH signalling cue to asymbiotic developmental events critical for host tracking and recognition.

The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores

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Two isolates of *Paenibacillus validus* (DSM ID617 and ID618) stimulated growth of the arbuscular mycorrhizal fungus *Glomus intraradices* Sy167 up to the formation of fertile spores, which recolonize carrot roots. Thus, the fungus was capable of completing its life cycle in the absence of plant roots, but relied instead on the simultaneous growth of bacteria. The supernatant of a mixed batch culture of the two *P. validus* isolates contained raffinose and another, unidentified trisaccharide. Among the oligosaccharides tested, raffinose was most effective in stimulating hyphal mass formation on plates but could not promote growth to produce fertile spores. A suppressive subtractive hybridization library followed by reverse Northern analyses indicated that several genes with products involved in signal transduction are differentially expressed in *G. intraradices* SY 167 when grown in coculture with *P. validus* (DSM 3037). The present investigation (1), while likely representing a significant step forward in understanding the arbuscular mycorrhizal fungus symbioses, also confirms that its optimal establishing and functioning might rely on many, as yet unidentified factors. Thi contribution also aims at reviewing, at least in part, the interactions between arbuscular mycorrhizal fungi, beneficial bacteria and growth and health of plants. (1) U. Hildebrandt, F. Ouziad, F.-J. Marner, H. Bothe, FEMS Microbiol. Lett. 254 (2006)258-267.

Hyphal healing mechanism in arbuscular mycorrhizal fungi

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In the last years, the study of anastomosis in arbuscular mycorrhizal fungi (AMF) has received increased attention. This mechanism, allowing protoplasm/cytoplasm continuity and genetic exchange, has been studied with spores as well as with the vegetative mycelium. Hyphal fusions indistinctly concern intact and damaged hyphae, the latter being described as a hyphal healing mechanism (HHM). However the significance of HHM for the functionality and integrity of the AM mycelial network has been poorly investigated. Here we report, describe and compare the HHM in strains, combining the non-destructive monoxenic culture system and the video-camera imaging. We demonstrated that the HHM is different between *Glomeraceae* and *Gigasporaceae*. With *Glomus* strains the HHM could increase the capacity of the fungus to colonize the roots due to the proliferation of new hyphae, while in *Gigasporaceae*, the HHM is presented as the most probably means of maintaining the viability of the hyphae in adverse conditions. We also demonstrated that under severe physical stress (i.e. multiple injuries and long distances separating cut sections), the HHM was supported by the capacity of the isolated sections to shelter from the surrounding environment, by rapid septa formation, and to preserve their vitality for several hours in the absent of any connection with a main hypha or a host. Our results suggest that the HHM has ecological implication for species survival, abundance, maintenance and biodiversity.

Mating type loci in the asexual arbuscular mycorrhizal fungi *Glomus intraradices*

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Unlike most fungal phyla, which have a known sexual cycle, arbuscular mycorrhizal fungi (AMF) are only known to reproduce through asexual mitotic processes. We sought insight into the evolution of this apparent difference by comparing different fungal genomes. Our results, in conjunction with an accompanying presentation (Alexandre Colard et al.), suggest that AMF may be capable of sexual reproduction. Sexual reproduction in fungi is governed, in part, by two different mating-type loci that establish sexual compatibility. In ascomycetes, one locus encodes a protein with a high mobility group (HMG) domain, whereas the other encodes a protein with an alpha box domain. In basidiomycetes, one locus encodes the two protein subunits of a heterodimeric homeobox protein whereas the other one encodes both peptide pheromones and transmembrane receptors. We identified genes from both mating type systems in *Glomus intraradices*. The consequences of this will be discussed with respect to the possibility of mating types and compatibility in AMF.

Phylogenetic evidences for horizontal gene transfer in arbuscular mycorrhizal fungi of the genus *Gigaspora*: occurrence of parasexual cycle in AMF

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Arbuscular mycorrhizal fungi (AMF) have been considered ancient asexual organisms (AAO) due to their long standing asexual reproduction. AAO are considered biological scandals because their evolutionary mechanisms to escape extinction are unknown. Recent research on AMF karyotic state have shown contrasting results. However, fungi can go through a parasexual cycle forming both homo- and heterokaryotic species. Parasexuality occurs, between genetically diverse but compatible fungi, through hyphal fusion "anastomoses", followed by nucleus exchange. Until now, only self-anastomoses has been reported in AMF, giving no support for parasexuality. Here we found evidence for reticulated evolution and recombination of rRNA genes in AMF, supporting the occurrence of parasexuality in AMF of the genus *Gigaspora*. We found more than one lineage of rRNA genes per genome, and that some lineages are polyphyletic across species boundaries, indicating hybrid species. Nevertheless, molecular clock estimates indicate parasexual events are rare. Parasexuality could explain both the homo- and the heterokaryotic states reported for AMF, and generate genetic variability and clear deleterious mutations from their genome. Our findings indicate that recombination is playing a significant role in shaping genetic diversity of AMF.

The first glomeromycotan monosaccharide transporter is characterized

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Geosiphon pyriformis is a fungus forming a unique endosymbiosis with cyanobacteria. Together with the arbuscular mycorrhizal (AM) fungi, *Geosiphon* belongs to the Glomeromycota. Many structural and functional similarities between the *Geosiphon-Nostoc* and AM symbioses support the interpretation that the *Geosiphon* symbiosis is an AM-like symbiosis with a photoautotrophic prokaryote. Due to this unique life style, the investigation of this symbiosis offers some fundamental advantages when compared to the AM. E.g., for gene expression studies the fungal poly(A) mRNA can be easily and specifically isolated from the symbiotic life phase of the fungus. In the AM and the *Geosiphon-Nostoc* symbioses, the nutriment exchange occurs at the symbiotic interface, where large amounts of plant-fixed CO2 are transported from the photobiont to the fungus as carbohydrates. Therefore, specific fungal carbohydrate-transporters have to be postulated. To characterize transporter genes involved in the nutrient exchange, we established methods to reproducibly isolate *Geosiphon* mRNA from the symbiotic stage. After reverse-transcription, cDNA was size fractionated, and directionally cloned into a yeast expression vector. The plasmid cDNA-library was used for functional complementation of a yeast mutant deficient for hexose uptake (EBY.VW4000). The approach led to the isolation and characterization of the first glomeromycotan monosaccharide transporter, GpMST1.

Symbiotic phenotype of transgenic *Medicago truncatula* plants with reduced amounts of the sucrose synthase MtSucS1

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The MtSucS1 gene encodes a sucrose synthase in the model legume *Medicago truncatula*. The MtSucS1 promoter directed reporter gene expression to the vasculature of different tissues, but primarily to roots. In arbuscular mycorrhizal (AM) roots, the MtSucS1 promoter mediated strongest expression in cortical cells harbouring arbuscules. Specifically in highly colonized sections, GUS-staining was furthermore detected in the surrounding cortical cells. In root nodules, strong promoter activity occurred in the infected cells of the nitrogen-fixing zone, but was additionally observed in uninfected cells, in the meristematic region, and the prefixing zone. Phenotypical studies were performed on p35S-driven MtSucS1-antisense lines inoculated either with *Glomus mosseae* or *Sinorhizobium meliloti*. In both endosymbioses, MtSucS1-antisense lines appeared to be impaired in terms of growth, leaf numbers, as well as flower and pod formation. Quantification of colonization rates of AM fungi or rhizobia mirror that these lines are infected in both mycorrhization and nodulation, ending up in a less efficient P or N acquisition. In GC/MS approaches, AM roots expressing the antisense construct showed higher levels of amino acids (AA) and derivatives of the AA biosynthesis pathway, whereas in root nodules these metabolites were significantly reduced. We suggest that MtSucS1 is involved in the establishment and maintenance of efficient root endosymbioses in *M. truncatula*.

Localization of acid invertases in ectomycorrhizal roots of *Populus* sp.

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Acid invertases, catalyzing the conversion of sucrose to glucose and fructose, are key enzymes of plant carbohydrate metabolism and they are required for the efficient distribution of sugars from source to sink tissues. Most ectomycorrhizal fungi are unable to use sucrose directly as a carbon source and require its prior conversion into hexoses. Plant invertases are required for this conversion, as ectomycorrhizal fungi do not possess such enzymes on their own. From previous studies it is suspected that sucrose is released into the apoplasmic interface between plant and fungus and then cleaved by a plant cell wall acid invertase. Using a peptide derived from a highly conserved domain of plant cell wall invertases, we have produced an antiserum and affinity-purified antibody which reacts specifically with plant cell wall invertases, with no significant cross-reaction to fungal cell wall proteins. In histological sections of an ectomycorhizal association of Populus tremula x P. tremuloides with Amanita muscaria, these antisera indicate the presence of cell wall invertases at the apoplastic innermost contact surface of plant and fungus. This zone is penetrated by flat fungal hyphae and is probably a zone of active metabolism and nutrient exchange in both organisms. Our results suggest that sucrose is cleaved apoplasmically The role of invertases in the regulation of nutrient exchange will be discussed.

Fungal specificity and phenology affects net carbon transfer through hyphal and soil pathways in the laboratory and in the field

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Interplant carbon transfer through hyphal and soil pathways has been suggested to vary with fungal species as well as source-sink patterns between plants. We examined the role of transfer pathways, fungal species, and plant phenology on the magnitude and direction of carbon transfer between ectomycorrhizal paper birch (*Betula papyrifera* Marsh.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the field and laboratory. In the laboratory, pot systems, root chambers, and isotopic pulse labeling were used to determine that carbon transfer was greater: 1) where hyphal connections were left intact than where severed, and that net transfer occurred from birch to fir, and 2) where birch and fir were inoculated with host-specific fungi (*Leccinum scabrum* or *Rhizopogon vinicolor*) or host-generalist fungi (*Cenococcum geophilum*) than where not inoculated. In the field, isotopic labeling of paper birch and Douglas-fir in spring, summer and fall showed phenology influenced both the direction and magnitude of net transfer. Net transfer occurred from paper birch to Douglas-fir in summer (4% of total fixation) and in the opposite direction in spring (12%) and fall (34%), corresponding with greater net photosynthetic rates of birch than fir in summer, and fir than birch in spring and fall. These findings provided evidence that both plant and fungal factors are important in regulating carbon transfer between plants.

Can the ectomycorrhizal fungus *Lactarius quietus* supply oaks with carbon during spring reactivation?

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The ECM morphotype formed by *Lactarius quietus*, a fungus specifically associated with *Quercus* sp., are abundant all the year long in a mixed oak forest (*Quercus robur, Quercus petraea*) in north-eastern France. Root and stem growth, starting before bud break in oaks, are strong carbon sinks mainly supplied with carbohydrates stored during previous years. We considered the hypothesis that *L. quietus* contributes to provide the trees with carbon at bud break through its exo-enzymatic activities, at a time when new carbon assimilation has not yet began. Eight exo-enzymatic activities, relevant to carbon cycling and the release of phophorus and nitrogen from soil organic matter (hydrolase and oxidase activities), were measured on *L. quietus* ECMs before, during et after bud break. Climatic and pedoclimatic parameters were also measured. Using a stepwise regression analysis, each enzymatic activity has been modelled according to climate and tree phenology. Laccase, glucuronidase, cellobiase and glucosidase activities proved to be significantly linked to tree reactivation or climate. All these activities can help forming new tissues by supplying carbon and other nutrients to the oak host. In complement to isotope and transcriptome-based approaches, the present one makes it possible to study the potential allocation of this carbon to the roots and to identify the fungal groups able to mobilize carbon from soil organic matter during bud break.

Carbon use, metabolic modeling and isotopic fractionation of ectomycorrhizal fungi

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Ectomycorrhizal fungi obtain most of their carbon from autotrophic host plants, with root-derived glucose being the main source. Both natural abundance and tracer levels of stable isotopes can be used to track carbon movement in fungi at different levels of resolution, ranging from measurements on whole organisms to specific compounds. In our study, ectomycorrhizal fungi were cultivated on solid agar media and carbon supplied as glucose individually labelled with ^{13}C at specific positions. The pattern of ^{13}C incorporation varied among fungal strains. The proportion of carbon derived from specific atoms demonstrated that fungi generally incorporate the individual carbon atoms in the preferred order, C4 (9%) < C1 (14%) < C5, C3, C2 (18-20%) < C6 (22%). By using these enrichment patterns and a simple model of fungal metabolic pathways, we calculated relative fluxes through the tricarboxylic acid, pentose phosphate, and glycolysis cycles. Further refinement of our metabolic model requires measuring isotopic labeling patterns in specific compounds extracted from five strains of cultured ectomycorrhizal fungi. Because phospholipid and neutral lipid fatty acids are valuable markers of different fungal classes, understanding their labeling patterns may be useful in estimating carbon fluxes. Our ongoing work will assess natural abundance and tracer ^{13}C enrichment patterns of neutral and phospholid fatty acids by gas chromatography.

Experimental and meta-analytical approaches in heavy metal phytoremediation

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We investigated the effectiveness of heavy metal (HM) phytoremediation and the role of the arbuscular mycorrhizal (AM) symbiosis using experimental and meta-analytical approaches. Our experimental results showed that 'wild tobacco' plants invest more in AM symbiosis than HM sequestration as soil HM concentration increases, as indicated by increasing AM root colonization levels. The HM uptake being lower in AM than non-AM plants suggests that AM fungi enhance plant tolerance by decreasing HM bioavailability. Our meta-analytical results revealed that indicators of actual plant HM uptake (e.g. HM content & concentration) are positively correlated with elevated soil HM concentrations. In contrast, indicators of relative plant HM uptake (e.g. HM bioconcentration factor & extraction yield percentage) and plant biomass are both negatively correlated with soil HM concentrations, suggesting a compromise between plant growth and HM uptake. The AM root colonization and its impact on plant HM uptake were negatively correlated with soil HM concentration increases, indicating lower HM content in AM than non-AM plants, especially at toxic soil levels. Overall, our findings show that HM phytoremediation declines upon reaching critical soil HM levels and that AM symbiosis prevents plant HM uptake, such as through metal-binding processes.

Responses of two poplar species, inoculated with arbuscular mycorrhizal fungi, to zinc or copper

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Reclamation of metal-contaminated areas by means of plant (phytoremediation) is an emerging technique. Poplar, due to its fast growth and large biomass, presents interesting features for remediation purposes. In addition, poplar roots can be colonized by AM fungi, especially in the first years of growth. The aim of this work was to verify if the inoculation with AM fungi could induce plant morphological or physiological modifications useful for phytoremediation. Plants of *Populus alba* and *P. nigra* were grown in a greenhouse, simulating field conditions, in soil contaminated with copper or zinc, and inoculated with *Glomus intraradices* or *G. mosseae*. Plant survival, biomass, mycorrhizal status, metal accumulation in the various organs, photosynthetic activity, and leaf polyamine content were measured after over 6 month growth. Metal treatments strongly inhibited plant growth, but inoculation with *G. mosseae* significantly alleviated all the negative effects induced by metals, including modifications in the polyamine pattern. Our results indicate that inoculation with some AM fungi can increase poplar tolerance to metals and improve their survival and growth, suggesting a high potential of the symbiosis in phytoremediation.

Another *Thlaspi* story –hyperaccumulation of Cd in embryos of *Thlaspi praecox*

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Brassicaceae are widely accepted as non-colonised by arbuscular mycorrhizal fungi (AMF). Nevertheless, *Thlaspi praecox* Wulf. was shown to form AM symbiosis (Regvar et al 2003). It successfully colonises Pb, Cd and Zn polluted area in Slovenia (Regvar et al. 2006) and it was demonstrated to accumulate up to 1.5% Zn, 0.6% Cd and 0.4% Pb in shoots (DW) (Vogel-Miku? et al., 2005). Recently, the functionality of its symbiosis was questioned under greenhouse conditions, which resulted in increased nutrient (e.g. P) and decreased Zn and Cd uptake (Vogel-Miku? et al., 2006). Improved nutrient and decreased Cd uptake can lead to production of a high quality seed reserves, thus enabling more successful reproduction at metal polluted sites. Therefore the strategy of its reproductive success was studied using X-ray fluorescence spectrometry (XRF). A hyperaccumulation of Cd in seeds was found, whereas seed Pb and Zn concentrations were low. The preferential Cd localisation in the embryonic tissues with the most intense signal in the epidermis of cotyledons was demonstrated by Micro-Proton Induced X-ray Emission spectrometry (micro-PIXE). Regvar M, Vogel-K, Irgel N, Wraber T, Hildebrandt U, Wilde P, Bothe H 2003. J. Plant Physiol. 160:615-626 Regvar M, Vogel-Mikus K, Kugonic N, Turk B, Batic F 2006. Environ. Pollut., in press Vogel-Mikuc K, Drobne D, Regvar M. 2005. Environ. Pollut. 133: 233-242 Vogel-Mikus K, Pongrac P, Kump P, Necemer M, Regvar M, 2006. Environ. Pollut.139: 362-371.

Heavy metal tolerance in mycorrhizal plants involves common features with other stress responses in plants

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Molecular mechanisms involved in heavy metal tolerance in mycorrhizal plants have not been clearly identified. The purpose of this work was to study such processes in *Medicago truncatula* interacting with *Glomus intraradices*. Tolerance to Cd was tested in non-mycorrhizal and mycorrhizal plants treated with 0, 2, 10 or 20 ppm of Cd. The highest level of Cd in which plants could grow in absence of AM fungi was 10 ppm. Tolerance of mycorrhizal plants to 2 and 10 ppm of Cd was evident 40 days after planting. Colonisation of plant roots by *G. intraradices* resulted in lower concentrations of Cd in shoots and roots as compared to non-mycorrhizal plants. This indicates that in *M. truncatula*, mycorrhization either inhibits Cd uptake by the plant or activates Cd extrusion. *G. intraradices* had a positive effect on P uptake by the plant and this was not inhibited by the presence of Cd. Moreover, non-mycorrhizal plants had a much reduced water content in shoots in the presence of Cd, whilst this was not observed in mycorrhizal plants, suggesting that water stress is induced by Cd and relieved by mycorrhization. Non-targeted (SSH library) and targeted approaches (RT-PCR for specific genes) were carried out in order to identify plant genes involved in heavy metal tolerance in mycorrhizal plants. Preliminary results indicate common features with processes of tolerance to biotic and abiotic stresses in other organisms. This work was financially supported by the French National Project ToxNuc.

GintABC1 and *GintMT1* are involved in Cu and Cd homeostasis in *Glomus intraradices*

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Control of the levels of heavy metals (HM) in any living being is of key importance for its fitness. Arbuscular mycorrhizal (AM) fungi, like other organisms, must have evolved a range of mechanisms, including binding to extracellular materials, regulation of transport processes and/or intracellular sequestration, to maintain the concentration of essential metals within a physiological range and control the toxicity of non-essential HM ions. Nevertheless, current knowledge on the molecular basis of HM tolerance in AM fungi is still in its infancy. At the genetic level, just two genes involved in HM homeostasis have been analysed in detail in AM fungi: the Zn transporter *GintZnT1* from *Glomus intraradices* and the metallothionein gene *GmarMT1* from *Gigaspora margarita*. We now present two new *G. intraradices* genes, *GintABC1* and *GintMT1*. *GintABC1* encodes a 434 amino acid polypeptide with homology to the N-terminal region of the Multidrug-Resistance-Protein (MRP) subfamily of ABC transporters, which is most likely involved in Cu and Cd detoxification. Expression analyses revealed that *GintABC1* transcript levels are induced both by long- and short-term exposure of the extraradical mycelium to Cu and Cd. *GintMT1* encodes a functional metallothionein which may play a role in the regulation of the redox status of the fungus and, therefore, in protecting the fungus against the oxidative stress caused by some heavy metals. Future work on these and other genes will further improve our knowledge on the mechanisms of metal homeostasis in AM fungi.

Role of arbuscular mycorrhizal fungi in plant adaptation to arsenic contaminated soil

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Arbuscular mycorrhizal symbioses play very important role in alleviation of heavy metal phytotoxicity, however, up to date limited information is available as for their role in plant adaptation to arsenic (As) contamination, also for the fungal functioning mechanisms. In a compartmented cultivation system that consists of two compartments separated by plastic board, 37 μm nylon mesh or kept un-separated, white clover (*Trifolium repens* Linn.) and ryegrass (*Lolium perenne* L.) were grown separately or together in a natural As contaminated soil for investigation on the influences of a AM fungus, *Glomus mosseae*, on growth, arsenic uptake, mineral nutrition of, and the possible interactions between the two plant species. Not as previous findings that ryegrass seldom show positive growth response to mycorrhizal colonization, both plant species were highly dependent on mycorrhizal symbiosis for surviving the arsenic contamination. Mycorrhizal inoculation essentially improved plant P nutrition, and on the other side markedly decreased shoot As concentrations and As translocation from root to shoot, as a result, plant dry weights were markedly increased. Based on present studies, we supposed that under As contamination AMF can enhance plant adaptation to As contamination by some kind of selective uptake and transfer of P over As to host plants. Mycorrhizal fungi may have the potential of accelerating ecological restoration of arsenic contaminated soils.

Degradation of DDT by four ectomycorrhizal fungi

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1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) are widely distributed in environment and relatively resistant to microbial degradation which listed in Stockholm Convention as one of the most POPs. However, a range of ECM fungi have been shown to degrade five major classes of environmentally important POPs . Out of the 42 species of ECM fungi screened so far, 33 have been shown to degrade one or more classes of the chemicals. In this study, 4 ectomycorrhizal fungi (ECMF) species, *Boletus edulis, Gomphidius viscidus, Laccaria bicolor* and *Leccinum scabrum* were selected with diphenylmethane as only carbon source. Biodegrading of DDT by the 4 ECMF was demonstrated by disappearance and hexane extractable metabolites from fungal mycelia identified by GC-MS included 1,1-DDD and DBP with retention times at 17.39 min and 14.89 min that shared identical mass spectra. During the 15-days incubation period, 57.30%, 60.03%, 52.93%, and 60.34% of the added DDT was converted to products by *B. edulis, G. viscidus, L. bicolour, G. viscidus* and *L. scabrum* respectively. Those results first clearly demonstrated that ectomycorrhizal fungi can degraded DDT, as well as may suggested that pathway for degradation is metaboliting DDT to DDD, then DBP which similar as the pathway proposed for white rot fungi. Bioaccumulation also occurred in mycelia as 23.29%, 20.49%, 27.54%, and 20.39% of the added DDT was absorbed by *B. edulis, G. viscidus, L. bicolour, G. viscidus* and *L. scabrum* respectively.

Ecological and biotechnological aspects of the edible ectomycorrhizal mushrooms of Mexico

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Mexican plant diversity includes more than 25000 species and 45 vegetation types. The country is an important reservoir of ectomycorrhizal (ECM) tree genera, holding for example the largest number of *Pinus* and *Quercus* species world-wide. Apart from this biological diversity Mexico has a great cultural diversity which includes more than 50 ethnic groups. As a result of this unique mixture, more than 270 wild mushrooms are eaten and more than one thousand traditional names, have been recorded. Annually, thousands of tones, of more than 100 species, are gathered and sold in popular markets. Despite the richness of native ethnomycological knowledge, along with dramatic deforestation rates, cultural erosion is being carried out at a rapid pace. Integral approaches in order to improve forest conservation, including enhanced knowledge of edible ECM mushrooms, are an urgent need in the country. Currently some ecological and biotechnological studies are being carried out including inoculation with ECM mycelium and spores of Pinaceae. Ecological studies have included the determination of physical and chemical characteristics in which external ECM mycelium proliferates, under natural conditions. The determination of ECM species in areas with different N deposition is also been carried out. ECM inoculum has been produced with more than 20 local species and successful inoculation of pines has been carried out using 10 to 1000 million spores per plant, under greenhouse conditions.

Population genetics, gene flow and spore rain of *Tricholoma matsutake* using traditional ecological knowledge and microsatellite markers

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Tricholoma matsutake has been consumed as a luxury edible mushroom for centuries. Recent declines in Japanese Matsutake have led to imports of this and other closely related taxa from throughout the northern hemisphere. In Shangri-la County, northwest Yunnan province, China, exporting *T. matsutake* comprises up to 60% of the county's income. This study examines the population structure and reproductive biology of *T. matsutake* and examines the perceived notion that over harvest of young mushrooms diminishes spore rain. Mycorrhizas and environmental data were collected from fifteen rhizospheres in each of three replicated (N=9) *Pinus densata*-dominated forest plots of distinct age brackets (old growth, ~50yo, and ~20yo) determined via participatory rural appraisal and tree ring counts. Using several moderately polymorphic microsatellite DNA markers, rhizosphere size, genetic diversity, and Mantel tests within populations are used to characterize reproductive strategy: whether spore germination or hyphal growth predominates in each forest age bracket. Geo-referenced mushrooms from surrounding populations will be extensively sampled and characterized by allele frequency to enable estimates of population structure and gene flow. Finally, *T. matsutake* spore rain will be compared among forest ages and various local conservation strategies using a Real Time PCR analysis of airborne basidiospores.

Assessing field-based competition between two *Rhizopogon* species and its effect on plant growth and survival using real-time PCR

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Interest in the ecology of ectomycorrhizal (ECM) fungi has increased tremendously in recent years, but little is known about interspecific interactions among ECM species. We examined effects of competition between two species, *Rhizopogon occidentalis* and *R. salebrosus*, in a field experiment at Pt. Reyes National Seashore, CA, USA. Species were grown on *Pinus muricata* seedlings in single- and two-species treatments, with the outcome of competition assessed using real-time PCR of ITS gene region. We found that *R. occidentalis* had significantly higher abundance than *R. salebrosus* on the seedlings in the two-species treatment, indicating it was the competitively superior species. Although *R. occidentalis* was competitively superior, we observed no differences in the survival, growth, or % leaf nitrogen of seedlings colonized with the two species, indicating that competition among ECM fungi may be fungal controlled. In conjunction with a previous lab experiment, these results suggest that when colonizing from spore, the timing of spore germination largely determines the outcome of competition among ECM fungi.

Ectomycorrhiza responses to thinning and burning in a *Pinus ponderosa* forest

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Our study investigated the impacts of mechanical thinning and prescribed burning of *Pinus ponderosa* forests on hypogeous sporocarp production and the abundance and diversity of ectomycorrhizal fungi (EMF). Nearly one hundred years of fire suppression in the southwest United States has greatly increased *P. ponderosa* tree densities, increasing their vulnerability to fire and outbreaks of insects and disease, and altering floral and faunal communities. Efforts are now underway to restore these forests to pre-settlement conditions by mechanically reducing tree densities and re-introducing a regular fire regime. We coupled sporocarp production surveys with morphological and molecular analysis of EM root tips in middle aged (35-40 dbh) *P. ponderosa* stands three years after restoration treatments. Our results indicate that thinning and burning in these forests may reduce the production and diversity of hypogeous sporocarps, as well as the overall abundances of fine roots and EM root tips. However, there were no significant differences in EMF diversity across treatments. The effect of treatment on the relative abundances of EMF differed markedly among species, including positive, negative, and neutral responses. We conclude that most EMF species persist or return within 3 years of treatment, but that reduced overall root tip and mycorrhiza abundances may result in lower sporocarp production by some species.

Functional diversity amongst mycorrhizas on Douglas-fir seedlings regenerating after wildfire or clearcutting

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Wildfires are common in Interior Douglas-fir forests of British Columbia. This ecosystem is also subject to commercial logging. In this study, we examined the function of ECM fungal communities on Douglas-fir seedlings developing in plots exposed to different kinds of disturbance: (i) unburned forest stands (control); (ii) stands where above-ground parts of understory vegetation and the forest floor were consumed by fire, but where trees survived (light burn); (iii) stands where trees were killed by the fire (severe burn); and (iv) stands that were clearcut logged in 2003. In May/June of 2004, Douglas-fir seeds were added to plots in four replicate sites of each disturbance type as well as control plots. In June and October 2005, ectomycorrhizas from several seedlings per site were assayed for external enzyme activity using fluorogenic substrates (4-methylumbelliferyl-phosphate for acid phosphatase, 4-methylumbelliferyl- β-glucoside for β-glucosidase, and 4-methylumbelliferyl-N-acetyl β-glucosaminide for chitinase) in 96-well plates. The ITS region of fungal rDNA was amplified from representative tips with NS11 and NLC2 or with ITS1 and ITS4B, and sequenced with ITS1 and NLB4 or with ITS1 and ITS4, respectively. Functional differences were evident amongst ECM fungi and amongst sites. Results will be discussed in the context of functional differences in ECM fungal communities following different kinds of disturbance and with respect to sustainable forest management.

Analysis of differential expression of cDNAs in *Glomus intraradices* mycorrhizal fungus using acetosyringone, a phenolic compound

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The signal molecules used for initial communication in symbiosis are not yet known, recent studies point to their existence [1]. A diffusible factor from AMF called "Myc factor" stimulate lateral root formation in legumes [2]. On the other hand, it's known that host roots release signalling molecules that trigger hyphal branching. This branching factor has been isolated from root exudates of *L. japonicus* and has been identify as a stringolactone [3,4]. To understand molecular communication between plant and fungus is essential identified the fungal signals that induce symbiosis-related gene expression in plants roots. Studies in our lab demonstrated the accelerated sporulation of *Glomus intraradices* in co-culture with transformed carrot roots and a phenolic compound; we analyzed further the differential gene expression in this condition using cDNA macroarrays. We constructed arrays with 1046 cDNA clones and found 40% differential expression. Clones up or down regulated were sequenced and compared to the non-redundant NCBI database. We are interest in a clone with a significant homology to the *G. versiforme* putative cruciform DNA binding protein (AF034574), which is up regulated in the hyphae producing spores. RACE technique was used to complete the gene. Data on the regulation are confirmed mediated RT-PCR analysis. 1. Harrison, M. 2005. ARM. 59:19-42 2. Oláh B. et al. 2005. PJ. 44:195-207 3. Akiyama, K. et al. 2005. N. Vol 435. Pp 824-827 4. Bécard, G. MPMI. In Press.

Dissecting fungal/host plant signalling that triggers root epidermal cell responses

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Arbuscular mycorrhizal (AM) fungi adhere to the surface of host roots through clusters of swollen hyphae called appressoria. Appressoria-contacted cells respond by organising a complex cytoplasmic structure, that we have called the pre-penetration apparatus, or PPA (Genre et al., 2005, Plant Cell 17, 3489-3499). PPA organisation initiates in contacted cells with the nucleus positioned at the contact site, accompanied by a focused reorganisation of cytoplasm. Subsequently, a second nuclear migration across the cell and away from the contact site leads to the organisation of the PPA, a dense cytoplasmic column across the cell lumen, where cytoskeleton, ER and plasma membrane presumably assembly the interface compartment through which the fungus will then traverse the cell. To further understand the specificity of these responses, transformed roots of *Medicago truncatula* expressing GFP tags for different cell components were challenged with pathogenic fungi and subjected to abiotic stimuli. These experiments were aimed at identifying AM-specific components of the local fungal signal and their related plant responses. Observations carried out on both wild type and non-mycorrhizal mutant roots have revealed stimulus-specific responses and will be discussed here with reference to the signalling pathway mediating fungal recognition in the AM symbiotic interaction.

Differential expression of fungal genes at preinfection and mycorrhiza establishment between *Terfezia boudieri* isolates and *Cistus incanus* hairy roots

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Changes in gene expression by isolates of *Terfezia boudieri* during mycorrhization with *Cistus incanus* hairy roots were followed. Four fungus-hairy root clone combinations were cultivated under two sets of conditions, in one of which the root and the fungus were separated by a cellophane sheet, the other allowing physical contact. One of the combinations produced endomycorrhizas, the other three solely ectomycorrhizas. Fragments isolated by cDNA-AFLP analysis from cellophane-separated cultures (pre-infection) were used to identify differentially expressed genes by reverse Northern analysis. Genes showing no homology to known sequences constituted the largest group at both growth conditions. It is shown that some fungal genes are expressed transiently while others exhibit altered expression patterns as conditions change from individually-growing through the pre-infection stage to mycorrhizas. Genes expressed exclusively under combinations allowing either ecto- or endo -mycorrhiza at a particular condition were detected. Our results point, for the first time, to some of the genes that may be involved in determining the type of association that will be formed – ecto- or endomycorrhiza.

Plant gene responses to *Glomus mosseae* in compatible and incompatible genotypes of *Medicago truncatula*

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Early recognition processes are crucial to successful root colonisation and subsequent microbial effects on plant processes. Part of our research focuses on molecular pathways that are activated prior to or during initial cell contact between arbuscular mycorrhizal symbionts in compatible (host) and incompatible (non-host) interactions. *Medicago truncatula* genes have been identified that are up-regulated as part of the plant response to appressoria formation by *Glomus mosseae* prior to root colonization. A sub-set of the plant genes, with predicted functions in signal transduction and transcription, have also been shown to be activated by diffusable signal molecules ('Myc factors') from the fungal symbiont. These recognition events are for the most part inactive when appressoria develop on an incompatible (non-host) *M. truncatula* genotype, mutated for a Ca^{2+}/calmodulin protein kinase gene (*DMI3/MtSYM13*). These results provide evidence that signal exchange between plant and fungal symbionts during arbuscular mycorrhiza interactions is essential to root colonisation and subsequent symbiosis development. They also point to a role of symbiosis-related plant genes in pathways for specifically sensing fungal signals by the host root. Furthermore, the fact that silencing of the *DMI3/MtSYM13* gene interferes with plant-fungal signalling events highlights the importance of calcium in arbuscular mycorrhiza interactions.

First comes, first served: Once a plant is mycorrhizal.....!

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Although, the establishment of the arbuscular mycorrhizal (AM) symbiosis is beneficial, plants developed a regulatory mechanism to control mycorrhization, that means, once roots are colonized by an AM fungus, further colonization is suppressed. Recently, we could show that an established mycorrhizal association systemically suppresses not only mycorrhization but also nodulation. Furthermore we found that a supernodulating mutant (supernodulating mutants lost their ability to autoregulate the nodule number and thus form a high number of nodules) not only lost its ability to autoregulate the nodule number but also its ability to autoregulate mycorrhization, pointing towards a defect of the autoregulatory mechanism not only for nodulation but also for mycorrhization. These data indicate a similar autoregulatory mechanism in both symbiotic associations. New data on mycorrhizal autoregulation are presented.

Multiple hormone act to mediate susceptibility of tomato to arbuscular mycorrhizal fungi

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Among all the microorganisms that form mutualistic symbioses with plants, the most widespread is the arbuscular mycorrhiza fungi (AMF). The AM symbiosis confers benefits to the host plant, mainly enhancing nutrient uptake and increasing host resistance to biotic and abiotic stresses. Despite increasing knowledge obtained about the genetic and molecular biology of AM not much is known about the biochemical and morphogenetic events mediated by phytohormones during AM formation. Certain roles for phytohormones in ecto- and arbuscular mycorrhiza have been suggested, and most of these putative functions are based on some experiments involving hormone application or measurements of hormone content in AM plants. Using wild type and mutant plants impaired in the perception or biosynthesis of phytohormones in the model plant *Lycopersicum esculentum*, we studied the relation between phytohormone signal molecules, their interaction, and their function in the development of the AM symbiosis. We first demonstrate that Abscisic Acid (ABA) increases susceptibility of tomato to AM infection. Furthermore, we demonstrated that Salicylic Acid, Jasmonic Acid and Ethylene all cause an increase in the resistance of the host plant to AMF, but each one could participate in different states of the symbiosis development. Our results suggest that ABA regulates the ethylene-dependent signalling in AM negatively.

Transcriptional patterns in the extramatrical mycelium of *Paxillus involotus* as response to various complexity of the nitrogen source

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The positive effects of ectomycorrhizal (ECM) fungi on plant nutrition have traditionally been attributed to the quantitative effect of the extramatrical mycelium on uptake of dissolved nutrients, such as nitrogen. In acid forest soils nitrogen is present either in inorganic (mainly as ammonium) or organic (mainly as amino acids, peptides and proteins) forms and nitrogen mobilization by hyphae from soil sources is directly linked to hyphal uptake capacities. Transcriptional programs for translocation and assimilation of various sources of inorganic and organic forms of nitrogen were investigated in the ECM association between *Paxillus involutus* and birch (*Betula pendula*). Nitrogen sources of various degrees of complexity, ammonium phosphate, ammonium sulphate, glutamine, chitin, bovine serum albumin, were provided as patches in peat microcosms in which the mycorrhizal mycelium was expanding from colonized roots. After establishment, the mycelium in these patches was harvested and total RNA was isolated. Global transcriptional analyses using a cDNA microarray containing approximately 3.800 *Paxillus* gene representatives revealed different transcriptional programmes relative the nitrogen source provided. Results from this study will be discussed.

Isolation and characterization of N transporters putatively involved in the *Hebeloma cylindrosporum-Pinus pinaster* ectomycorrhizal association

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Studies of nitrogen metabolism in ectomycorrhizas have demonstrated that the fungal symbiont plays a fully integrated role in plant root metabolism and participates actively in the assimilation and transfer of newly absorbed nitrogen compounds by transferring amino acids. In symbiotic associations, such as mycorrhizas, location of the symbiosis ensures that mycelial growth of the fungus into soil provides access to nutrients in the soil solution. Changes in the distribution and activity of membrane-bound transport proteins in response to symbiotic interactions need to be investigated. In recent years several genes from *H. cylindrosporum* and *P. pinaster*, putatively involved in the nitrogen uptake and/or transfer, have been isolated or characterised by functional complementation of yeast mutants. The current state of the art will be presented.

Identification of a deamination enzyme, L-amino acid oxidase, from ectomycorrhizal fungi of genera *Hebeloma* and *Laccaria*

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Amino acids hold a central position in the N metabolism of cells. They are also major N components in soils. We determined whether *Hebeloma* and *Laccaria* expressed the enzyme L-amino acid oxidase (LAO), which catalyzes oxidative deamination of α-amino group of L-amino acids. Crude extracts of fungal mycelia converted model L-amino acid substrate to the expected reaction products, showing that *Hebeloma* and *Laccaria* expressed LAO-enzymes. LAO of *Hebeloma* and *Laccaria* were able to utilize most proteinogenic L-amino acids as substrates, showing that the enzymes had broad substrate specificities. LAO of *Hebeloma* was expressed in all culture conditions analysed. The highest activities were detected in nitrogen-rich growth conditions, suggesting that LAO expression is upregulated when the fungus encounters nutrient-rich environments. Also, isoenzyme patterns, K_m and pH optima of LAO-enzymes from crude extracts of *Hebeloma* were determined. Our study is the first detailed description of LAO enzyme activity from basidiomycete fungi. We suggest that LAO is a major enzyme catalyzing deamination of amino acids in *Hebeloma* and *Laccaria*.

How does an ectomycorrhizal fungus manipulate its host-plant metabolism?

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The mycorrhizal association leads to changes of the metabolic activities expressed by the 2 partners. Concerning these changes two hypotheses can be tested: The first one explains the metabolic modifications as an integral part of the reprogramming of the cellular activities leading to the differentiation of a mycorrhiza. In a second hypothesis we can consider that the control of the metabolic pathways results from the modifications of the fungal hyphae and plant cells micro-environment at the Hartig net level in terms of of nutrients available in the intercellular spaces. We tested these hypotheses for the nitrate pathway in the association between *Hebeloma cylindrosporum* and *Pinus pinaster*, both partners being able to use NO_3. Quantification of plant and fungal nitrite reductase mRNA was performed in mycorrhizas formed by either wild-type or nitrate reductase deficient fungal strains. In the presence of NO_3, the plant gene is down-regulated in the association with the wt strain but expressed at a high level in mycorrhizas formed by the mutant. In the case of NO_3 assimilation, these opposite results obtained with wt and mutant strains support the 2nd hypothesis of regulation of this pathway by the nutrients available. Plant assimilation is repressed only when the fungus is assimilating NO_3. We suggest that reduced N compounds transferred from the fungus to the cortical cells at the Hartig net level could repress the plant NO_3 assimilation pathway.

Enzymatic evidence for the key role of arginine in nitrogen translocation by arbuscular mycorrhiza fungi

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The involvement of arginine in nitrogen translocation by arbuscular mycorrhizal (AM) fungi was studied using key enzymes of the urea cycle and ^{15}N labelling patterns of arginine. Mycorrhizas were established between transformed carrot (*Daucus carota* L.) roots and *Glomus intraradices* in two-compartment Petri dishes. Three ammonium levels were supplied to extra-radical mycelium (ERM) in the root free compartment. Time courses of specific enzyme activities were obtained for glutamine synthetase, argininosuccinate synthetase (ASS), arginase and urease in ERM and in AM roots. $^{15}NH_4^+$ was used to study the dynamics of nitrogen incorporation into and turnover of arginine. The absence of external N or presence of L-norvaline, an inhibitor of arginine synthesis, both prevented the synthesis of arginine in the ERM and decreased activity of arginase and urease in the AM root. The catabolic activity of the urea cycle in the roots therefore depends on arginine translocation from ERM. The ^{15}N labelling of arginine in the ERM was very fast and the combined analysis of its time course and isotopomer pattern allowed estimation of the arginine translocation rate along the mycelium as 0.13 µg Arg mg^{-1} FW h^{-1}. The results highlight the synchronization of the spatially separated reactions involved in the anabolic and catabolic arms of the urea cycle, a prerequisite for arginine to be a key component in N translocation along the AM mycelium.

From ROC to AM-P *in vitro* culture systems for arbuscular mycorrhizal fungal transport studies

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Since the first reports involving root-organ cultures (ROC), many criticisms have been formulated on the suitability of this cultivation system to study arbuscular mycorrhizal (AM) fungal transport. In particular, the nature of the host (an excised root), and therefore the lack of photosynthetically active tissues (a shoot), has restricted the observations to the uptake and translocation of elements by the extraradical fungal mycelium, without being able to take into consideration the transfer from the fungal cells to the plant cells. As a result, many questions remained unresolved and most results the object of skepticism. Thus, the necessity to develop an *in vitro* system, associating an autotrophic plant with an AM fungus was highly requested. In the present work, we developed two new AM-plant (AM-P) *in vitro* culture systems with *Medicago truncatula* and *Solanum tuberosum*. We demonstrated that both systems were adapted to culture AM fungi in association with autotrophic plants under *in vitro* conditions and to perform transport studies. We observed (1) the production of several thousand spores, an extensive extraradical mycelium and an abundant root colonization, (2) the capacity of the produced spores to colonize new plantlets under the same conditions, and (3) the ability of AM fungi to transport P and Cs to their host. These systems are therefore proposed as models to conduct AM fungal transport studies, but also to investigate AM fungal-plant associations, in general.

The *rmc* mutation in tomato does not prevent colonisation by disease-causing organisms, but increases *Fusarium* wilt and root knot nematode development

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Mutations affecting arbuscular mycorrhiza (AM) development in non-legumes, such as the *r*educed *m*ycorrhizal *c*olonisation (*rmc*) mutant in tomato, may lead to identification of AM-related genes that are not also involved in nodulation. The recessive *rmc* gene has been mapped to chromosome 8 and close flanking markers identified, which will enable positional cloning. We are characterising interactions of *rmc* with different AM fungi and pests and pathogens, compared to wild type tomato (76R). Results show that: a) *rmc* allows different AM fungi to colonise to different extents, providing evidence that a single plant gene can influence host-specificity; b) prior inoculation of *rmc* with an AM fungus that can colonise the root cortex and form a functional mycorrhiza does not predispose the plant to be colonised by a second fungus that does not normally penetrate beyond the epidermis; c) a range of different pests and pathogens (*Rhizoctonia solani*, binucleate rhizoctonia, bulb and potato aphid, root knot nematode and Fusarium wilt (*Fol*)) are all able to infect roots of both *rmc*and 76R; however, d) *rmc* showed increased disease development of *Fol* and reproduction of root knot nematode, compared with the wild-type parent. Quantitative differences in development of AM fungi, *Fol* and nematodes in *rmc*, not observed in wild-type tomato, indicate that *rmc* has the capacity to discriminate between different strains of the invading organisms and may be involved in both symbiosis and disease.

The inhibition of *Phytophthora nicotianae* in tomato induced by AMF is not related to the modification of root exudation

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The mechanisms implicated in the biocontrol of the pathogen *Phytophthora nicotianae* Breda de Haan in tomato plants colonized by arbuscular mycrrohizal fungi (AMF) were investigated using a soil compartmented system. Plants were inoculated with the AMF *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe or *G. intraradices* Schenk and Smith, or daily soaked with exudates collected from plants colonized with the same AMF. Non-colonized controls received root exudates from non-colonized plants or pure water. Plants were infected by inoculating *P. nicotianae* in a neighbouring soil compartment, separated by a 35 Ìm membrane. An acetate separation was used to block the growth of *P. nicotianae*, in non-infected treatments. AMF inoculation and root exudates application did not modify root or shoot weights and root necrosis. Root infection with *P. nicotianae* was significantly reduced in AMF inoculated plants in comparison to non-inoculated plants but not in plants that received root exudates from AMF inoculated plants, as assessed with DAS-ELISA. These results do not support that a change in root exudation caused by AMF colonization is a significant factor in the biocontrol induced by AMF on tomato plants. A PCR-DGGE approach is currently used to amplify and separate the bacterial 16s rDNA from soil to assess the effects of AMF inoculation and root exudates application on soil bacterial taxa that could be implicated in this biocontrol.

Role of arbuscular mycorrhiza-associated bacteria from the genus *Paenibacillus* in biocontrol of *Pythium*

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Several studies have shown that arbuscular mycorrhiza (AM) can reduce root diseases caused by *Pythium*. Antagonism from bacteria in the mycorrhizosphere has been proposed as a mode of action. In the present study, the *Pythium* biocontrol features of seventeen strains of *Paenibacillus* spp. from the rhizosphere of mycorrhizal or non-mycorrhizal cucumber plants or from the hyphosphere of *Glomus intraradices* were examined. Thirteen strains significantly increased the percentage of seedling emergence of seeds inoculated with *P. aphanidermatum*. The two best strains of *Paenibacillus macerans* not only reduced pre-emergence damping-off incidence with 73%, but also gave full protection against *P. aphanidermatum* so that 68%-82% of the emerged seedlings remained healthy seven days after sowing. The two best strains of *Paenibacillus macerans* and the best strain of *Paenibacillus polymyxa* also significantly increased the percentage of seedling emergence after inocuation with 10^5 zoospores of *P. aphanidermatum*. In conclusion, our results demonstrate a potential among bacteria from *Paenibacillus* spp. to control pre- and post emergence damping off in cucumber caused by *Pythium* and indicate that mycorrhiza-assocaited bacteria may play a significant role in biocontrol of root pathogens in the mycorrhizosphere. Interactions between these *Paenibacillus* strains and AM, and their combined effects on *P. aphanidermatum* is investigated in an on-going experiment with cucumber grown in rock-wool.

Insights into the control of root-feeding nematodes by arbuscular mycorrhizal fungi

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In spite of the research about AMF as controlling agents of nematode pest in agriculture, we know very little about the interaction between AMF and root-feeders in natural systems. This study explores the interactions between the pioneer dune grass *Ammophila arenaria*, an inoculum of native arbuscular mycorrhizal fungi (AMF) obtained from the rhizosphere of this plant and the root-feeding nematode *Pratylenchus penetrans*. The interactions between root-feeding nematodes and *A. arenaria* are very well studied, therefore it is a good system to search for mechanisms that control nematode populations. Our objectives were a) to determine whether AMF can suppress nematode infection and reproduction, b) to explore the mechanisms of nematode control by AMF. Two experiments were performed to analyse the interactions between AMF and *P. penetrans*, a sequential inoculation experiment and a split-root experiment. Root infection and multiplication of *P. penetrans* were significantly reduced by the native inoculum of AMF in both experiments. We also detected a significant effect of plant pre-inoculation with AMF in the reduction of nematode performance. With the information obtained in the split-root experiment we concluded that nematode suppression by AMF did not occur through a systemic plant response but through locally operating mechanisms. Our results suggest that AMF are key organisms in the control of populations of root-feeding nematodes in natural systems.

Consequences of invasion by *Phytopthora ramorum* on ectomycorrhizal root and external soil colonization in tanoak (*Lithocarpus densiflorus*) forests.

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Sudden oak death caused by *Phytophthora ramorum* is an emergent plant disease that threatens the biodiversity and ecological integrity of native coastal forests in California and the Pacific Northwest of the US. We have implemented a study to examine the whether the effects of carbon reduction caused by canker formation by *P. ramorum* reduces the soil and root mycelia of tanoak (*Lithocarpus densiflorus*) ectomycorrhizal fungi. A randomized block design was implemented and trees were physically girdled to reduce carbon flow by cutting a circular gouge to prevent translocation of the phloem (fully girdled) or cutting a gouge half of the circumference of the tree (half girdled) for comparison with untreated control plots. Roots and soil were collected, extracted and DNA was quantified by quantitative PCR for three genera (*Cenococcum*, *Sebacina* and *Tricholoma*) at 5, 9 and 13 months after girdling. We compared the effects of girdling across genera and sample interval and found no reduction of ectomycorrhizal DNA from roots suggesting that fungi maintain root colonization by accessing stored carbon reserves. In contrast, fully girdled plots exhibited a reduction in extracted soil DNA as early as five months. Given that the external soil mycelia is the functional site of nutrient uptake, it is not improbable to expect that reductions in ectomycorrhizal mycelia may affect nutrient balance in tanoak forests after invasion by *P. ramorum*.

Role of *Periconia macrospinosa*, a tallgrass prairie endophyte in improved pathogen resistance

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Our recent studies suggest that root-colonizing, nonmycorrhizal fungal endophytes may equal or exceed arbuscular mycorrhizal fungi in abundance in a tallgrass prairie ecosystem. *Periconia* spp. are the most commonly isolated endophytes from dominant forbs and grasses. The function of these endophytes has remained elusive, although many possibilities have been proposed including improved pathogen resistance of host plants. This hypothesis was tested by following the susceptibility of *Periconia macrospinosa* inoculated and uninoculated *Arabidopsis thaliana* to a necrotrophic fungal pathogen, *Botrytis cinerea* and biotrophic bacterium, *Pseudomonas syringae* infection. Susceptibility of *Arabidopsis* to bacterial infection was unaltered by *Periconia* colonization. However, the endophyte inoculated plants showed lesser susceptibility to *B. cinerea* infection with fewer necrotic leaves than uninoculated plants. This experiment suggests a role of *P. macrospinosa* in improved resistance to fungal pathogens in native tallgrass prairie plants. Role of possible defense pathways and molecular evidence for the same will be presented.

Ectomycorrhizal communities associated with a *Pinus radiata* plantation in New Zealand

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The study investigated above- and belowground ectomycorrhizal (ECM) communities in a *Pinus radiata* plantation in the North Island of New Zealand in five stands of different age, using morphological and molecular identification methods (PCR, RFLP and direct sequencing). The ITS region (rDNA) of sporocarps and root tips was amplified and subject to RFLP analysis, patterns of ECM root tips were compared to those of sporocarps. Amplification products were direct sequenced, and searched through NCBI-GenBank. Both, sporocarp and ECM root tip species diversity increased from nursery to harvest, with species diversity being higher for ECM root tips at each age group. Sporocarps morphologically identified as *Hebeloma crustuliniforme*, *Tricholoma pessundatum* and *Laccaria laccata*, have been reported to be mycorrhizal associates of *P. radiata* in New Zealand. Sequence analyses have not confirmed these results; work is underway to clarify these species labels. The sporocarp survey included *Lactarius rufus*, not reported from New Zealand to date. *Wilcoxina sp.*, *Phialophora sp.* and *Pseudotomentella sp.* were collected in the ECM root tip survey, but have not previously been reported in association with *Pinus radiata* in New Zealand. The results imply that the underground ECM community is more diverse than what the sporocarp survey suggests.

Spatial ecology of Scots pine ectomycorrhizal communities

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Ectomycorrhizal fungi are an important component of forest ecosystems yet relatively little is known about how ectomycorrhizal communities are spatially structured. By investigating the spatial distribution of ectomycorrhizal fungi we may be able to determine how species in an ectomycorrhizal community interact with each other and the surrounding environment. A combination of structured grid-based sampling, morphotyping, molecular biology and geostatistical techniques were used to study ecological interactions, in below-ground Scots pine ectomycorrhizal communities. Overall community structure was assessed using similarity matrices, whilst cluster analysis was used to examine spatial patterns between species within the community. The abundance of black mycorrhizas was strongly correlated with total root tip number, suggesting that these species are uniformly distributed across the site wherever roots occur. However, for other species (*Clavulinaceae* sp., *Cortinarius* spp.) factors other than overall root distribution appeared to be important in determining spatial pattern. At the scale studied no overall structure was found within the ectomycorrhizal community as a whole. However, associations and dissociations were found between pairs of species, suggesting that positive and negative biological interactions may be taking place.

Community structure of ectomycorrhizal tree islands: Size matters

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Habitat size and isolation play a key role in our current understanding of the processes generating and maintaining patterns of biological diversity. Ecologists have attempted to formalize the effects of habitat size and isolation through theories such as the species area relationship, island biogeography, and metapopulations. Aside from its theoretical value, this body of work has provided important guidance for conservation planning in the modern period of habitat destruction and fragmentation. While these theories have been successfully applied to macroorganisms, there is currently strong debate about whether microbial biogeographic patterns are governed by fundamentally different processes. Our study examines the richness of ectomycorrhizal fungi on "tree islands" located within a non-ectomycorrhizal vegetation matrix. We show that ectomycorrhizal richness is significantly reduced on smaller and more isolated tree islands. Moreover, the slope of the relationship between richness and island size (the z-value) is similar to those reported for macroorganisms. Isolation reduced richness as well, but only on the largest islands. Ectomycorrhizal community composition was highly nested, with small island communities always drawn from a predictable subset of larger island communities. Because mycorrhiza are critical drivers of many ecosystem processes, the effect of habitat size and isolation on these communities is of great importance for both science and society.

Effect of clear-cutting and fire on ectomycorrhizal community in a *Pinus oaxacana* forest in a tropical mountain ecosystem

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This study was conducted to evaluate the effects of increasing levels of site disturbance after application of different forestry practices on ectomycorrhizal (EM) fungal communities in a secondary pine-oak forest. Above and bellow-ground EM fungi were analyzed in terms of species richness by examining ectomycorrhizal root tips and sporocarps in a control stand, a clear-cut stand, and a clear-cut plus burning stand. The fungal region ITS1-5.8S-ITS2 from single EM root tips was digested and compared to the ITS-RFLP of the identified sporocarps of the same stands. Diversity of total EM fungi showed to be higher in terms of ITS-RFLPs of the root tips than that of the sporocarps. Numbers of fungi were not affected by clear-cutting and by fire, but species composition was different among stands. Several sporocarps and RFLP-taxa were found only in the control stand and others only in the treated stands, *Russula emetica, Laccaria laccata* and *Lactarius crysorheus* were found in the 3 stands. The characteristically ectomycorrhizae formed by the ascomycete *Cenoccocum geophylum* were also found in the 3 stands. The treatment clear-cut showed to have the highest diversity of the root colonizing fungi as well as EM sporocarps, 22 RFLP-taxa and 16 sporocarp species, whereas the most disturbed stand showed to have 19 RFLP-taxa and 10 sporocarp species. Sixteen RFLP-taxa were found in the EM root tips of trees and 12 EM sporocarp species in the soil of the control stand.

Scots pine bait seedling performance *vs.* ectomycorrhizal fungal community dynamics before and over four years after forest clear-cut logging

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Scots pine seedling performance and ectomycorrhizal (ECM) fungal community dynamics in a typical Scots pine dominated stand one season before and over four seasons after forest clear-cut logging were investigated. The effects of planting location and time were assessed in relation to subsequent seedling performance and active ECM diversity, based on morphotyping and ITS-fingerprinting (RFLP and sequencing). Advanced regeneration, where seedlings were planted (June 1997) one growing season before the clear-cut event (February 1998), resulted in increased seedling growth in the clear-cut area over the subsequent 4 years. Counterparts planted in the interface zone in 1997, between the future uncut stand and the clear cut area, exhibited poorer growth compared to those planted after clear-cut harvesting. Seedlings planted into the interface zone and clear-cut area after clear-cutting exhibited an identical, positive growth response. ECM community structures in the interface zone and clear-cut area were particularly influenced by changing environmental conditions at the site. The interface zone generally harboured the highest diversity although a large number of ECM taxa were present on Scots pine seedling roots in the treatment zones over the 5 year sampling period. Instead of dramatic changes in total taxa numbers, community dynamics was better related to changes in 'exploration types' of mycorrhiza with a trend towards less rhizomorphic types in the clear-cut treatment.

Mechanisms by which CMNs mediate forest plant competition: evidence of overstorey-understorey resource flows and network effects on EMF communities

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A large-scale field experiment in a New England forest provided the first direct test of the hypothesis that common mycorrhizal networks (CMNs), independently of direct root interactions, limit the negative effects of competition by overstorey trees on understorey seedling growth and survivorship (Booth 2004). Here I present results of research on two potential, non-exclusive mechanisms by which CMNs were observed to have positive effects on pine seedling performance in that study: 1) resource flows (C, N) from overstorey trees to seedlings via CMNs and 2) differences in ectomycorrhizal fungal (EMF) communities on networked and non-networked seedlings. Regarding the first mechanisms: comparisons of seedlings' C and N isotope ratios with those predicted to result from resource-flows in CMNs suggest that neither C transfer nor increased N availability were related to positive CMN effects on seedlings. Correspondence between low $\delta 13C$ values, high seedling growth, and low N content was more consistent with the hypothesis of dedicated water transfer to seedlings via CMNs. Regarding the second mechanism: a combination of molecular typing, EMF community comparisons, and ordination show that CMNs were essential for maintenance of EMF diversity and community structure on seedlings. This suggests that successional differences in EMF communities correspond to differences in life-history strategies, which are likely to affect the terms of trade between EMF and individual plant hosts.

Mycorrhizal fungi as producers of novel and well-known hydroxamate siderophores

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Under iron-limiting conditions, most fungi release siderophores as chelating agents which form soluble complexes with Fe^{3+} with very high stability constants, thus solubilizing ferric iron. Ericoid mycorrhizal fungi produce as main siderophores ferricrocin and fusigen, respectively. Ferricrocin was also demonstrated to represent the main siderophore of the ectomycorrhizal fungus *Cenococcum geophilum*, some E-strain fungi forming ectendomycorrhizae, and *Phialocephala fortinii* as typical dark septate root endophyte. Arbuscular mycorrhizal fungi appear to enhance iron uptake rates and the leaf tissue iron content of associated host plants which is taken as an indication that mycorrhizal siderophores may be involved. However, so far the structure of siderophores released by arbuscular mycorrhizal fungi is unknown. Orchidaceous mycorrhizal fungi were shown to synthesize as main siderophores both, well-known hydroxamates or a siderophore with a novel and only recently elucidated structure named basidiochrome.

Molecular phylogeny and ultrastructural research of *Tulasnella* in epiphytic orchids of a tropical mountain rain forest in southern Ecuador

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The rapid loss of habitats enforces the understanding of ecology and symbiotic relationships of Ecuadorian orchids in order to improve efficiency of conservation efforts. Root samples of 77 adult individuals of the epiphytic orchids *Stelis hallii*, *Stelis superbiens*, *Pleurothallis lilijae*, and *Stelis concinna* were collected in a tropical mountain rain forest of southern Ecuador. Isolation of mycobionts was combined with ultrastructural evidence of symbiotic interaction and molecular sequencing of fungi directly from the mycorrhizas. Three different *Tulasnella* isolates were obtained in pure culture. Ultrastructural analyses displayed vital orchid mycorrhizas formed by fungi with an imperforate parenthesome and cell wall slime bodies typical for the genus *Tulasnella*. Phylogenetic analysis of the mycorrhiza forming Tulasnellales and the isolates based on coding regions of the ribosomal large subunit (nucLSU) and the 5.8S subunit, including parts of the internal transcribed spacers, yielded seven distinct *Tulasnella* clades. *Tulasnella* mycobionts in *Stelis concinna* were restricted to two *Tulasnella* clades. All *Tulasnella* sequences were new to science and distinct from known sequences from mycobionts of terrestrial orchids.

Mycorrhizal symbiosis in an achlorophyllous orchid, *Epipogium roseum*

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Epipogium roseum is an achlorophyllous orchid species distributing from Japan to tropical region. In the beginning of July, underground organs were collected in colonies of this orchid at the three geographically separated sites, in Tokyo, Kyoto and Nara. Mycorrhizal fungi were isolated from coiled hyphae colonizing rhizomes using a modified Czapek Dox agar medium. All the fungi were identified to be *Psathyrella* spp. or *Coprinus* spp. in Coprinaceae by a phylogenetic analysis based on the sequences of ITS region of nuclear rDNA. This is the first study to reveal the involvement of the fungi in Coprinaceae in orchid mycorrhiza. Large colonies of this orchid were frequently found around tree stumps or fallen logs. Because the fungi in Coprinaceae are known as saprobes, these decaying wood materials would be used as large and persistent carbon source for the growth of this orchid. Furthermore, a series of cultivation of this orchid, from seed germination to flowering, was successfully achieved under a symbiotic culture condition using a mycorrhizal fungus that is closely related to *Coprinus disseminatus*. According to the developmental process observed in the culture, it was revealed that this orchid has an efficient vegetative propagation system to produce many inflorescences from one seed with the mycorrhizal symbiosis.

Comparison of mycorrhizal colonization frequency of *Bromus tectorum*; (L.) and native grass species at Antelope Island State Park, Utah, U.S.A.

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Bromus tectorum; (L.)—cheat grass, is a non-native species that affects ecosystems by altering plant community structure. Though much is understood about the role of symbionts in the ecology of native species, little is known about the role of symbionts and invasive species establishment. The objective of the study was to compare the colonization frequency of root symbionts (arbuscular-mycorrhizal and dark septate endophytes) of *Bromus tectorum*; and several species of native grasses. Samples from eighty individual grasses were collected from four test sites at Antelope Island State Park—an island in the Great Salt Lake of northern Utah. Samples were examined for root fungal structures and identified. Preliminary results show no AM-mycorrhiza and a higher colonization frequency of an unidentified dark-septate-endophyte (DSE) in B. tectorum relative to native grass species. We found differential distribution of an unknown Chytridiomycete in the non-native species, *B. tectorum*;, when compared to the native grass species. Inoculum potential of the soil was determined by growing *Zea mays*; (L.) and the native species in soil collected from the treatment sites.

Fungi and fungal laccase genes from ericoid mycorrhizal roots of *Rhododendron*

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Rhododendron maximum (Rm) thickets suppress overstory regeneration in hardwood forests of the southern Appalachian mountains, USA. They also alter N cycling and soil extracellular enzyme activities; we previously found that soil polyphenol oxidase activity was greater under Rm compared to hardwood stands without Rm. Ericoid mycorrhizal (ERM) fungi associating with Rm may contribute to local differences in polyphenol oxidase activity. We characterized the ERM community in two ways. First, we collected fungal cultures from ERM roots, and PCR-amplified and sequenced the ITS region. Second, we cloned and sequenced the PCR-amplified ITS region of fungal DNA from ERM roots. Fungal cultures were dominated by *Oidiodendron* spp., and also included *Rhizoscyphus ericae*, and taxa in the Xylariaceae. Most of the same taxa were observed via root-cloning methods, with the addition of sequences similar to *Sebacina* and *Capronia* spp., ectomycorrhizal fungi, and several unknown ascomycetes. Laccase belongs to the group of polyphenol oxidases whose activity is greatest under Rm. We amplified a portion of the laccase gene from cultured fungi. All fungi possessed at least one and as many as 3 unique laccase gene sequences. The production of laccase by ERM fungi may be an important mechanism for nutrient acquisition in polyphenol-rich soils. Documenting laccase gene sequences is an important step towards determining laccase gene expression levels in ERM fungal communities.

Antarctic dark septate root fungal endophytes: diversity and function

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Mycorrhiza are widespread in most terrestrial ecosystems but appear rare in Antarctic plant communities. This research is the first to investigate the taxonomic status and possible ecological role of dark septate endophytic (DSE) fungi in two vascular plant species native to Antarctica, *Deschampsia antarctica* (Poaceae) and *Colobanthus quitensis* (Caryophyllaceae). Microscopy studies showed DSE to be common colonists of *D. antarctica* and *C. quitensis* roots across their latitudinal range. DSE isolated from *D. antarctica* and *C. quitensis* roots grouped into eight distinct morphotypes with phylogenetic analyses of nuclear ribosomal internal transcribed spacer region and partial 28S large subunit sequences placing all but one morphotype group within the Helotiales. Strikingly, the most commonly isolated DSE morphotypes differed between the two species, providing evidence for host preference. A group of putative *Mollisia* species were most commonly isolated from *D. antarctica* roots whereas a Pleosporales species and a group of *Leptodontidium* and *Cadophora* species were most commonly isolated from *C. quitensis* roots. The possible functional significance of *D. antarctica* root-associated DSE isolates was investigated through growth experiments, which showed for the first time that DSE can improve the growth of *D. antarctica* plants when organic, but not inorganic, nitrogen was supplied.

Demonstration of the host specific action on fungal polyphosphate hydrolysis and P efflux from ectomycorrhizal fungi

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The hypotheses which have been proposed to explain the transfer of P from the fungal cell into the common cell wall space harboring the host- and fungal cells are based mainly on the hydrolysis of fungal polyphosphate followed by the passive efflux of phosphate from the cytoplasm. We measured P effluxes from the ectomycorrhizal fungus *Hebeloma cylindrosporum* by using ^{32}P-orthoposphate (^{32}Pi). After a labelling period, the mycelia were incubated in a medium without Pi, and with or without a plant. Plants were either maritime pine or maize, that are respectively compatible and non-compatible host-plant. In parallel, we followed the dynamic of polyphosphate in fungal cells using ^{31}P-NMR. The data obtained demonstrated that a significant efflux of ^{32}P occurs in the medium only when pine roots were present in the solution. ^{31}P NMR spectra recorded in parallel experiments showed also that the accumulation of NMR-visible polyP in the mycelia occurs only after incubation of *H. cylindrosporum* with the host-plant. Our hypothesis is that the roots of maritime pine release a signal in the solution which initiates and maintains the hydrolysis of the large amounts of insoluble polyphosphate (non-visible NMR-polyphosphates) previously accumulated in the fungal cells.

Phosphorus uptake in a non-responsive host plant: deciphering the mycorrhizal pathway

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The role of arbuscular mycorrhizal fungi (AMF) in nutrition of non-responsive plants is gaining increasing attention. Conventional wisdom that non-responsiveness results from a dysfunctional symbiosis is no longer considered realistic. In order to better manage AMF in agricultural systems there is a need to improve our understanding of the 'silent' nutrient exchange that occurs in colonised but non-responsive plants such as barley. Under field conditions barley in South Australia can be extensively colonised. However in glasshouse trials mycorrhizal barley typically exhibits depressions in growth and P uptake relative to control non-mycorrhizal plants. An AM plant has two possible pathways for the uptake of P from the soil: the direct root uptake pathway and the mycorrhizal pathway. We have used ^{32}P labelling in compartmented pots to demonstrate that, despite the lack of responsiveness, the mycorrhizal pathway contributes nearly 100% of total plant P. In addition real time PCR was used to examine expression of the root epidermal P transporters, *HvPT1* and *2* and the mycorrhiza-specific *HvPT8*. Despite the reliance on the mycorrhizal uptake pathway, the epidermal P transporters, *HvPT1* and *2*, were not down-regulated but a significant up-regulation of *HvPT8* was observed. *HvPT8* is localised to cortical cells containing arbuscules. Our ^{32}P flux experiments with excised barley roots over-expressing *HvPT8* revealed this gene to be a high-affinity P transporter.

Organelle involved in polyphosphate translocation in arbuscular mycorrhizal fungi: polyphosphate-synthesizing activity associated with membrane

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Inorganic polyphosphate (polyP) is a linear polymer of phosphate linked by high-energy bonds. It has been demonstrated that arbuscular mycorrhizal (AM) fungi accumulate polyP for long distance translocation of P. Biochemical processes involved in polyP-synthesis, -compartmentation and -depolymerization, however, are largely unknown in eukaryotic microorganisms. The objectives of the present study are to isolate and characterize organelles involved in polyP accumulation in AM fungi. *Glomus* sp. HR1 was cultured on river sand using marigold as a host using a 37 μm nylon mesh bag under P-deficient conditions. Twenty-four hour prior to harvest, 1 mM Pi was applied to the medium to maximize polyP content in extraradical hyphae. The hyphae were harvested from the outside of the mesh bag, homogenized on a mortar and fractionated by the density gradient centrifugation. PolyP in the fractions was quantified by the polyphosphate kinase/luciferase method. PolyP-synthesizing activity was determined based on an increase in polyP after incubation at 30°C for 30 min in the presence or absence of ATP. About 60-80% of polyP was found in the supernatant of the density gradient centrifugation, suggesting that majority of polyP is accumulated as a soluble form in fragile organelles. PolyP-synthesizing activity was found in the fraction 1 (density: 1.06-1.10 g mL^{-1}) in the presence of ATP. Evaluation of the purity of the fraction by the marker enzymes and microscopic observations is required.

Phosphate delivery by MtPT4 is a key component of the arbuscular mycorrhizal symbiosis

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The ability of the mycorrhizal fungus to deliver significant amounts of phosphate to the plant is of major agronomical and environmental importance. The objective of this work is to dissect the mechanisms by which the plant obtains phosphate from the fungus. Previous studies in *Medicago truncatula* led to the identification of a unique phosphate transporter, MtPT4, induced in response to colonization by arbuscular mycorrhizal fungi. This transporter is expressed only in the periarbuscular membrane. This highly specific expression pattern suggested that MtPT4 should play a role in the acquisition of phosphate released from the fungus. This hypothesis is being investigated by reverse genetics. We identified RNAi lines and EMS mutants (identified by TILLING) that show an inhibition of the expression of MtPT4. In response to colonization by a mycorrhizal fungus, control plants show a higher phosphate content in the leaves as well as an improvement in growth. This stimulation is not observed in the *MtPT4* mutant lines. Surprisingly, the fungal hyphae and arbuscules are degenerate in the mutant lines but not in the control lines. A detailed analysis of initial invasion of the root and formation of the first arbuscules indicates that arbuscule degeneration is triggered prematurely in the mutants. Altogether our data point out an essential role of the phosphate transport at the arbuscular interface.

Arbuscules are not an absolute requirement for P transfer in AM symbiosis: evidence from ^{32}P transfer.

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Carbon (C) and phosphorus (P) transfer is believed to occur across the arbuscular interface that develops in the plant cortical cells in normal AM colonisation. In the *r*educed *m*ycorrhizal *c*olonisation (*rmc*) tomato mutant the formation of arbuscules by *Scutellospora calospora* is blocked (phenotype Pen+ Cort-). We hypothesise that P transfer will not occur in Pen+ Cort- interactions due to lack of arbuscules, but that it will occur in Myc+ in the wild type tomato 76R. ^{32}P transfer to plants by fungal mycelium was followed in a compartmented pot system with one harvest at 6 weeks. The AM fungal contribution to P uptake was quantified using P concentrations and ^{32}P specific activities in plants and soil. Plant dry weight and fungal colonisation were also determined. ^{32}P was transferred to the plants regardless of AM colonisation phenotype, although transfer to *rmc* was about 2/3 of that transferred to 76R. This shows that the Pen+ Cort- phenotype in *rmc* was able to transfer P even though arbuscules were absent. The results will be discussed in relation to the importance of the intraradical hyphae in AM symbiosis.

Preferential allocation of resources to beneficial mycorrhizal fungi by *Allium cernuum* and the maintenance of a plant-fungal mutualism

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Mutualisms, beneficial interactions between different species, are common. However, mutualisms are predicted by evolutionary theory to be unstable when participants are transmitted horizontally, as they are in mycorrhizal mutualisms. Assuming that benefits from the mycorrhizal fungi are costly to produce, fungi that receive carbon from plants but do not reciprocate should reproduce faster than fungi that benefit plants. We hypothesized that spatial structure of fungal species in the soil may allow plants to allocate resources to more benefical fungi, which would maintain the stability of the mutualism. Using a split-root pot design, we tested whether spatial structure affected the ability of *Allium cernuum*, to preferentially allocate resources towards *Scutellospora fulgida*, the more beneficial fungus, over *Glomus claroidium*.

The use of fluorescent proteins to investigate the attachment of *Azospirillum* strains to AM roots and fungal structures

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Arbuscular Mycorrizal (AM) fungi are known to interact with other components of the rhizosphere microbiota to benefit plant fitness and soil quality. The PGPR *Azospirillum* spp., are target bacteria in this context. The plant stimulatory effects exerted by *Azospirillum* have been attributed to an increased plant nutrient, mainly N, uptake by changing the rooting pattern, as mediated by the production of plant growth-promoting substances, mainly indol-3-acetic acid (IAA). There is an increasing interest of using genetically modified (GM) *Azospirillum* to improve its beneficial characteristics with the GM modification relaying on enhancing the production of IAA. The attachment of co-inoculated *Azospirillum* cells to root and AM fungal structures seems critical for growth promotion in AM-plants. Accordingly, the aim of this study was to compare the adhesion and distribution of GM *vs.* wild type (WT) *A. brasilense* on AM-root and fungal structures, by using fluorescent proteins and confocal scanning microscopy approaches. AM monoxenic cultures were established. These cultures were further inoculated with either GM or WT *Azospirillum* labelled with fluorescent proteins. Root fragments and mycelium containing spores were extracted from these cultures. A preferential attachment to root and mycelium was detected while spore surface appeared free of bacteria. No differences were observed between both types of strains (GM vs. WT) in that concerning the number of attached bacteria.

Colonisation by ectomycorrhizal fungus *Suillus bovinus* modifies archaeal and bacterial populations of forest soil and pine roots

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Ectomycorrhizas and sporocarps formed by particular fungi appear to harbour distinct types of bacteria, but the overall effect of soil colonisation by ectomycorrhizal external mycelia on bacterial populations has most often been negative. The effect of ectomycorrhizal colonisation on root or soil Archaea has not been reported previously, although Archea have been found from mycorrhizospheres. To study the mycorrhizosphere effect on soil prokaryotes, samples were collected from different Scots pine-*Suillus bovinus* mycorrhizosphere compartments grown both in boreal forest and in microcosms. Colonisation frequency of bacteria was investigated by plating and sequencing while direct DNA extraction, PCR-DGGE and sequencing was used to identify the Archaea. Bacterial numbers, particularly of those of Proteobacteria were highest in uncolonised short roots and external mycelia, whereas the Archaeal frequency was highest in the mycorrhizal samples. Without colonisation of mycorrhizal fungi, the Scots pine roots harboured a high percentage of Proteobacteria of all culturable bacteria but hardly any Archaea. Uncolonised humus contained clearly different bacterial and archaeal populations compared to the mycorrhizosphere populations.

Physiological and molecular characterisation of bacterial populations associated with ectomycorrhizas of *Salix* sp. in heavy metal contaminated soils

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Both ectomycorrhizal and arbuscular mycorrhizal fungi can be affected beneficially by different bacterial species. Bacteria can colonize the surface of mycorrhizal roots, and inter- and intra-cellular locations within the ectomycorrhizal mantle and Hartig net. This work focuses on the culturable bacterial populations associated with ectomycorrhizas of *Salix caprea* growing at heavy metal contaminated sites. To assess the effects of high heavy metal concentrations in soils on the diversity of ectomycorrhizal-bacterial communities, culturable bacteria were isolated from identified ectomycorrhizas of *S. caprea* growing at former mining areas with different levels of contamination. Bacterial strains were enumerated (colony-forming units) and characterized on the basis on morphological characters of bacterial colonies, which were determined both macroscopically and microscopically. Isolates were characterized using the metabolic fingerprinting of the BIOLOG system. Amplified ribosomal DNA restriction analyses of bacterial strains were made. Analyses of distances between obtained sequences were done for phylogenetic analysis using the parsimony (PAUP) program. The bacterial communities retrieved from different morphotypes of ectomycorrhizal roots of *S. caprea* were dominated by Proteobacteria. *Pseudomonas* was the most common genus isolated.

Effects of fungal characteristics on the predation by soil microarthropods

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Microarthropods represent a major trophic group of decomposers and may directly influence fungal communities through grazing on mycelium. Several studies on VAM, saprotrophic fungi and few studies on ECM reported a selective grazing behaviour. It seems that saprophytes and dark pigmented fungi were preferred. Toxic fungi were rejected. Some fungi form crystals at the hyphal surface, which are supposed to function as grazing protection. However, a systematic test on parameters affecting grazing preference is still missing. In different in vitro food choice experiments we investigated the interaction between the Collembolan *Folsomia candida* and several fungal species (ECM and saprophytes). Species were selected considering poisonous metabolites like amanitin or muscarine, crystal structures and pigmentation. Grazing preference and vital parameters of the Collembolan like oviposition were observed. *F. candida* preferred pigmented mycelium and was repelled by species with toxic metabolites or crystalline deposites at the hyphal surface, which indicates their ecological function as protection against predators. Furthermore, grazing preference and oviposition of were correlated. Grazing reduced the mycelial growth in all experiments. However, some fungi gain advantage under grazing pressure in a competitive situation. The results may help to explain the composition of fungal communities in soil and their influence on plant growth.

Below and aboveground multitrophic interactions through spatial and temporal scales in tropical and temperate regions

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Understanding of the linkages between below and aboveground organisms is important issue in ecology since they influence each other, which in turn would produce biological diversity, although our understanding of the interactions is still incomplete, tropical regions in particular. Here, we reveal that the effect of arbuscular mycorrhizal (AM) colonization on diversity of arthropods through plant diversity at Kalimantan and Jawa islands in Indonesia in addition to Tsuruoka in Japan. Vegetation at the experimental plot (5 m x 5 m) was removed by herbicide, then fungicide (benomyl) was applied on soil surface at each plot every two weeks as a treatment, while water was applied to the plot as a control through a year in 2004 at the three sites mentioned above. In general, plant and insect samplings were obtained every two months except for the sampling in Japan, while AM colonization was measured twice through the year. Fungicide application decreased the AM colonization of three plant species, while the effect of fungicide application tended to be smaller in other species. Belowground organisms are likely to influence differently on the functional groups of arthropods through plant diversity across spatial and temporal scales.

Insect-plant-mycorrhizal interactions at the community level

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There is now good evidence that the growth and survival of both foliar-and root-feeding insects can be affected by mycorrhizas. However, the vast majority of studies have been conducted in controlled conditions, using one plant species, one insect and one or two fungal species. How realistic are these studies, considering that plants in the field may be colonized by a community of mycorrhizal species? In this presentation, I will first examine species-specific effects of mycorrhizas on insects, with the aim of showing how variable these interactions can be. The critical question is whether laboratory effects translate into effects in the field at the population or community level. Results of the first field experiments to address this question will be presented, in which mycorrhizas were manipulated and the abundance of insect herbivores and their predators recorded.

Biodiversity and community structure of AMF in pasture and primary forest in brazilian Amazon

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This work is part of a global project to understand the impact of land use systems upon below ground biodiversity of several functional important groups of soil biota. The objective of this study is to assess diversity and community structure of AMF under pasture and tropical Amazon forest in Brazil. Within a 300 x 300 m^2 grid, 13 soil samples were collected in pasture and 17 in primary Amazon forest; distance between sampling points was 100 m. A total of 61 AMF morphotypes were found, 32 in pasture and 43 in forest. Average spore numbers was significantly higher in pasture (343) than in forest (56) although mean species richness was not statistically significant between pasture (7.7) and forest (7.5). Shannon index of diversity was significantly higher in forest than in pasture with values of 1.72 and 2.37, respectively and Sorenson similarity index was 0.37. AMF species were ranked according to their abundance in both LUS and correlation was established on their abundance yielding a $r^2 = 0.91$ ($P < 0.0001$), indicating that community structure is highly similar between both LUS. In pasture, the most frequent species were *Entrophospora colombiana* (92%) and *Acaulospora delicata* (85%) while in forest was a *Glomus* sp. and *Acaulospora* sp., both with 76% of frequency. Our results suggest that alpha diversity is high in both systems and the disparity between plant communities in both systems seems to have a low influence on species richness and mycorrhizal community structure.

Community structure and host preference of arbuscular mycorrhizal fungi in tropical forest ecosystems

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Arbuscular mycorrhizas are the dominant mycorrhizal types in tropical forest ecosystems and arbuscular mycorrhizal (AM) fungi are known to influence composition and competitive ability of host plants. Molecular investigations have confirmed that diverse arbuscular mycorrhizal fungal communities colonize short pieces of host plant roots. We have developed a nested PCR protocol to amplify AM fungal SSU rDNA from mycorrhizas that allowed subsequent RFLP typing, phylogenetic and community analyses. Our current studies on community structure of AM fungi associated with host tree species in natural and plantation forests in Afromontane forest ecosystems in Ethiopia, revealed the presence of distinct AM fungal communities and a specific host plant - AM fungal community interaction. Our results suggest that tropical forest ecosystems that are characterized by their unique plant biodiversity and richness could also be rich in distinct AM fungal community.

Arbuscular mycorrhizal fungal community associated with indigenous and non-indigenous plants in and around Phoenix, Arizona USA

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Arbuscular mycorrhizal (AM) fungal biodiversity from 30 sites throughout the Phoenix, Arizona USA metropolitan area were compared. Soil collected from non-indigenous and indigenous plants at urban sites and from indigenous plants at desert sites were used to obtain spores for identification. The total number of species detected, number of species per plant and species richness were greater at the desert sites in comparison to urban sites, but was similar between urban sites with indigenous plants and those with non-indigenous plants. There was a significant overlap in the species composition between desert and urban sites. Differences between the Phoenix, Arizona urban sites and the surrounding desert sites appear to be a much stronger influence on below ground diversity than the influence of indigenous and non-indigenous plants. Certainly the disturbance caused by the process of urbanization may still be a reasonable explanation for the differences observed.

Do mycorrhizas control invasiveness?

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Many plant species have expanded their natural range through human activity. Mutualisms such as the arbuscular mycorrhizal (AM) fungi have been proposed as factor that could explain the ability of some plant species to exploit new habitats. The genus *Acer* (the maples) has a circumpolar distribution, and many species have been introduced into the UK. A few have naturalised, but the majority are restricted to ornamental planting. We hypothesised that the native species *A. campestre* has a more diverse community of AMF than the other taxa. If the ability to interact with the native AMF is related to establishment, then the naturalised species would be predicted to have an AM community more similar to that of the native. Ten *Acer* species were sampled from the Castle Howard Arboretum in April, June and October 2004. The community of AM fungi colonising the roots was profiled using tRFLP. Seasonality was the most significant factor: the AMF community changed dramatically between April and June without a change in overall diversity. The community then increased in diversity by October and that of the native *A. campestre*, was highest. In the April, the native and naturalised species had a community profile that was distinct from the introduced taxa. It is remarkable to detect a signal like this in a field system where the signal:noise ratio is low. We suggest this is evidence that established native and naturalised species share a common AM community.

Composition of root-colonizing AM fungal communities in ecosystems around the globe, with an insight to a boreal forest

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A survey is presented based on publications that report on the occurrence of natural root-colonizing AM fungi identified using rDNA region sequences. Comparable data of AM fungal taxon richness and community composition across 36 host plant species and 25 locations involved 95 fungal taxa (SSU rRNA gene sequence groups), 49 of which were recorded from >1 study site, and 65 from >1 host plant species. The number of AM fungal taxa per host plant species differed between habitat types: 18.2 in tropical forests, 8.3 in grasslands, 5.6 in temperate forests and 5.2 in habitats under anthropogenic influence. AM fungal communities exhibit differing compositions in broadly defined habitat types: tropical forests, temperate forests and habitats under anthropogenic influence. A number of AM fungi had a global distribution, including sequence groups related to the *Glomus intraradices/fasciculatum* group, *G. mosseae*, *G.* sp. UY1225 and *G. hoi*, as well as the *Glomus* and *Scutellospora* types of unknown taxonomic affiliation. Widespread taxa occur in both natural and anthropogenic (disturbed) habitats, and may show high local abundance. However, new data from a herb-rich boreo-nemoral spruce forest in Estonia uncover challenging AM fungal richness, positively associated with aboveground plant richness. Complex fungal community patterns in this ecosystem can be linked to the effects of forest management intensity and host plant identity.

Establishment and functioning of synthetic arbuscular mycorrhizal fungal communities

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Arbuscular mycorrhizal fungal (AMF) communities in soils mostly consist of more than one species. This also holds true for plant monocultures such as encountered in some agroecosystems. It is unknown how this fungal diversity is regulated and what are its functional consequences for the plants. However, it appears important to know whether multispecies AMF communities in plant roots bear any advantage to the host plant compared to single AMF species. Also, with regard to expanding mycorrhizal technologies, aiming almost exclusively at inoculation of soil with selected strains of AMF, we need to understand how the competition and survival of AMF is determined and how the inoculated strains perform within a community. We propose using strains of AMF with known phenology and functional properties to uncover links between (functional) diversity of AMF community and its effects on plant nutrient acquisition and growth. An overview of methodologies for quantitative analysis of the AMF communities will be given here, including signature fatty acid analysis, quantitative PCR, and immunochemistry approaches. Evidence for functional complementarity among AMF species sharing the same root system will be presented.

Community-level and species-specific responses of arbuscular mycorrhizal fungi in a long-term global change experiment are temporally dynamic

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Anthropogenic enrichment of CO_2 and N affects trading partnerships between plants and arbuscular mycorrhizal fungi (AMF). We investigated the effects of CO_2 and N enrichment and host diversity (0, 1 or 16 plant species) on AMF communities after 2 and 7 years in a field experiment in MN, USA. After 7 years, the species composition of spore communities was altered by enriched CO_2 and host diversity, but not by N, while total AMF abundance and richness were not altered by CO_2, but were by N and host diversity. Extramatrical hyphal lengths were also affected by CO_2, N, host diversity and time. AMF responses to treatment varied by fungal species depending on host diversity and were temporally dynamic. AMF community composition after 2 years of treatment was significantly different than after 7 years. Some species responses were the same, while others varied with time. For example, the abundance of *Archeaospora trappeii* increased with N after 2 years and decreased with N after 7 years, whereas, the abundance of *Glomus clarum* increased with CO_2 after both 2 and 7 years. Other species responded to host diversity. *Gigaspora gigantea* proliferated in 16 species plots, whereas *Acaulospora laevis* was most abundant in single species plots. These results indicate that AMF responses to global change factors and host diversity are species-specific and dynamic, and some important responses may be missed by short-term studies.

AM fungi ameliorate high temperature soil stress

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Yellowstone National Park contains active geothermal sites characterized by poorly developed soils with temperatures that increase with depth. Root distribution is shallow to avoid high temperatures, and roots are colonized by arbuscular mycorrhizae (AM). In the greenhouse, survival and growth for some host plant species in elevated soil temperatures is dependent on mycorrhizae, and there is an increase in extraradical hyphae (ERH) in high temperature soils, along with a decline in root biomass. In the field, hyphae occur at greater depths, and hence higher temperatures, than plant roots. We are assessing mycorrhizal adaptation to temperature by measuring fungal growth (AM colonization and extraradical hyphae growth) across soil temperatures in the greenhouse. AM fungi were collected from alpine, nonthermal and thermal soils, and grown with plants at soil temperatures of 15, 25 or 40 °C. In the field, we are measuring carbon allocation to AM fungi across temperature gradients, with the hypothesis that host plants depend on AM fungi to augment root function in high temperature soils. Although geothermal soils are relatively unique systems, elevated soil temperatures, seasonally and diurnally, are a common stress. Our work suggests that AM fungi increase host plant success by replacing roots in parts of the habitat that are inhospitable for root growth.

Elemental composition of AM fungal spores from a saline soil in Tunisia

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Arid and saline soils are a great problem for our planet's ecosystems and especially for agriculture. The United Nations Environment Programme estimates that circa 20% of all agricultural used land is salt-stressed, which represents a major limiting factor in crop production. Arbuscular mycorrhiza (AM) can be found under severe salinity and is known to improve plant growth even under salt-stress conditions. In order to maintain growth under salinity, plants need to discriminate the toxic sodium against required nutrient ions such as K. The ratio of K (or other plant nutrients)/Na in the surrounding soil is usually much lower than in the plant tissue, indicating that plants selectively take up nutrients in favour of Na. Very little is known about the role that AM fungi play in this selective uptake. To estimate the capacity of AM fungi to discriminate against Na in favour of plant nutrients we extracted AM fungal spores from a saline and xeric soil in Tunisia and analysed them with proton induced x-ray emission (PIXE). With this technique it is possible to quantify and locate elements of a tissue at a resolution of 1µm and thereby to produce elemental maps. The elemental composition of the spores will be compared to the extractable ions from the soil and to the elemental composition of AM fungal spores grown under optimal conditions in axenic cultures. We will present our interesting and even aesthetical elemental maps with estimates of elemental concentrations in AM spores.

Ectomycorrhizal fungi alleviate deicing salt stress in urban trees

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Salinity is a serious problem for the roadside vegetation in urban areas that are affected by winter applications of deicing salt. In our earlier studies, we found that some ectomycorrhizal associations reduced root salt uptake and restricted root-to-shoot transfer of sodium by their host plants. In the present investigation, we used *Hebeloma crustuliniforme* and *Laccaria bicolor* to inoculate the following tree species: American elm (*Ulmus* americana), columnar aspen (*Populus tremula* 'Erecta'), bur oak (*Quercus macrocarpa*), Siberian larch (*Larix sibirica*). We examined: 1) the effectiveness of ECM fungi in increasing salt tolerance of trees planted along busy streets where salt is used for road deicing and 2) the effects of soil compaction on salt resistance of ECM and non-ECM trees. In salt-affected compacted and non-compacted sites, the mortality of ECM American elm and columnar aspen was reduced and the seedlings broke bud dormancy earlier in spring. The roadside planted ECM trees had higher net photosynthetic rates and chlorophyll contents compared with the non-ECM trees. The effects of ECM on sodium and chloride uptake varied between the studied tree species.

Ozone induced responses on ectomycorrhizal beech roots

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In Kranzberg forest near Freising (Germany) a novel "Free-Air Canopy O_3 exposure" (http://www.casiroz.de) system has been employed for analysing O_3-induced responses that are relevant for carbon balance and CO_2 demand of 60-years old beech trees (*Fagus sylvatica*) and on 6-years old beech seedlings exposed for two years in sun or in shade. The below ground ectomycorrhizal community was studied in 9 soil cores (270 ml each) throughout vegetation seasons 2003 and 2004. In seedlings, root systems were analysed using WinRhizo programme. Types of ectomycorrhizae were determined after anatomical and molecular characteristics (restriction fragment length polymorphism of ITS regions in rDNA database and GenBank sequence comparison) and quantified. Patterns of nitrogen (N) isotope composition, total N contents and cytokinins were determined in leaves and ectomycorrhizal roots of adult beech trees. The number of vital ectomycorrhizal root tips increased, the mycorrhizal community structure and cytokinin contents changed and the specific rate of inorganic N-uptake by the roots was reduced in 2 x O_3. The relative indicators of root growth and ectomycorrhizal community structure in adult and young trees was not comparable. Acknowledgement The study was part of the Project "CASIROZ" (5FW EU project EVK2-2002-00165). The Slovenian partner was co-financed by the Ministry for Higher Education, Science and Technology through the research programme P4-0107.

Root hydraulic conductance in AM bean plants is less dependent on aquaporin activity and more tolerant to osmotic stresses than in non-AM ones

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Several stresses cause an inhibition of root hydraulic conductance (L), which is governed by aquaporins. Almost all plants in the nature make a symbiosis with arbuscular mycorrhizal fungi (AMF), which improve plant stress tolerance. Here, we examined the effects of AM symbiosis on root hydraulic properties and aquaporin expression in *Phaseolus vulgaris* plants under several stress conditions (drought, salinity or chilling). In control conditions, non-mycorrhizal (NM) plants had more L than mycorrhizal (M) ones. L was decreased by these three stresses in NM plants, while in M ones it was almost unchanged. Root expression analysis of three aquaporin genes were carried out. The most significant results were: 1) Salinity raised the expression of the three genes in both NM and M roots. 2) A general decrease of aquaporin gene expression was caused by chilling, being more pronounced in NM roots. 3) Drought caused a general rise of aquaporin gene expression on NM roots while no trend was observed in M roots. In control conditions, M roots had 80% less aquaporin protein abundance than NM roots. However, all the stresses decreased aquaporin protein abundance in NM roots, while no changes were observed in M roots. From these results we conclude that root water transport in M roots is less dependent on aquaporins activity and more tolerant to abiotic stresses than in NM roots.

High-throughput TILLING to identify symbiosis-related plant gene function in arbuscular mycorrhiza

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A large number of plant genes have recently been reported to be modulated during the establishment and development of arbuscular-mycorrhiza (AM). However, knowledge of molecular mechanisms involved in this symbiosis still remains limited. We have identified *M. truncatula* genes that are highly or only expressed during mycorrhizal interactions, using suppressive-subtractive hybridization and cDNA microarrays. A subset of these genes was selected to evaluate their possible role in AM formation and/or function using the reverse genetics approach of high-throughput TILLING (Targeted Induced Local Lesions In Genome), which is based on screening mutagenised plants for mutations in the selected genes. Genomic DNA, extracted from a second self-cross generation of *M. truncatula* plants obtained from EMS-mutagenised seeds, was pooled to screen a wide number of individuals (4500). Genes were PCR-amplified, cleaved by CelI enzyme digestion, products were analysed by LiCor gel-electrophoresis to detect mutations and sequences validated. First screening targeted a gene coding a protease inhibitor, which is uniquely expressed in AM roots. A missense mutation was detected which does not significantly affect the morphological aspects of the symbiosis. Expression of phosphate transporter and H^+ATPase genes are being profiled to assess whether the mutation interferes with symbiotic functions. Other mycorrhiza-related *M. truncatula* genes are presently being screened using the TILLING strategy.

Functional genomics in pea using virus induced gene silencing

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Viruses induce a defence system in plants, which targets the viral genomic RNA for degradation. Insertion of a host gene fragment into the virus genome, results in degradation of both the recombinant virus and the targeted host mRNA. This phenomenon, Virus Induced Gene Silencing (VIGS), has been shown to be a powerful tool for functional genomics as an alternative to transformation by RNAi constructs. It allows the analyses of essential genes and can be employed in plant species, which cannot be transformed. *Pisum sativum* and the tobravirus, *Pea early-browning virus* (PEBV) were chosen as model system, to transfer this technology to legumes. One of the advantages of developing a VIGS system for legumes is that it facilitates functional analyses of genes involved in the legume-rhizobium and legume-mycorrhiza symbioses. The PEBV-VIGS system was initially shown to cause efficient down-regulation of three non-symbiotic genes (1) and the nodulation reference gene, *PsSym35*, in pea (Constantin et al, work in progress). We are currently testing the reference genes, *PsSym9* (dmi3) and *PsSym19* (dmi2) of the common pathway for rhizobial and mycorrhizal symbioses, to study the potential of the PEBV-VIGS system to interfere with plant genes involved in mycorrhization. The PEBV-VIGS system and *PsSym9*, *PsSym19* silencing results will be presented. 1) Constantin et al., 2004, Plant J, 40, 622-631.

Suppression subtractive hybridization as a tool for identifying genetic diversity between mycorrhizal fungal genomes

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The Suppression Subtractive Hybridization (SSH) applied to genome is a technique allowing the isolation of DNA fragments that are present in one genome (tester) but not in another (driver). Genomic SSH has been widely used to detect genetic differences among bacteria. By contrast, to our knowledge, this technique has not yet been applied to mycorrhizal fungi. The aim of this work was to use such a technique to identify genetic diversity between mycorrhizal fungal genomes. First, to identify genomic regions involved in the evolution of the genus *Tuber*, we performed three experiments : 1) SSH experiments with phylogenetically non related species (*T. melanosporum-T. borchii*); 2) SSH experiments with phylogenetically more related species (*T. magnatum-T. borchii*) and 3) SSH experiments with two isolates of the same species (*T. magnatum* from Croatia and Piedmont). Second, to identify specific genomic regions linked to metal resistance in the endomycorrhizal ericoid fungus *Oidiodendron maius* we realized SSH experiments between a metal tolerant isolate and a non metal tolerant isolate. We demonstrate that SSH can be used to identify specific genomic regions between mycorrhizal fungal genomes at inter- and intra-specific level and in different experimental systems. In addition, results suggest that retrotransposons might have played a role in truffle evolution.

Fluorescent *in situ* RT-PCR used to localise gene expression in the ectomycorrhizal fungus *Hebeloma cylindrosporum*

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Mycelium of the ectomycorrhizal fungus *Hebeloma cylindrosporum* was used to study mycorrhizal fungal gene expression. *In situ* RT-PCR allowed us to localise gene expression and to compare expression patterns in response to phosphate treatment. Expression was visualised after staining with the commonly used NBT/BCIP or with a fluorescent substrate called ELF-97. A gene encoding elongation factor-1 alpha (*EF1α*) was constitutively expressed. Under phosphate starvation a clear growth reduction of the mycelium was observed and the expression levels of *EF1α* were slightly decreased when visualised with ELF-97, but this could be explained by a loss of vitality of the mycelium under this condition. The expression of a mycorrhizal fungal specific phosphate transporter (*HcPT1*) showed, however, a clear response to the phosphate treatment. Under phosphate starvation *HcPT1* was expressed in most of the mycelium, whereas when phosphate was available, only a small part of the mycelium showed expression. Fluorescent *in situ* RT-PCR clearly showed to be suitable for localisation of gene expression in the ectomycorrhizal fungus *H. cylindrosporum*. The use of the ELF-97 substrate made it possible to detect the expression with epifluorescence microscopy and enabled localisation at the level of the individual hyphae.

Rapid classification of arbuscular mycorrhizal fungi (Glomales) in roots by Quartz Crystal Microbalance

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Arbuscular mycorrhizal (AM) fungi living symbiotically with plant roots can be analysed by microscopy, PCR or Restriction Fragment Length Polymorphism (RFLP), but it is not possible using these methods to obtain a rapid and/or explicit identification of these fungi. Here we present a method for rapid detection of AM fungi DNA in an AM fungi/plant root DNA mix using a Quartz Crystal Microbalance (QCM). This method can also be used to classify these fungi. Detection of AM fungi-DNA using QCM is achieved through immobilization of AM fungi specific oligonucleotides by avidin–biotin binding onto the surface of quartz crystals. The sequence of the oligonucleotides is specific for AM fungi sequences that are not present in the genomic DNA of the host plant. When present in the sample, AM fungi-DNA hybridizes with the oligonucleotides causing an increase of the mass on the QC-surface, which in turn decreases frequency of QC vibration (the signal). The difference between the signals before and after the hybridization reaction correlates with the concentration of AM fungi-DNA in the sample. By using species-specific *Glomus* oligonucleotides it is possible to detect individual *Glomus* species.

Quantitative detection of extraradical soil mycelium of *Lactarius deliciosus* by Real-Time PCR. Application in the study of interspecific competition

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Real-Time PCR has been applied to quantify extraradical soil mycelium of *Lactarius deliciosus* in an interspecific competition experiment under greenhouse conditions. *Pinus pinea* seedlings were inoculated with either *L. deliciosus* or *Rhizopogon roseolus*. Two one-year-old plants from different inoculation treatments were transplanted 10 cm apart into 4l pots filled with two types of soil at the following combinations: *L. deliciosus-R. roseolus*, *L. deliciosus*-Control (non-inoculated) and *R. roseolus*-Control. Soil cores equidistant from both plants were taken at three and six months after transplantation. Each soil core was divided in two depths and DNA was extracted to perform Real-Time PCR analysis using specific primers and a TaqMan® MGB probe designed in the ITS1 rDNA region of *L. deliciosus*. Six months after transplantation, root colonization and plant growth were monitored. No significant effect of the accompanying plant was detected in the final percentage of mycorrhizas of *L. deliciosus*. However a significant effect of the soil type was observed. Soil mycelium was successfully detected in one type of soil and was significantly affected by the sampling time and soil depth factors. Competition of the accompanying plant was not significant. Also, extraradical soil mycelium of *L. deliciosus* was significantly correlated with the final percentage of mycorrhizas. Plant growth was only affected by the soil type.

Influence of mycorrhization on biomass production and active compounds of endangered species *Arnica montana* L.

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Arnica montana L. is a rare plant fully protected by Polish law. Regarding its' medicinal value, this endangered species has been commonly used in pharmaceutical and cosmetic industries. Information on optimal condition for growing of *A. montana* should improve efficiency of its' cultivation and cut down illegal harvesting from natural stands. In our study *A. montana* grown in laboratory were inoculated with various strains and species of mycorrizal fungi, or cultivated without inoculum. Survival of seedlings was monitored as well as plants growth, and fluorescence of photosynthesis. Development of arbuscular mycorrhiza in roots (microscopic evaluation of mycorrhiza frequency, mycorrhizal colonisation and arbuscule richness) was investigated. Mycorrhizal fungi are know to influence secondary metabolites of plants. These compounds participate in interactions of a plant with its biotic and abiotic environment, and are also responsible for medicinal properties of plants. For these reasons we analysed selected active metabolites in *Arnica montana*, collected from nature, and cultivated in laboratory conditions with various arbuscular mycorrhizal fungi. The present work was supported by Polish Ministry of Education and Science Grant PB 2P04G10928.

Analysis of the mycorrhizal status and diversity of AM fungi associated to representative endangered plant species in Andalucía, Spain

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The universally-recognized crucial importance of arbuscular mycorrhizal (AM) fungi for the establishment, development and proliferation of plants in stress situations envisaged a fundamental role of these fungi in the conservation of endangered flora. Therefore, to assess the impact of native AM fungi for conservationist purposes is an issue of current outstanding interest. Accordingly, an evaluation of the AM status of target threatened species and the analysis of AM fungal diversity in the rhizosphere of these plants growing in their native habitats were carried out. Particularly, four representative taxa belonging to the "Red List" of Andalusian Flora, Ilex aquifolium, Laserpitium longiradium, Sorbus hybrida and Atropa baetica were selected. These are listed as in a "critical danger status", according to following UICN (2001) categories. It was first demonstrated the AM-status of these target species. Trap plant cultures were then established and the isolated spores were morphologically characterised. Pure cultures have been established. The genetic characterization of most representative AM fungal isolates has been carried out by PCR amplification and sequence analysis of their SSU rRNA. A germoplasm bank of the isolated AM fungi has been established. This is the first step for exploiting the AM potentiality, as a biotechnological tool, for recovering endangered plant species and for the preservation of populations of threatened plants.

Mycorrhizal colonization of endangered plants in fen meadows

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AMF species are known to improve the fitness of different plant species to various extents. Information on the soil micro-flora known to improve plant fitness and soil health is thus of utmost importance for endangered plant species considered to be introduced into their former environment. Hence, this project focuses on the analysis of the mycorrhizal status of different plant species which are locally endangered in the county of Salzburg, Austria. Root samples of *Allium carinatum* (Liliaceae), *Betoncia officinalis* (Lamiaceae), *Filipendula vulgaris* (Rosaceae), *Gladiolus palustris* (Iridaceae) and *Serratula tinctoria* (Asteraceae) were collected at different stages of plant development throughout the vegetation period of 2003. AMF and DSE colonization intensities were quantified. The AMF species colonizing the plant roots were identified by partial amplification of rDNA (SSU) followed by cloning, RFLP and sequencing. Soil parameters like pH, water content and plant available phosphorus were determined as well. In addition, AMF spores were extracted from rhizosphere soil, grouped according to morphological features, and identified. Results obtained through application of both classical as well as molecular methods will be discussed.

Mycorrhizal and root endophytic fungi of *Pedicularis* L. from the northwest of Yunnan Province, China

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Plants of *Pedicularis* L. (Orobanchaceae) display great ornamental values as well as biological significance owing to their dramatic morphological diversity and special pharmacological effects. Unfortunately, they have a reputation for being uncultivable, which hinders their exploitation to a large extent. Once considered key limiting factors to cultivation, host plants have been applied but can hardly overcome the difficulties encountered. Speculating that mycorrhizal fungi might play a more important role, we examined the colonization status of root associated fungi in 14 *Pedicularis* species from the northwest of Yunnan province, southwest of China. These species included: *P. gracilis*, *P. longipes*, *P. axillaris*, *P. cephalantha*, *P. tenuisecta*, *P. tapaoensis*, *P. likiangensis*, *P. dichotoma*, *P. yui*, *P. rhinanthoides*, *P. rex*, *P. longiflora*, *P. siphonantha* and *P. oxycarpa*, among which 9 are endemic to China. Three types of fungi were observed, namely arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (EMF) and dark septate endophytic fungi (DSEF), with DSEF as the most common colonizers. Unexpected high colonization level was detected in this genus. Of the 19 sampling sites examined, 10 presented colonization frequency of above 50% and 6 showed colonization index of more than 50. Heavy colonization suggests a significant ecological role of these fungi and their potential to be applied to successful cultivation of these hard-to-deal-with plants.

Arbuscular mycorrhiza colonization is comparatively high in habitats with extremely elevated soil salinity

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The aim of the study was to establish a possible adaptive significance of arbuscular mycorrhizal symbiosis for endangered and rare plants from coastal habitats - sand dunes, salt marshes and coastal grasslands. Characteristic features of these habitats are irregular nutrients availability, drought or wet and salinity. Root samples of several plant species were collected from coast of Baltic sea and Riga Sea bay in territory of Latvia. In total roots of 28 plants species were decolorized in 10 % KOH and stained with 0.05 % trypan blue. The roots were washed and stored in lacto-glycerol. Results indicate that plant species from salt marshes (soil salinity up to 4358 mg kg^{-1} Na) had high rate of infection with arbuscular mycorrhiza ranging from 65.5 % (*Aster tripolium*) and 51.5 % (*Plantago maritima*) to 24.3 % (*Trifolium fragiferum*). Arbuscules were abundant in roots of *A. tripolium* and *P. maritima*. Infection intensity of arbuscular mycorrhiza for plant species from sand dunes and coastal grasslands varied from 47.8 % (*Eryngium maritimum*) to < 2 % (*Centaurium litorale*). In most of all plant species various forms of vesicules were observed. A possible significance of arbuscular mycorrhizal symbiosis as adaptive biological feature is discussed. This research is a part of the project of University of Latvia „Biological basis of conservation of rare and protected plant species in Latvia: coastal zone".

Mycorrhiza in well preserved native and in replanted and disturbed Brazil Pine forests

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Ecosystems with predominance of *Araucaria angustifolia*, the Brazil Pine, are endangered of extinction. Although being a conifer, this tree species is not ectomycorrhizal. However, it is very dependent on arbuscular mycorrhizal (AM) fungi; knowing their diversity is fundamental for the sustainability of Brazilian preservation and reforestation programs. Soil and root samples were collected twice a year at PETAR, a State Park, localized close to Apiaí, São Paulo State, Brazil. Two ecosystems were studied: 1) well preserved native forest, composed of a mixed vegetation of numerous tree and shrub species, and 2) reforested area with a pure standing of *Araucaria angustifolia*, and high anthropic impact. Total no. of spores, species richness, root colonization rates, Shannon's diversity index and Simpson's dominance index were determined. By using Canonic discriminating analysis and cluster analysis we could discriminate between both areas. Shannon's diversity index was the ecological indicator with the highest contribution to this discrimination. At all 27 AMF species were identified, and the native forest presented much higher spore numbers, root colonization rates and diversity index than the replanted forest. The applied multivariate statistical tests proved to be very useful tools in allowing for a correct separation between areas, with the help of several biological indicators.

Linking land use change, soil quality, and biodiversity in the dry tropics: are mycorrhizal associations important for forest regeneration?

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Primary and secondary vegetation, and agricultural land are mixed in a dynamic mosaic in the tropics. Secondary vegetation cover increases, mainly due to the abandonment of agricultural land. Considering the policies derived from the Kyoto protocol (paying for carbon emissions by investing in tropical forests), it is increasingly relevant to understand the key factors involved in the regeneration of tropical vegetation. We conducted a series of studies to understand the role of mycorrhizal associations in forest regeneration in the dry tropics (42% of the tropical forests), where the strong water limitation makes regeneration very difficult. We hypothesize that in this context mycorrhizal associations play a more important role as soil quality enhancers (rehabilitating long-term nutrient and water retention mechanisms in degraded soil) than as plant growth promoters. We measured 1) physical, chemical and biological properties to evaluate the status of the soils, 2) mycorrhizal development parameters in soil and roots, and 3) seedling growth and water relations. Our results indicate that mycorrhizal colonisation, inoculum potential and glomalin was similar in the soils. Secondary forest soils have recovered, compared to agricultural land, in many soil quality variables and diversity, and were the best seedling growth substrates. However, despite being close to primary forests in several processes, secondary forests still lack important water and nutrient retention mechanisms.

Land-use history and ectomycorrhizal fungi richness and diversity patterns in managed European Mediterranean oak woodlands: Alentejo region, Portugal

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During centuries most European Mediterranean oak woodlands (EMOWs) were converted into agro-silvo-pastoral (ASP) systems combining soil productivity and biological conservation. Over the 20th century strong changes in management planning strategies have conducted to the landscape simplification, both by intensification of land-use practices and abandonment. Ectomycorrhizal (ECM) fungi have been showed to be sensitive indicators of agroforestry response to environmental disturbances. To study whether land-use activities influence ECM fungi richness and diversity patterns in managed oak woodlands, we examined the ECM associated to cork oak (*Quercus suber* L.) over spring, summer and autumn, in 15 plots (9 farm units) under different ASP exploitation, in the Alentejo region (Portugal). In total, 751 810 cork oak root tips were assembled from 720 soil cores (84.96 dm3) in the 15 plots, and 120 ECM taxa (85 below-ground) were identified using morphotyping and direct DNA sequencing. Apart from *Cenococcum geophilum*, the ECM communities below-ground were dominated by tomentelloid fungi (15%) and members of the genus *Russula* (25%), *Lactarius* (9%) and *Cortinarius* (5%). Our results showed that ECM fungi richness and diversity patterns were significantly influenced by: 1) land-use history, 2) composition of vegetation cover, 3) edaphic conditions, and 5) geographical location. Implications of cork oak woodlands management in ECM biodiversity descriptors are discussed.

Spatial arbuscular mycorrhizal fungal community composition of soybean crop and hybrid poplar in temperate agroforestry system in south-west of Quebec

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To the best of our knowledge, it is the first study where the AM (arbuscular mycorrhizal) fungal community is assessed in order to highlight both qualitative and quantitative differences in agroforestry systems and conventional forestry systems. The goals of the work are to identify the AM fungal species involved in root colonisation of soybean crop (*Glycine max*) and poplar (*Populus nigra x maximowiczii*) growing in arable fields in the south-west of Quebec, using RFLP-PCR amplification of SSU rRNA genes, and to determine whether their distribution reflects host species and the location. In addition, we took a look at AM spores abundance and distribution. We hypothesize that 1) the pattern of AM fungal community composition in the arable crop exhibits spatial changes correlated with distances from poplar, 2) in the agroforestry system, arable crop affects the AM fungal community composition of the tree component compared to the forestry system, and 3) co-occurring crop and tree roots share a similar AM fungal community composition. We tested the relationships between the AM spores abundance and distribution, the AM fungal community composition, crop and tree yield, canopy opening, and soil chemical and physical properties. The first results revealed that in agroforestry plots the spore abundance and crop yield increased significantly with distance from poplar, light transmittance and soil P content, while spore abundance and soil P content were homogenous in forestry plot.

Effect of soil management and weed control on the diversity and establishment of arbuscular mycorrhizal colonisation of wheat in Mediterranean climate

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Soil tillage may markedly reduce or delay the rate of arbuscular mycorrhiza (AM) establishment by breaking up the living AM fungal mycelium in the soil, and restrict the desirable rapid and effective AM colonization of fall-sown crops under Mediterranean conditions. Weeds roots may help to initiate the colonization process by allowing the development of an already well established and active mycelium network by the time the crop germination occurs. Pot experiments were developed to assess the timing during the cropping season of AM colonization in wheat roots from disturbed and undisturbed soil subject to different weed control methods. Results indicate that colonization occurred first in undisturbed soil, where the extra-radical mycelium (ERM) is preserved. In wheat roots, 3 weeks after seedling emergence, AM colonization in undisturbed soil was double than in sieved soil, showing that ERM survived over the Mediterranean summer, however not leading to an improved plant performance. These results still held when herbicides (contact or systemic) were used to control weeds over the late summer/early fall. To gain a global overview of the diversity of Glomeromycota under the 2 cultivation systems, fungi rDNA sequences were amplified from DNA extracted directly from field soil. In total 87 sequences were analysed and the results support the view that AMF are differently vulnerable to soil disturbance, not only in terms of diversity, but also in terms of the community structure.

Effect of organic and mineral P fertilizations on mycorrhizal biodiversity in a maize-soybean rotation.

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Beneficial impacts of the ubiquitous symbiosis between plants and arbuscular mycorrhizal fungi (AMF) on nutrient cycling, soil structure, environmental and pathogen stresses have been documented in several ecosystems. In agriculture, AMF may have more impact on plant nutrition due to the limited availability of phosphorus (P). Although intensively managed agricultural soils are fertilized each year, little is still known about the effects of P-inputs on AMF diversity and relation with nutrient cycling. The evaluation of mycorrhizal diversity based on identification of spores is highly time-consuming and biased toward sporulating taxa. The use of culture-independent DNA-based techniques has help to overcome these technical constraints. In this study, total soil DNA was extracted and a PCR-Denaturing Gradient Gel Electrophoresis approach was used to profile the dominant mycorrhizal ribotypes within 2 years of a maize/soybean rotation with regards to plant phenology and fertilization with organic or mineral P sources. Over the 90 samples studied, 15 distinct ribotypes were identified, with only a few present in less than 5 cases. Between 1 and 3 ribotypes were observed in most samples. Community structure varied within and between fertilization treatments, indicating that specific taxonomic units are not related to the type of fertilizer. Changes in ribotype assemblage were also noted according to time but without a clear seasonal trend of the AMF community.

Advances on management of effective arbuscular mycorrhizal symbiosis in tropical ecosystems

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In the last 10 years, a lot of advances related with mycorrhizal symbiosis had been obtained in Cuba on the knowledge of different factors which determine the effective management of symbiosis trough the inoculation of efficient strains, the development of novel mycorrhizal products applied in low doses and its application ways. All of this had allowed the use of the symbiosis on big land extensions in a grand number of important crops, different soils and production ways, not way restrained to marginality conditions. The main results related with: culture inoculation with efficient strains by soil group, strain - culture low specificity, the influence of mineral or organic fertilizer supply on symbiosis effectiveness, the join application with others biofertilizer and the influence of culture sequences are presented in this work. The validations results in different crops, kind of soils and production ways are showed too. The application of mycorrhizal inoculants trough recovering seeds could be successfully integrated with the agricultural machinery on big agricultural systems production and also with manual applications on small agricultural systems production. Beginning from the universality of the AM symbiosis and from different results obtained emerges the concept of efficient AM cropping systems which are constructed around the model of the effective mycorrhizal plant, through inoculation with effective strains and management practices optimizing their efficiency.

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