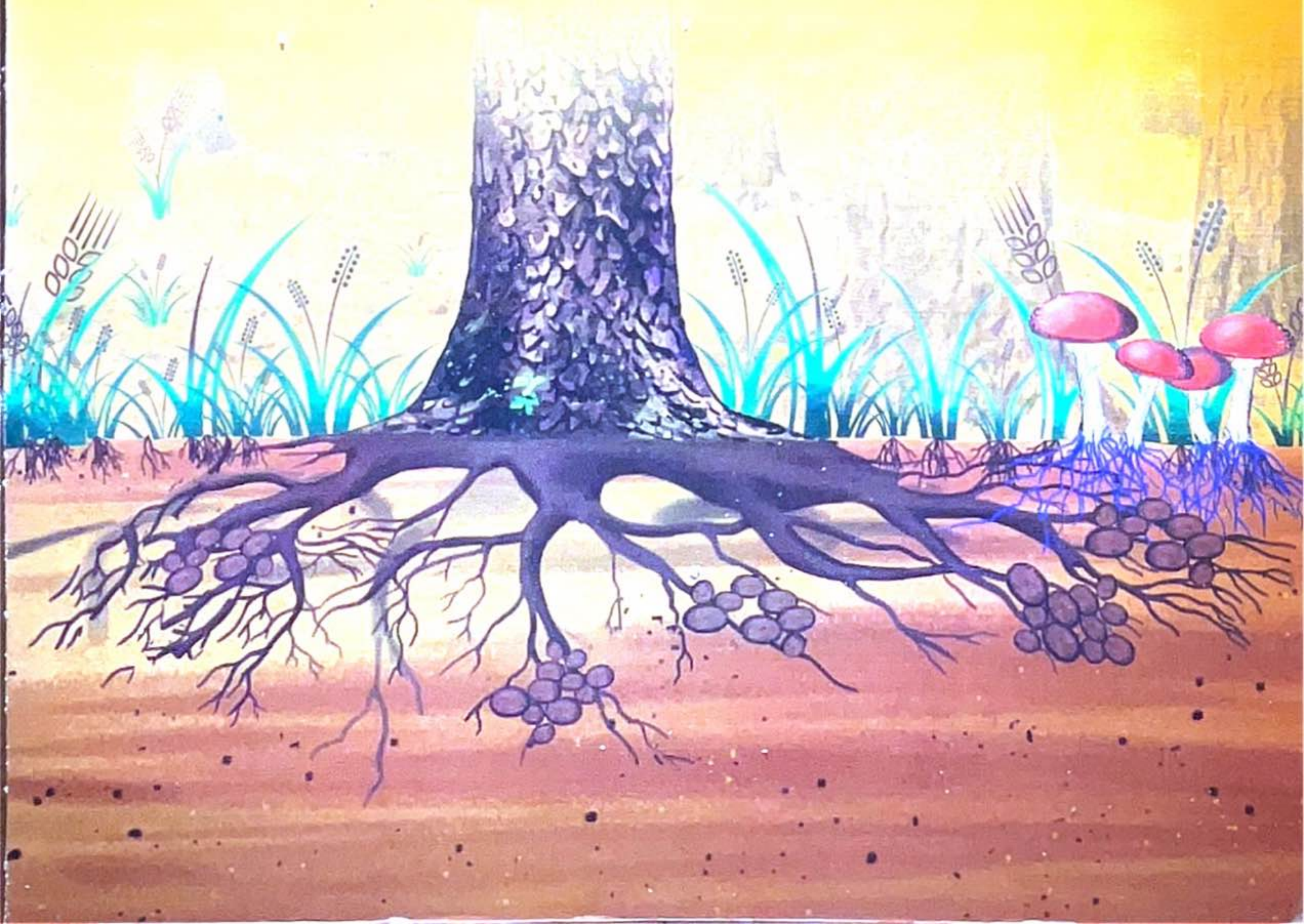
ICOM 7

ABSTRACTS

7th International Conference on Mycorrhiza

"Mycorrhiza for All: An Under-Earth Revolution"

6-11 January 2013, New Delhi, India



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"Mycorrhiza for All: An Under-Earth Revolution"
6-11 January 2013, New Delhi, India

सत्यमेव जयते

Department of Biotechnology
Ministry of Science and Technology
Government of India

teri
The Energy and Resources Institute

iMS
International Mycorrhiza Society

MYCORRHIZA NETWORK

PREFACE

It is my pleasure to welcome you all to the Seventh International Conference on Mycorrhiza, which is being organized in Asia for the first time. We are proud to be hosting this global forum for interaction of researchers on the scientific and applied areas of the mycorrhizal symbiosis. It is indeed a pleasant sight to observe the interest and initiative taken by scientists, technological experts, and students towards developing the knowledge and application base on mycorrhiza, for the benefit of the world.

The theme of this conference, "Mycorrhiza for All: An Under-Earth Revolution" aptly represents the spirit of change. The mycorrhizal revolution has the capability to address the uncontrolled use of chemical fertilizers, thus, managing the associated environmental footprint. The ongoing research on this subject has vital implications for agriculture, energy, and environment as a whole. Thus, it is in the interest of many groups—technologists, scientists, agriculturists, regulators, and policy-makers—to continue the enthusiasm to explore more, sharing and disseminating knowledge through various channels. The conference intends to provide one such platform to contribute to the existing resource on mycorrhiza through circulation of ideas and research anecdotes of relevance. This interaction would equip the international community with a roadmap on refining and enriching future research in this area.

We sincerely hope that the conference will be both pleasant and productive, for all of you. I and my colleagues would facilitate this conference in every possible manner to ensure that it is an enriching experience.

I wish to take this opportunity to thank all the Individuals, Institutions and Sponsors who have contributed towards the conference. I express my gratitude and appreciation to all the members of the local organizing committee, without whose hard work and dedication, the conference would not have been possible.

I also extend my appreciation and recognition to those, who helped in choosing India, as the first Asian Venue for the ICOM.

Wishing you a very Happy and Prosperous New Year 2013"!

Dr Alok Adholeya

ICOM SERIES AND ITS IMPORTANCE

The International Conference on Mycorrhiza (ICOM) provides an international forum for researchers in all scientific and applied areas of the mycorrhizal symbiosis. The conference facilitates participants to present their current research status to the international community in the form of either oral presentations or posters. In addition, symposia and workshops covering topical issues related to the basic biology of the symbiosis, forestry, agronomy, and horticulture form an essential part of the conference.

Besides providing a forum for education, knowledge transfer, and professional development, the ICOM intends to facilitate interaction between different scientific working groups in the industry to discuss the state of mycorrhizal research around the world, and sharing of local and international experiences. The Conference provides exposure for students to newer horizons and countries to newer collaborations. The function set of the participants ranges from area experts to students desirous of learning more about mycorrhiza.

ABOUT ICOM7

The theme of the 7th International Conference on Mycorrhiza, ICOM7, "Mycorrhiza for All: An Under-Earth Revolution" has been aptly chosen. The logo has been carefully thought out, to reflect this idea. The Banyan Tree is of extreme importance to the cultural heritage and legacy of India for ages. Its roots are mycorrhized by golden honey dew drops resembling mycorrhizal spores. The symbiotic association is providing protection to the plant and nutrient security, represented by the protective arc over the tree. The theme focusses on the latest research and development in the field and efforts made to spread the benefits to the common man. It has been so chosen, such that it may prove to be the epicentre of a new revolution of which our planet is in dire need. A change that would help minimize the usage of chemical fertilizer on soil and hence leave the least environmental footprint.

ABOUT TERI

The Energy and Resources Institute (TERI) was formally established in 1974. With the headquarters in New Delhi, it has created an environment that is enabling, dynamic, and inspiring for the development of solutions to global problems in the fields of energy, environment, and sustainable development. It has been ranked 20th in the list of top global think tanks on environment by Think Tank and Civil Societies Program, University of Pennsylvania.

The Biotechnology and Management of Bioresources Division

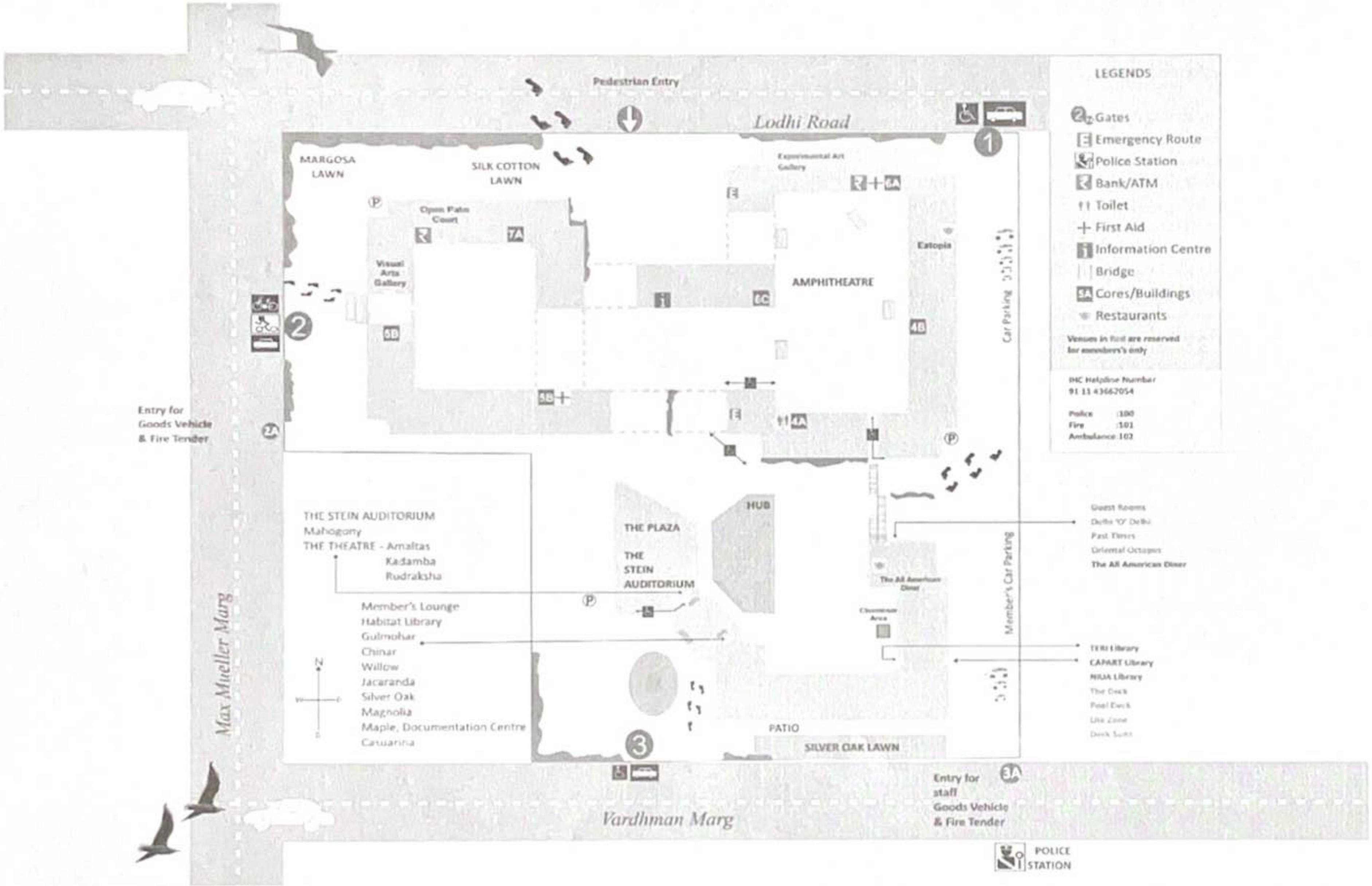
The concern for food security is increasing rapidly. With recent projections of global population revised to 10 billion by 2050, the pertinent questions that arise are: How do we feed these numbers without degrading the environment further? How do we meet the demand for biofuels and other biomass in a carbon constrained world? Sustainable agriculture and land utilization hold the key. The activities of the Division focus both on increasing the productivity and productive capacity of all types of land (including degraded lands) using cost-effective, eco-friendly microbial resources known as mycorrhizal fungi, and on large-scale propagation of high-quality plant material using modern biotechnological techniques. The new TERI-Deakin Nano Biotechnology Research Centre will substantially improve fungicides and pesticides so as to enhance the resource efficiency of agricultural ecosystems. The division's ongoing activities also include genetic improvement of plant material (with food and fuel potential) and facilitate subsequent large-scale production of high quality tissue culture plants in TERI's facilities (ISO 9000 certified) for global dissemination. TERI is currently seeking to establish a bio-refinery (using wheat and rice residues as feedstock) with a capacity of 500,000 tonnes/year of feedstock, with the objective of maximizing ethanol production as a substitute for petrol. Efforts are also being made to collect, screen, and characterize the microalgal gene pool with an aim to optimize the growth conditions of superior strains (with 52% oil content) for potential biodiesel production.

The Centre for Mycorrhizal Research

In view of the stark reality of increasing demand for food production, food security, and nutrient deficiency, Mycorrhiza will in the future play a key role owing to its contribution to the plant, and as a result to phosphorus nutrition in agriculture. Mycorrhiza, with its mutualistic, symbiotic association, is of great relevance and significance to problems, such as nutrient deficiency and marginal lands. With its main focus on basic and applied research using microbial resources for biotechnological intervention to address agriculture, energy, and environment-related issues, the Centre for Mycorrhizal Research at TERI, spearheaded by Dr Alok Adholeya, has core competence in mycorrhizal technology and bioremediation.

The Centre started its activities in 1986 with documentation, characterization, and conservation of mycorrhizal germplasm. Later, a decade's research on several plants in different agro-climatic regions confirmed the beneficial and application-oriented role of these fungi, and led to the development of technologies.

Mass production technology for the first-ever mycorrhiza product from *in vitro* technology was developed in 2000 and other technologies involving environmental remediation (of fly ash overburdens, sites laden with chlor alkali, sites used for disposing of distillery effluent) using selected mycorrhizal organisms were ready a few years later. The *in vitro* mass production technology developed by TERI has claimed two patents, received many awards, and has been transferred to seven industries, couple of which produces 2,000 tonnes of mycorrhizal biofertilizer annually.


Lodhi Road
Pedestrian Entry
Max Mueller Marg
Vardhman Marg
Entry for Goods Vehicle & Fire Tender
2A
Entry for staff Goods Vehicle & Fire Tender
3A
POLICE STATION
1
2
3
MARGOSA LAWN
SILK COTTON LAWN
Open Palm Court
7A
Visual Arts Gallery
5B
6B
Experimental Art Gallery
6A
6C
AMPHITHEATRE
Eatopia
4B
4A
Car Parking
Member's Car Parking
HUB
THE PLAZA
THE STEIN AUDITORIUM
The All American Diner
PATIO
SILVER OAK LAWN
THE STEIN AUDITORIUM
Mahogony
THE THEATRE - Amaltas
Kadamba
Rudraksha
Member's Lounge
Habitat Library
Gulmohar
Chinar
Willow
Jacaranda
Silver Oak
Magnolia
Maple, Documentation Centre
Casuarina
N
Guest Rooms
Delhi 'O' Delhi
Past Times
Oriental Octopus
The All American Diner
TERI Library
CAPART Library
NIUA Library
The Deck
Pool Deck
Lite Zone
Deck Suite
LEGENDS
Gates
Emergency Route
Police Station
Bank/ATM
Toilet
First Aid
Information Centre
Bridge
Cores/Buildings
Restaurants
Venues in Red are reserved for members's only
IHC Helpline Number
91 11 43662054
Police :100
Fire :101
Ambulance :102

VENUE MAP

GENERAL INFORMATION

Apex Committee

Dr Lalji Singh, Vice Chancellor, Banaras Hindu University
Dr P L Gautam, Chairperson. Protection of Plant Varieties and Farmers' Rights Authority
Dr R S Paroda, Former Director General, Indian Council of Agricultural Research
Dr S Ayyappan, Director General, Indian Council of Agricultural Research
Dr T Ramasami, Secretary, Department of Science and Technology
Dr R K Pachauri, Director-General, The Energy and Resources Institute

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Dr Tom Bruns, University of California, USA
Dr Alok Adholeya, The Energy and Resources Institute, India

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Prof. B L Jalali, Member, Commission of Agricultural Reforms, Govt. of Jharkhand
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Prof. C Manoharachary, Emeritus Scientist, CSIR
Prof. D J Bagyaraj, Chairman, Centre for Natural Biological Resources and Community Development
Dr H S Gupta, Director, Indian Agriculture Research Institute
Prof. K V B R Tilak, Emeritus Scientist, CSIR

Local Organizing Committee

ICOM 7 President: Dr Alok Adholeya

Conference Logistics, Hospitality And Accommodation Committee: Sudheer Katoch, I I Jose, K S Vasan, R Venkatesan, Sonal Bajaj, Sonali Baswani

Conference Registration Committee: Megha Parashar, Vartika Gaur, Ritu Ghai, R Venkatesan, K Muralidharan

Conference Programme Committee: Reena Singh, Rahul Singh, Pushplata Singh, Ekta Jain

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Exhibition Committee: Chandrakant Tripathi, Gagandeep Sassan, Deepak Kumar

Invited Speakers

Keynote Speakers

Dr Francis Martin, INRA Nancy, France
Prof. Uwe Nehls, Bremen University, Germany
Dr Ian Dickie, Landcare Research, New Zealand
Dr J André Fortin, Université Laval, Canada

Plenary Speakers

Dr Natalia Requena, Karlsruhe Institute of Technology, Germany
Dr Anders Tunlid, Lund University, Sweden
Dr Guillaume Bécard, University of Toulouse III, France
Dr Toby Kiers, Vrije Universiteit, The Netherlands
Dr Edith C Hammer, Freie Universität, Germany
Dr Bingyun Wu, Tokyo University, Japan

Dr Maarja Öpik, Tartu University, Estonia
Dr Thorunn Helgason, University of York, The United Kingdom
Dr Alok Adholeya, The Energy and Resources Institute, India

Workshop Convenors

Prof. Luisa Lanfranco, University of Turin, Italy
Prof. Yoram Kapulnik, Volcani Center, Israel
Prof. Leho Tedersoo, University of Tartu, Estonia
Dr Arthur Schüssler, University of Munich, Germany
Dr Björn Lindahl, Swedish University of Agricultural Sciences/SLU, Sweden
Prof. François Buscot, Helmholtz Centre for Environmental Research (UFZ), Germany
Dr Tania Wyss, University of Lausanne, Switzerland
Dr Maarja Öpik, Tartu University, Estonia
Dr Aurélie Deveau, INRA, France
Dr Mika Tarkka, Helmholtz Centre for Environmental Research GmbH - UFZ, Germany
Prof. Akiyoshi Yamada, Shinshu University, Japan
Dr Chantal Hamel, Semiarid Prairie Agricultural Research Centre, Canada
Dr Dipankar Maiti, Central Rainfed Upland Rice Research Station (ICAR), India
Prof. Erik Hobbie, University of New Hampshire, The USA
Dr Rupam Kapoor, Delhi University, India
Dr Courty Pierre-Emmanuel, University of Basel, Switzerland
Dr Sara Hortal, Hawkesbury Institute for the Environment, UWS, Australia
Dr Kazuhide Nara, University of Tokyo, Japan
Prof. A. Manjunath, University of Agricultural Sciences, India
Prof. C Manoharachary, Osmania University, India
Dr Marc-André Selosse, Centre for Evolutionary and Functional Ecology, France
Prof. D. Lee Taylor, University of Alaska Fairbanks, The USA
Prof. John Klironomos, University of British Columbia, Canada
Dr Maman Turjaman, FORDA, Indonesia
Dr Reena Singh, The Energy and Resources Institute, India
Prof. Melanie Jones, University of British Columbia, Canada
Prof. D J Bagyaraj, CNBRCD, India

ICOM Awards

The organizers of ICOM7 express their sincere gratitude to the donors of prizes that will be awarded on this occasion to students making oral and poster presentations, as recognition of the quality of their contribution to mycorrhiza research.

The IMS / New Phytologist J L Harley Medal

Awarded by IMS to the **best paper** presented by a graduate student at ICOM. The medal is accompanied by prize money of £200 (GBP).

Barbara Mosse – Laval Université Prize

Given by: Laboratoire de mycologie, Centre d'étude de la forêt, Université Laval

Awarded to a graduate student for the second best oral presentation on any aspect of mycorrhiza research. The prize will consist of a certificate and US$100.

Mycorrhiza Prize

Given by: The Journal Mycorrhiza

Awarded for the best poster presentation by a graduate student on any aspect of mycorrhiza research. The prize will consist of a certificate and voucher for US$250 towards the purchase of any book available at www.springer.com.

Larry Peterson Anatomy Prize

Given by: Larry Peterson

Awarded to a graduate student for the best poster presentation with emphasis on the structure of mycorrhizas. The prize will consist of a certificate, US$100 and a copy of the book, Mycorrhizas: Anatomy and Cell Biology.

Ultimate Mycorrhiza Application for Harnessing Agriculture Resources Integration Award (UMAHARI Award)

Given by: Dr Alok Adholeya

Awarded to a best poster and a best oral presentation emphasizing application research of mycorrhiza towards product or inoculants. The awards will consist of US$100 each besides a certificate and citation.

MCRC Awards

BioSyneterra Prize

Given by: Peter Moutoglis of BioSyneterra Solutions Inc.

Awarded for the best oral presentation by a graduate student with emphasis on applied research and innovative/industrial applications with mycorrhizas. The prize will consist of a certificate and US$100.

Biotisa Prize

Given by: Gerard Catroux of Biotisa

Awarded to a graduate student for the best poster presentation dealing with applications of mycorrhizas. The prize will consist of a certificate and US$100.

Hall of Fame Awards

International Mycorrhiza Society (IMS) in its attempt to honour the work of early Mycorrhizologists (living or posthumous) have institutionalized awards under "Hall of Fame" category. This edition of ICOM would be the first to witness it.

7TH INTERNATIONAL CONFERENCE ON MYCORRHIZA (ICOM7)

6- 11 January 2013
India Habitat Centre (IHC), Lodhi road, New delhi-110003

AGENDA

SUNDAY, JANUARY 6, 2013	
14:00 – 18:00	**Registration** *Charminar, IHC*
MONDAY, JANUARY 7, 2013	
8:00 – 9:00	**Registration** *Stein Side walk Area, IHC*
9:00 - 9:45	**Inauguration Ceremony** *Stein Auditorium, IHC*
9:45 - 10:15	**Tea** *The Hub, IHC*
10:15 - 11:00	**PLENARY SESSION 1** Developmental, Functional and Environmental Genomics of Mycorrhiza *Stein Auditorium, IHC* KEYNOTE SPEAKER **Dr. Francis Martin**, INRA Nancy, France "Exploring The Genome Diversity Of Mycorrhizal Fungi To Understand The Evolution And Functioning Of Symbiosis"
11:00 - 13:00	**PLENARY SESSION 1** Symposia *Stein Auditorium, IHC* **Prof. Anders Tunlid**, Lund University, Sweden "Examining The Saprotrophic Ability Of Ectomycorrhizal Fungi Using Genomics, Transcriptomics And Spectroscopy" **Dr. Guillaume Bécard**, University of Toulouse III, France "Do strigolactone and Myc LCO signals participate in a true molecular dialogue?" **Dr. Natalia Requena**, Karlsruhe Institute of Technology, Germany "Lessons For Living Together In Harmony From The Arbuscular Mycorrhizal Symbiosis"

Prof. François Buscot, Helmoltz Centre for Environmental Research (UFZ), Germany
"Trophinoak: A Controlled System With Ectomycorrhizal Oak Microcuttings To Study Gene Expression Multitrophic Interactions"

Dr. Laurence Fraissinet-Tachet, Lyon 1 University, France
"A New Functional Metagenomics Approach Allows Discovering The Forest Soil Eukaryotic Heavy-Metal Resistome"

13:00 - 14:00 **Lunch**
The Hub, IHC

14:00 - 15:30 Poster Session
Charminar, IHC

15:30 - 16:00 **Tea**
Stein – Gulmohar bridge area, IHC

16:00 - 18:00 **WORKSHOPS** (3 concurrent)
Workshop 1
Developmental, functional & comparative genomics
Stein Auditorium, IHC

Speakers
Dr. Hinanit Koltai, ARO Volcani Center, Israel
"Strigolactones As Coordinators Of AM Symbiosis And Root Development: Comparative Genomics And Bioinformatics Study"

Prof. Luisa Lanfranco, University of Turin, Italy (Convenor and Speaker)
"Looking For Fungal Molecular Determinants Of Arbuscule Differentiation"

Prof. Philipp Franken, Leibniz-Institute of Vegetable and Ornamental Crops, Germany
"Tit For Tat: Control Of Nutrient Supply And Exchange In Arbuscular Mycorrhiza"

Prof. Yoram Kapulnik, Volcani Centre, Israel (Convenor)
Dr. Minna Kemppainen, National University of Quilmes, Argentina
"Positive-Acting GATA-Factor Controls Nitrate Growth In *Laccaria bicolor*"

Dr. Sylvie Cranenbrouck, Université Catholique de Louvain, Belgium
"The Mycorrhizal Donor Plant *In Vitro* Culture Sytem: A Key Tool To Study The Mycorrhizal Symbiosis Establishment And Plant Defense Mechanisms"

Mr. Tao Li, Chinese Academy of Sciences, China
"First Cloning And Characterization Of Two Functional Aquaporin Genes From An Arbuscular Mycorrhizal Fungus *Glomus intraradices*"

Mr. Xian an Xie, Huazhong Agriculture University, China
"Functional Analysis Of The Novel Mycorrhiza-Specific Phosphate Transporter Aspt1 And PHT1 Family From *Astragalus sinicus* In The Early Period Of The ArbuscularMycorrhizal Symbiosis"

Dr. Yolande Dalpé, Agriculture and Agri-Food, Canada
"Protein encoding genes as reliable tools for the molecular identification of *Glomus* species"

Workshop 2

Evolution, phylogeny, taxonomy and biogeography of mycorrhizal fungi and symbioses

Gulmohar, IHC

Speakers

Dr. Arthur Schüßler, University of Munich, Germany (Convenor and Speaker)
"Phylotaxonomy Of Glomeromycota (AM Fungi) - " A Consensus Amongst Leading Scientists"

Prof. Leho Tedersoo, University of Tartu, Estonia (Convenor and Speaker)
"Biogeography Of Ectomycorrhizal Fungi"

Ms. Erica Sterkenburg, Swedish University of Agricultural Sciences, Sweden
"Shift In Functioning Of The Ectomycorrhizal Fungal Community Along A Natural Nutrient Gradient"

Ms. Lily Ishaq, Murdoch University, Australia
"Mycorrhizal Fungi And *Eucalyptus gomphocephala* Decline In Southwestern Australia"

Dr. Marc Ducousso, CIRAD, France
"*Sporocarpia gigantea* gen. nov., spec. nov., the most spectacular arbuscular mycorrhizal fungus"

Dr. Masahide Yamato, Tottori University, Japan
"Effect Of Environmental Gradient On Community Of Arbuscular Mycorrhizal Fungi In Coastal Vegetation"

Dr. Nadia Nadejda Soudzilovskaia, Vrije Universiteit, The Netherlands
"Global Patterns Of Mycorrhizal Colonization Intensity Explained By Temperature And Soil Conditions"

Ms. Nathalie Diagne, Institut de recherche pour le développement, Senegal
"Ectomycorrhizal Diversity Enhances Growth And Nitrogen Fixation Of *Acacia mangium* Seedlings"

Workshop 3

Advances in ecosystem ecology
Jacaranda, IHC

Speakers

Dr. Björn Lindahl, Swedish University of Agricultural Sciences, Sweden (Convenor)

Prof. François Buscot, Helmoltz Centre for Environmental Research (UFZ), Germany (Convenor)

Prof. Erik Hobbie, University of New Hampshire, USA
"Fungal Functioning In A Pine Forest: Evidence From A 15N-Labeled Global Change Experiment"

Dr. Karina Clemmensen, Swedish University of Agricultural Sciences, Sweden
"Mycorrhizal Fungi Drive Long-Term Carbon Sequestration In Boreal Forest"

Dr. Tesfaye Wubet, UFZ-Helmholtz-Centre for Environmental Research, Germany
"Arbuscular Mycorrhizal Fungal Communities In Grassland Ecosystems Are Influenced By Land Use, Geographic Location And Vegetation Cover"

Dr. Ana Corrêa, CSIC, Spain
"Shedding Light Into Nutrient Responses Of AM Plants: Nutrient Interactions May Lead To Unpredicted Outcomes Of The Symbiosis"

Dr. Laura M. Suz, Royal Botanic Gardens, UK
"Mycorrhizas In Europe's Oaks: Belowground Indicators And Their Environmental Drivers"

Ms. Sannakajsa Velmala, The Finnish Forest Research Institute, Finland
"Ectomycorrhizal Fungi On Norway Spruce: Genotypic Effects On Fungal Colonization And Root Characteristics In Relation To Host Tree Vitality And Growth"

Dr. Yongjun Liu, Lanzhou University, China
"Influences Of 4 Yr Of Shading And Fertilization On Plants And Their Associated Arbuscular Mycorrhizal Fungi In An Alpine Meadow Ecosystem"

Prof. Yu Liang, CAS, China
"Impacts Of Plant Functional Group Removal On AMF Diversity In Plant Roots In A Grassland Ecosystem"

18:00 – 18:30 **Hall of Fame Awards Ceremony**
Stein Auditorium, IHC

- Recognition of Early Pioneers of Mycorrhizal Research
- Acknowledgement of recently passed mycorrhizal scientists

18:30 onwards Cultural Extravaganza-"**Confluence**" followed by Dinner
Stein Auditorium, IHC

TUESDAY, JANUARY 8, 2013

9:30 - 10:15 **PLENARY SESSION 2**
Stein Auditorium, IHC
Physiology including carbon and nutrient exchange between symbionts and the saprotrophic/biotrophic continuum

Keynote Speaker
Prof. Uwe Nehls, University of Bremen, Germany
"Physiology Of Carbon And Nutrient Exchange Between Symbionts And The Saprotrophic/Biotrophic Continuum"

10:15 - 12:45 **PLENARY SESSION 2**
Symposia
Stein Auditorium, IHC

Dr. Bingyun Wu, Tokyo University, Japan
"14C-Photosynthate And 33P-Nutrient Translocation Between Extraradical Mycelia Of Ectomycorrhizas Via Mycelial Anastomosis"

10:45 – 11:15	**Tea** *The Hub, IHC*
11:15 – 11:45	**Dr. Edith C. Hammer**, Freie Universität, Germany "Multilateral Belowground Deals - " Foraging And Nutrient Dynamics Amongst Multiple Mycorrhizal Partners" **Dr. Toby Kiers**, Vrije Universiteit, The Netherlands "Reciprocal Rewards Help Stabilize Cooperation In The Mycorrhizal Symbiosis" **Ms. Alice Vayssières**, INRA, France "Reprogramming Of Root Development By The Ectomycorrhizal Fungus, *Laccaria bicolor*: Involvement Of Auxin Pathways" **Dr. Maryline Calonne**, University of Littoral, France "Insight The Biochemical And Molecular Mechanisms Involving In PAHs Impact On The AMF Development And The Establishment Of A Functional Symbiosis"
12:45 - 13:30	**Lunch** *The Hub, IHC*
13:30 - 15:00	**Poster Session** *Charminar, IHC*
15:00 - 15:30	**Tea** *The Hub, IHC*
15:30 - 17:30	**WORKSHOPS** (4 concurrent) **Workshop 4** Metagenomics and population genomics *Stein Auditorium, IHC* **Speakers** **Dr. Maarja Öpik**, University of Tartu, Estonia (Convenor) **Dr. Tania Wyss**, University of Lausanne, Switzerland (Convenor and Speaker) "Investigating Intra-Specific Genetic Diversity In A Population Of *Rhizophagus irregularis* Using High Throughput Sequencing Techniques" **Dr. Christina Hazard**, The University of Aberdeen, United Kingdom of Great Britain "Role Of Environment And Geographical Distance On The Distribution Of Arbuscular Mycorrhizal Fungi At The Landscape-Scale"

Ms. Jennifer Forsythe, University of British Columbia, Canada
"A Field Study Of Specificity In The Arbuscular Mycorrhizal Symbiosis"

Dr. Jones Nirmalnath, University of Agricultural Sciences, India
"Molecular Diversity Of Arbuscular Mycorrhizal Fungi And Their Influence On Grapevine (*Vitis vinifera* Cv. *Cabernet sauvignon*)"

Mr. Lucas Villard, University of Lausanne, Switzerland
"Large Spatial-Scale Metagenomic Analysis Of Arbuscular Mycorrhizal Fungi Communities In Western Switzerland"

Dr. Manuela Krueger, Swiss Federal Institute of Technology, Switzerland
"Single Molecule Real-Time (SMRT) Sequencing Of Natural And Manipulated Arbuscular Mycorrhizal Fungal Assemblages In The Field"

Ms. Mengxuan Kong, University of Montreal, Canada
"Diversity Of Abscular Mycorrhiza Fungi In Extreme Petroleum Hydrocarbon Contaminated Site"

Ms. Rajalakshmi Muralidharan, Department of Biotechnology (GoI), India
"Molecular Characterization Of Seven AMF Hyphal Fusion Progenies And Their Eight Parents Using Amplified Fragment Length Polymorphism (AFLP)"

Workshop 5
Multitrophic interactions, including interactions with prokaryotes and soil fauna in the mycorrhizosphere as well as above-ground trophic interactions.
Gulmohar, IHC

Speakers

Dr. Aurélie Deveau, INRA, France (Convenor and Speaker)
"Do Bacterial Communities Associated With The Black Truffle Contribute To The Life Cycle Of The Ectomycorrhizal Fungus?"

Dr. Mika Tarkka, Helmholtz Centre for Environmental Research GmbH – UFZ, Germany (Convenor)

Ms. Florence Kurth, Helmholtz Centre for Environmental Research - UFZ, Germany
"Plant Responses To Mycorrhization Helper Bacterium *Streptomyces* Sp. Ach 505"

Ms. Sabine Recht, Helmholtz Centre for Environmental Research – UFZ, Germany
"Gene Expression Patterns And C- And N- Allocation During Combined Mycorrhizal Symbiosis And Pathogen Attack On *Quercus roburL*"

Dr. Bleddyn Hughes, University of Sheffield, UK
"Arbuscular Mycorrhizas As Inducers Of Crop Defence In Agriculture"

Dr. Katie Field, University of Sheffield, UK
"Atmospheric CO_2 And The Functional Symbiosis Between Basal Land Plants And Endogonales"

Ms. Shubhangi Sharma, Amity University, India
"Analysis Of The Bacteria Colonizing The Root Endophyte *Piriformospora indica*"

Prof. Thomas Kuyper, Wageningen University, The Netherlands
"Impact Of Arbuscular Mycorrhizal Fungi And Earthworms On Aggregate Stability"

Ms. Yadugiri V T, National Centre for Biological Sciences, India
"Host Plant Investment In Multiple Mutualists And The Effect Of Herbivory"

Workshop 6
Advances in agricultural applications of mycorrhizae
Jacaranda, IHC

Speakers
Prof. Akiyoshi Yamada, Shinshu University, Japan (Convenor and Speaker)
"Pure Culture Establishment And Mycorrhization Of Asian Chanterelles"

Dr. Chantal Hamel, Semiarid Prairie Agricultural Research Centre, Canada (Convenor and Speaker)
"Reduced Diversity Of Arbuscular Mycorrhizal Fungi In Annually Cropped Fields Of The Canadian Prairie"

Dr. Dipankar Maiti, Central Rainfed Upland Rice Research Station (ICAR), India (Convenor and Speaker)
"Exploitation Of Native Arbuscular-Mycorrhizal (AM) Resources Under Rainfed Upland Rice (*Oryza sativa* L.) Based Cropping Systems"

Dr. Jan Jansa, Academy of Sciences of the Czech Republic, Czech Republic
"Carbon Expenditure And The Economy Of Arbuscular Mycorrhizal Symbiosis"

Dr. Fernandez Felix, SYMBORG:SL, Spain
"Management Of Mycorrhizal Inoculant Mycoup Applied Through Irrigation Systems Localized Under Intensive Agriculture Conditions"

Dr. Ibrahim Ortas, University of Cukurova, Turkey
"Mycorrhizal Inoculated Horticultural Seedling Production And Transplantation Models To Field Conditions"

Dr. Lu-Min Vaario, Fiinnish Forest Research Institute, Finland
"Does *Tricholoma matsutake* Live Between Symbiotic And Saprophytic-A Vertical View Of Matsutake Shiro"

Dr. Mahaveer P Sharma, Directorate of Soybean Research (DSR-ICAR), India
"Cultivars As Determinant Of Mycorrhizal Dependency And Nutrient Uptake In Soybean Grown In Vertisols"

Workshop 7

Global scenario on regulations and practices facilitating mycorrhizae application across borders and islands
Video Conference Room 4th Floor, TERI, IHC

Convener
Dr. Alok Adholeya, The Energy and Resources Institute, India

19:00 onwards **Dinner**
The Hub

Wednesday, January 9, 2013

09:00 onwards **Paid Excursion trip** (Taj Mahal/ Field Trip/Delhi Darshan)

Thursday, January 10, 2013

9:30 - 10:15 **PLENARY SESSION 3**
Population, Community and Physiological Ecology of Mycorrhiza
Stein Auditorium, IHC

Keynote Speaker
Dr. Ian Dickie, Landcare Research, New Zealand
"Mycorrhizas In Changing Ecosystems"

10:15 – 10:20	IMS Announcements *Stein Auditorium, IHC*
10: 20 – 12:30	**PLENARY SESSION 3** Symposia *Stein Auditorium, IHC* **Speaker** **Dr. Maarja Öpik**, University of Tartu, Estonia "Communities Of Arbuscular Mycorrhizal Fungi - The DNA Story" **Dr. Thorunn Helgason**, University of York, UK "Spatial And Temporal Variation In The Composition And Structure Of Arbuscular Mycorrhizal Communities: Do AM Fungi Show Biogeographic Patterns?" **Prof. Jonathan Leake**, University of Sheffield, UK "The Evolution Of Mycorrhizas And Global Biogeochemical Cycles" **Dr. Martina Janouškova**, CAS, Czech Republic "Coexistence And Competition Of Arbuscular Mycorrhizal Fungal Genotypes: Lessons From Inoculation Experiments" **Prof. Nancy Johnson**, Northern Arizona University, USA "Glomeromycota Of The Serengeti: Abundance And Community Composition Are Influenced By Rainfall And Soil Properties"
12:05 – 13:00	Lunch *The Hub, IHC*
13:00 - 14:30	**Poster Session** *Charminar, IHC*
14:30 - 16:30	**WORKSHOPS** (3 concurrent) **Workshop 8** Physiological ecology *Stein Auditorium, IHC* **Speakers** **Dr. Björn Lindahl**, Swedish University of Agricultural Sciences, Sweden "Ecophysiological Properties Of Mycorrhizal Symbiosis Link C And N Availability With Organic Matter Turn-Over In Boreal Ecosystems"

Dr. Courty Pierre-Emmanuel, University of Basel, Switzerland (Convenor and Speaker)
"Expression of phosphate transporter genes in Sorghum and Linum: Developmental aspects and regulation in a common mycorrhizal network"

Prof. Erik Hobbie, University of New Hampshire, USA (Convenor)

Dr. Karin Pritsch, Helmholtzzentrum Muenchen, Germany
"Functional Plasticity Of Ectomycorrhizae - What Have We Learned From Studies Based On Potential Extracellular Enzyme Activities"

Dr. Rupam Kapoor, Delhi University, India (Convenor and Speaker)
"AM Symbiosis And Synthesis Of Terpenoids In Aerial Parts Of Plant"

Dr. Hairu Jin, Zhejiang Normal University, China
"Arginine Accumulation In The Different Parts Of Arbuscular Mycorrhizal Symbiosis After Supplied With Different Forms Of Exogenous Nitrogen And Carbon Sources"

Prof. Heike Bücking, South Dakota State University, USA
"The Role Of Carbon In Fungal Nutrient Uptake And Transport: Implications For Resource Exchange In Common Mycelial Networks"

Mr. Kevin Garcia, INRA, France
"Localization And Analysis Of K+ Transport Systems Of The Ectomycorrhizal Model Fungus *Hebeloma cylindrosporum*"

Dr. Mark Smits, Hasselt University, Belgium
"Ectomycorrhizal Fungi And SOM Degradation"

Workshop 9
Population and community ecology
Gulmohar, IHC

Speakers

Ms Carrie Van Dorp, University of British Columbia, Canada
"Inter And Intraspecies Spatial Interactions Of *Rhizopogon vesiculosis* and *R. vinicolor*; Sister Species Share Space, But Siblings Have Boundaries"

Dr. Kazuhide Nara, University of Tokyo, Japan (Convenor and Speaker)
"Ectomycorrhizal Fungal Communities In Japanese Douglas-Fir Forests"

Dr. Sara Hortal, Hawkesbury Institute for the Environment, UWS, Australia (Convenor and Speaker)
"Beech Roots Are Simultaneously Colonized By Multiple Genets Of The Ectomycorrhizal Fungus *Laccaria amethystina* Clustered In Two Genetic Groups"

Dr. Tatsu Ezawa, Hokkaido University, Japan
"Responses Of Arbuscular Mycorrhizal Fungal Communities To Soil Acidity: Acid-Tolerant Fungi Are The Generalists That Occur In A Wide Range Of Soil Ph"

Dr. Irena Maček, University of Ljubljana, Slovenia
"Impact Of Long-Term Soil Hypoxia On Arbuscular Mycorrhizal Fungal Communities In Mofette Areas (Natural CO_2 Springs)"

Prof. Marcel van der Heijden, Agroscope Reckenholz Tanikon, Switzerland
"Facilitation And Suppression By Mycorrhizal Networks"

Mr. Mohammad Bahram, Tartu University, Estonia
"Spatial Structure Of Ectomyorrhizal Fungal Communities Across Different Scales"

Dr. Laura B. Martinez-Garcia, Landcare Research, New Zealand
"120,000 Years Of Change: The Response Of Arbuscular Mycorrhizal Fungal Communities To Post-Glacial Ecosystem Development And Retrogression"

Workshop 10
Systematics and ecology of mycorrhizal communities in Austral – Asia
Jacaranda, IHC

Speakers
Prof. A. Manjunath, University of Agricultural Sciences, India (Convenor and Speaker)
"Soil solution phosphorus level as a basis for determining VAM dependency or VAM responsiveness of plant species"

Prof. Manoharachary Chakravarthula, Osmania University, India (Convenor and Speaker)
"Biodiversity, Taxonomy, Conservation And Ecology Of Arbuscular Mycorrhizal Fungi - Indian Scenarlo"

Prof. Airong Li, Chinese Academy of Agriculture, China
"Direct And Indirect Influences Of Arbuscular Mycorrhizal Fungi On Phosphorus Uptake By Two Root Hemiparasitic *Pedicularis* Species: Do The Fungal Partners Matter?"

Prof. Bishwanath Chakraborty, University of North Bengal , India
"Activation Of Defense In Tea And Mandarin Plants Against Fungal Pathogens Triggered By AMF, PGPR And PGPF"

Prof. Hui Lin, Huazhong Agricultural University, China
"Functional analysis of a phosphate transporter gene GigmPT in Arbuscular Mycorrhizal fungus Gigaspora margarita"

Dr. S Krishna Sundari, Jaypee Institute of Information Technology, India
"Anti-Oxidative Compounds From Edible Ectomycorrhizal Fungi And Their Ability To Protect Cells From Oxidative Damage"

Dr. Yaron Sitrit, The Jacob Blaustein Institute for Desert Research, Israel
"Pre-Symbiotic Interactions Between *Terfezia boudieri* And *Helianthemum sessiliflorum* Reveal A Novel Role For Auxin: Redirection Of Roots Growth By Intervention In Gravitropism"

Prof. Yi Huang, Peking University, China
"Secretion Of Thermostable Proteins Is Induced In Ectomycorrhizal Fungi And Inoculation Pine By Excessive Copper And Cadmium"

16:30 – 18:00 Transfer from IHC to TERI Gram, Gual Pahri, Haryana

18:00 onwards '**Wines of the World**' along with Dinner
TERI Gram

FRIDAY, JANUARY 11, 2013

9:00 - 9:45 **PLENARY SESSION 4**
Mycorrhizae in agriculture and horticulture, including inoculation of seedlings, inoculum production and policy development
Stein Auditorium, IHC

Keynote Speaker
Dr. J André Fortin, Université Laval, Canada
"Arbuscular Inocula: Production, Formulation, Application And Results"

9:45 – 12:15	**PLENARY SESSION 4** Symposia *Stein Auditorium, IHC* **Speakers** **Dr. Alok Adholeya**, The Energy and Resources Institute, India "Application of AMF's in Global farming interventions: Towards a full circle" **Dr. Kelly Craven**, The Samuel Roberts Noble Foundation, USA "Promoting Low-Input Agriculture Through Symbiosis"
10:45- 11:15	**Tea** *The Hub*
	Prof. Mohamed Hijri, Universite de Montreal, Canada "GlomusIrregulare Controls Mycotoxin Production In A Virulent Fungal Pathogen On Potato" **Prof. Anabela Marisa Azul**, University of Coimbra, Portugal "Project Valuemicotruf: Prospecting, Productivity And Add Value To Native Ectomycorrhizal Fungi And Truffles Supported By Criteria Of Quality And Indicators Of Sustainable Production" **Dr. Joyce Mnyazi Jefwa**, CIAT-Kenya "Commercial Mycorrhizal Inoculants As Agricultural Inputs: Quality, Efficacy And Adoption"
12:15- 13:00	**Lunch** *The Hub, IHC*
13:00 -14:30	**Poster Session** *Charminar, IHC*
14:30 -15:00	**Tea** *The Hub, IHC*
15:00 -17:00	**WORKSHOPS** (3 concurrent) **Workshop 11** Mycoheterotrophy and mixotrophy *Stein Auditorium, IHC* **Speakers** **Prof. D. Lee Taylor**, University of Alaska Fairbanks, USA (Convenor and Speaker) "Is it A Ghost Or A Thief? Evidence For Nitrogen Theft In The Epiphytic Orchid *Dendrophylax lindenii*"

Dr. Marc-André Selosse, Centre for Evolutionary &Functional Ecology, France (Convenor and Speaker)
"On The Evolutionary Meta-Stability Of Mixotrophy In Orchids"

Dr. Duncan Cameron, University of Sheffield, UK
"How Do Plants Parasitise Fungi? Resolving The Biochemical Basis For Mycoheterotrophy"

Prof. Keitaro Tawaraya, Yamagata University, Japan
"Mutualism And Parasitism Between *Allium fistulosum* And Arbuscular Mycorrhizal Fungi At Different Soil Phosphate Availability"

Prof. Maria Catarina Megumi Kasuya, Universidade Federal de Viçosa, Brazil
"Endophytic And Mycorrhizal Communities Associated With Roots Of Endangered Native Orchids From The Atlantic Forest"

Mr. Rafael Schneider, Zurich University of Applied Sciences, Switzerland
"Mycorrhiza On Green Roofs – Spontaneus Establisment Of Native Orchids On Roofs In Switzerland"

Prof. Yukari Kuga, Hiroshima University, Japan
"Cellular Acquisition And Allocation Of Carbon And Nitrogen In Orchid Symbiotic Protocorm Unveiled By Isotope Microscopy"

Dr. Yuki Ogura-Tsujita, Tohoku University, Japan
"Shifts In Mycorrhizal Fungi During The Evolution Of Autotrophy To Mycoheterotrophy In *Cymbidium* (Orchidaceae)"

Workshop 12
Mycorrhizae in extreme or polluted environments/ restoration
Gulmohar, IHC

Speakers

Prof. John Klironomos, University of British Columbia, Canada (Convening and Speaking)
"Plant Growth Response To Mycorrhizal Fungi Is Stronger Under Extreme Abiotic Conditions"

Dr. Maman Turjaman, FORDA, Indonesia (Convenor and Speaker)
"Bio-Reforestation: Mycorrhizal Fungi Promoted Plant Growth In Degraded Tropical Peat Swamp Forests And Post Mining Lands"

Prof. Anissa Lounès Hadj Sahraoui, ULCO, France
"Arbuscular Mycorrhizae For Green And Sustainable Remediation Of Polluted Soils: A Feedback From Laboratory To Field Scale Experiments"

Prof. Graziella Berta, Univerità del Piemonte Orientale, Italy
"Effects Of Arsenic And Arbuscular Mycorrhizal Fungi On The Fern PterisVittata: From Proteome Analyses And Molecular Characterization To Field Experiments"

Dr. Katarzyna Turnau, Jagiellonian University, Poland
"Mycorrhizal Fungi As Modulators Of Secondary Metabolite And Antioxidant Production Under Extreme Conditions"

Prof. Mark Tibbett, Cranfield University, UK
"Rapid Colonisation Of Soils And Mining Wastes By Roots And Mycorrhizas In Landforms Restored After Mining"

Prof. Neera Garg, Panjab University, India
"Superiority Of *Glomus intraradices* In Enhancing Proline Biosynthesis And Imparting Tolerance To Pigeonpea Plants Under Salt Stress"

Ms. Shivani Srivastava, The Energy and Resources Institute, India
"Mycorrhized root cultures as continuous biological and biochemical factories"

Workshop 13
Human impact on mycorrhizae
Jacaranda, IHC

Speakers
Ms. Claudia Krueger, LMU Munich, Germany
"Arbuscular Mycorrhizal Fungi For Reforestation In South Ecuador"

Ms. Marie Merrild, University of Copenhagen, Denmark
"Root Associated Fungi In A Changing Climate: Impact Of Elevated CO_2, Drought, And Warming"

Prof. D.J. Bagyaraj, CNBRCD, India (Convenor and Speaker)
"Can Agricultural Practices Affect AM Fungal Population And Diversity?"

Prof. Melanie Jones, University of British Columbia, Canada (Convenor)

Dr. Jennifer K.M. Walker, Lewis and Clark College, USA
"Does Resource Partitioning Influence Community Assembly By Ectomycorrhizal Fungi In Clearcuts And Forests?"

Dr. Reena Singh, The Energy and Resources Institute, India (Convenor and Speaker)
"Mycorrhiza-Assisted Reclamation Of Abandoned Ash Pond And Its Carbon Sequestration Potential"

Dr. Andrew Taylor, The James Hutton Institute, UK
"Impacts Of Above-Ground Grazing On Ectomycorrhizal Communities Associated With Birch Trees Are Minimised By Mycelial Networks Linking Grazed And Ungrazed Trees"

Ms. Luise Olbrecht, Agroscope Reckenholz-Tänikon, Switzerland
"Agricultural Management Practices Affect Ecosystem Services And Plant Productivity By Changing Arbuscular Mycorrhizal Fungal Communities"

Prof. Mohamed Al-Yahya'ei, University of Basel, Switzerland
"Native arbuscular mycorrhizal fungi as prospective biotechnological tool for sustainable agriculture and conservation in the Arabian Peninsula"

17:00- 18:00 **Valedictory Ceremony**
Stein Auditorium, IHC

ORAL PRESENTATIONS

KEYNOTE LECTURES

Exploring the genome diversity of mycorrhizal fungi to understand the evolution and functioning of symbiosis

Francis Martin*

*The Mycorrhizal Genomics Initiative Consortium, Lab of Excellence ARBRE, Department of Tree-Microbe Interactions, INRA, France, E-mail: fmartin@nancy.inra.fr

Genomics has introduced an important new dimension into mycorrhizal research by establishing data to serve as a new and fundamental resource for genetics and molecular biology of the symbiosis formation. With the current genomic view of ectomycorrhizal (EM) fungi that we have, a possible scenario suggests that (1) irreversible losses of lignocellulose decomposition pathways play a key role in the evolutionary stability of the ectomycorrhizal mutualisms and (2) that each major EM fungal clade has subsequently and independently designed symbiotic molecular toolboxes each time the mycorrhizal lifestyle has arisen in the tree of life. This hypothesis would predict that symbiotic toolboxes are tailor made for each major fungal clade (e.g., Agaricales, Sebacinales) and may be tuned according to specific plant hosts. To further our understanding of the evolution of these symbioses, the Mycorrhizal Genomics Initiative targets a set of 30 fungal mycorrhizal species. Taxa have been selected for (1) their phylogenetic novelty, (2) their ability to establish different types of mycorrhizal symbioses, and (3) their taxonomic relationships with already sequenced EM genomes. Several target species are capable of forming different types of intracellular colonizing structures, and this plasticity depends on plant host. Several species are dominant fungus in their ecological settings and others are currently used in the commercial forestry industry to inoculate conifer or hardwood seedlings for lumber, bioenergy and landscape trees. I will discuss how the comparative analysis of mycorrhizal genomes has, and will continue, to shed light on the evolution of mycorrhizal symbioses.

Physiology of carbon and nutrient exchange between symbionts and the saprotrophic/biotrophic continuum

Uwe Nehls*

*University of Bremen, Dept. of Ecology/Botany, Germany, E-mail: nehls@uni-bremen.de

Essential for mycorrhizal symbiosis is the exchange of plant-derived carbohydrates for fungus-based nutrients. Fungal carbohydrate uptake experiments with mycelia and studies combining gene expression and protein function data indicated hexoses to be a main fungal carbohydrate source. Depending on the environmental conditions, soil- and symbiotic interface-located importers contribute to fungal carbon nutrition in ectomycorrhizal (ECM) symbiosis. Until recently, sucrose has been discussed as carbon source that is most likely exported by the host plant. However, novel data are questioning this view. In ECM and VAM model plants, members of a gene family encoding glucose facilitators are induced upon mycorrhiza formation. Together with a clear preference of fungal hexose importers for glucose, glucose must be supposed as a major carbohydrate delivered by the host plant. Sugar transport is, however, only one level of fungal carbon nutrition in symbiosis. The mechanisms of carbohydrate allocation by plant cells at the symbiotic interface and the fast distribution of sugars within fungal hyphae are remaining open questions. Essential for host plants is an optimal nitrogen support. The delivered nitrogen source by fungal hyphae and the mechanism by which nitrogen is transferred are in the spotlight of actual research. Ammonium is discussed as an integral part of fungus-based plant nitrogen nutrition in symbiosis as in poplar ammonium importers and in two *Laccaria bicolor* aquaporins, conferring plasma membrane ammonia permeability in yeast, are induced upon symbiosis. In this contribution, the current view of molecular mechanisms by which carbon and nitrogen exchange is established will be discussed.

Mycorrhizas in changing ecosystems

Ian Dickie*

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Whether ectomycorrhizal (EM) ecosystems are functionally different from arbuscular mycorrhizal (AM) ecosystems remains an open question. Transitions in dominant mycorrhizal type as ecosystems change provide an unique opportunity to answer it. EM ecosystems tend to have more soil organic matter and low plant diversity compared to AM ecosystems, but mechanisms are unclear. I explore four major hypotheses. (1) Lower foliar nutrients in EM plants may drive soil organic matter. We tested for foliar differences using a global plant traits database and phylogenetic independent contrasts. We found no consistent effect of mycorrhizal status on plant foliar nutrients after accounting for phylogeny. (2) Positive plant-soil feedback has been proposed as a mechanism decreasing plant diversity and appears more common in EM than AM systems. However, a spatially explicit simulation incorporating parameters derived from field studies suggests positive feedback and is unlikely to be the main driver of low diversity of EM systems. (3) Direct access to minerals in rocks has been reported in EM systems and could drive differences in mineral nutrient cycles. However, we find only limited evidence of differences between AM and EM forest ecosystems in rock dissolution, with tunnels in mineral rock grains under both. (4) Organic nutrient utilization by EM fungi. Using an ecosystem carbon and nutrient model, we find the decoupling of carbon and nutrient stoichiometry in EM decomposition may be an important ecosystem driver. These findings have direct applications in understanding ecosystem transitions in dominant mycorrhizal status, including belowground legacies of EM weed invasion into AM ecosystems.

Arbuscular inocula: production, formulation, application, and results

André J. Fortin*

*Laval University, Forest and Wood Science Dept., Canada, E-mail: j.andre.fortin@videotron.ca

Mycorrhizal symbiosis is a fundamental and universal phenomenon in the evolution and functioning of terrestrial plants and ecosystem. All agricultural principles and technologies have been conceived and are applied as if mycorrhizae were not existing. This suggests we should revise all agricultural principles and practices. Secular practices and even more so modern ones have profoundly altered agricultural soil biology. Among other causes, frequent soil works for weed control and seed bed preparation including soil compaction, excessive use of fertilizers, and others have altered mycorrhizal flora in term of biodiversity and quantities. To rebuilt mycorrhizal flora, different approaches are possible; one of them being soil inoculation with AMF. Different production methods have been developed, with two main avenues: on whole plants in clean soils and on aseptically grown root-organ cultures. In Canada, up to now, only aseptically produced inoculums can be sold. This was a big challenge to meet that forced industry to develop a most sophisticated product. At this date two companies met this challenge with success: the Energy and Resources Institute (TERI), India, and PremierTech, Canada. Until recently, few species of AMF could be produced *in vitro*. New species have been successfully cultivated and especially several isolates of *Glomus irregulare*. Once inocula have been produced, formulations adapted to crops, and different requirements must be met. We will illustrate some aspects of aseptic inoculum production, formulation, and application, as well as results in terms of productivtiy and product quality.

SYMPOSIA

Examining the saprotrophic ability of ectomycorrhizal fungi using genomics, transcriptomics, and spectroscopy

Anders Tunlid[1]*, Francois Rineau[2]

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A large part of the nitrogen in forest soils is found in recalcitrant organic matter-protein complexes. Ectomycorrhizal fungi are thought to have a key role in the decomposition and mobilization of nitrogen from such complexes. The knowledge on the functional mechanisms of these processes, and how they are regulated by carbon from the host plant and the availability of more easily available forms of nitrogen sources are limited. We examined how the ECM fungus *Paxillus involutus* degrade organic litter material using spectroscopy and transcriptome profiling. The fungus partially degraded polysaccharides and modified the structure of polyphenols. The observed chemical changes and the expressed transcriptome were consistent with a hydroxyl radical attack, involving Fenton chemistry similar to that of brown-rot fungi. The set of enzymes expressed by *P. involutus* during the degradation of the organic matter was similar to the set of enzymes involved in the oxidative degradation of wood by brown-rot fungi. However, *P. involutus* lacked transcripts encoding extracellular enzymes needed for metabolizing the released carbon. Further experiments have shown that the decomposition and assimilation of nitrogen from organic litter material are triggered by adding glucose. Addition of easily available forms of nitrogen (i.e., ammonium) had minute effects on these processes. Our experiments demonstrate that the saprotrophic activity of *P. involutus* has been reduced to a radical-based biodegradation system that can efficiently disrupt the organic matter-protein complexes and thereby mobilize the entrapped nutrients. We suggest that the host photosynthate can firmly regulate these activities.

Lessons for living together in harmony from the arbuscular mycorrhizal symbiosis

Natalia Requena*

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Microorganisms are permanently challenged with hazardous environmental conditions that restrict their potential for survival and reproduction. To overcome this threat many of them evolutionarily opted for a life in symbiosis. Fungi from the Glomeromycota phylum engaged in a life in mutualistic symbiosis with plant roots more than 450 million years ago. Since then, plants provide fungi with carbohydrates in return for an improved inorganic fertilization. The arbuscular mycorrhizal (AM) symbiosis is a fine-tuned regulated process where the fungal colonization is limited to the root cortex, contrasting to fungal parasitic interactions that usually invade the vascular cylinder. This is remarkable

considering that AM fungi are obligate biotrophs and need to feed on photoassimilates during their *in planta* growth for the completion of their life cycle. To do that AM fungi have to first sort out the defence barriers of the host during colonization and then use carbon resources allocated to the root without provoking a parasitic situation. The molecular mechanisms of how plant and fungi recognize each other to achieve an almost perfect relationship will be the focus of this talk.

Trophinoak: a controlled system with ectomycorrhizal oak microcuttings to study gene expression multitrophic interactions

François Buscot[1]*, Lasse Feldhahn[1&4], Sabine Recht[1], Florence Kurth[1], Ivo Grosse[4], Tesfaye Wubet[1], Oguzhan Angay[2,3], Frank Fleischmann[2], Thorsten Grams[3], Mika Tarkka[1], Sylvie Herrmann[1]

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Plant functioning depends on beneficial and detrimental biotrophic interactions affecting internal processes, such as the endogenous rhythmic development with alternating shoot and root growth flushes of oak trees (*Quercus robur* L.). Such flushes impact on the formation of ectomycorrhiza (EM). In an interdisciplinary project (www.TrophinOak.de), micro-propagated oaks mycorrhized with the EM fungus *Piloderma croceum* and in addition interacting with biotrophic partners such as mycorrhization helper bacteria, pathogen microorganisms, and herbivores on leaf and roots are currently compared at the two levels gene expression regulation and resource allocation, in a common standardized experimental set-up. The methods include 13C and 15N labelling experiments and transcriptomics using high throughput sequencing technology. The final intent is to bridge observed gene expression patterns for metabolic pathways dealing with C and N allocation and shifts in C and N allocations. Here, we report on the molecular analyses in this frame since oak genome has not yet been sequenced. We first constructed a *de novo* hybrid assembly from 18 cDNA libraries to obtain a reference contig library specific to our micro-propagated oak clone DF159: OakContigDF159. Mycorrhiza-related gene expression was characterized by analysing Illumina Libraries obtained from mycorrhizal and non-mycorrhizal short roots. The gene expression data will be presented with a particular focus on pathways dealing with C and N allocation. Ongoing Illumina sequencings are comparing gene expression profiles at different developmental stages in additional biotrophic interactions in the frame of TrophinOak.

A new functional metagenomics approach allows discovering the forest soil eukaryotic heavy-metal resistome

Laurence Fraissinet-Tachet[1]*, Frédéric Lehembre[1], Didier Doillon[2], Sandrine Perrotto[1], Elise David[1], Laurent Vallon[1], Jan Colpaert[3], Michel Chalot[2], Damien Blaudez[2], Roland Marmeisse[1]

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Heavy metal-polluted soils are colonized by microorganisms which have developed different resistance mechanisms to these pollutants. Knowledge of eukaryotic resistance mechanisms often results from studies on a limited number of model species which are not representative of the soil biological diversity. It is, therefore, necessary to gain insights into resistance mechanisms developed by all the soil microorganisms even still unknown or uncultivable. The objective of this study was to develop a new experimental strategy to identify the diversity of eukaryotic heavy-metal resistance genes really expressed in soils by eukaryotic microorganisms. For this purpose, we have implemented the functional metatranscriptomics approach. This approach is based on the direct extraction of eukaryotic polyadenylated messenger RNAs from Pine forest soil samples (heavy metal-contaminated or not) followed by their conversion into cDNAs which are cloned in a yeast expression vector. These environmental cDNA libraries were used to complement three distinct yeast mutants sensitive to either Zn or Cd and resistant genes were recovered, sequenced and analysed. Several genes conferring Cd or Zn resistance in yeast were recovered and grouped into nine gene families. Four gene categories could be distinguished: (i) novel genes with no homologs in databases; (ii) novel gene families whose encoded protein structure suggests a biological function (e.g., metal chelation); (iii) genes belonging to known families but unknown to be implicated in heavy metal resistance; and finally, (iv) genes already known and implicated in heavy-metal resistance mechanisms. Several of these genes could originate from soil fungi including ectomycorrhizal ones.

Do strigolactone and Myc-LCO signals participate in a true molecular dialogue?

Virginie Puech-Pages[1], Jean-Philippe Combier[2], Mathilde Malbreil[1], Luz Gilbert[1], Nianwu Tang[3], Coline Balzergue[1], Dominique Lauressergues[1], Soizic Rochange[1], Christophe Roux[1], Guillaume Bécard[1]*

[1]Toulouse University, [2]CNRS, [3]Huazhong Agricultural University

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Strigolactones (SLs) are important root excreted molecules for the establishment of AM symbiosis (Gomez-Roldan et al. 2008). One possible reason is that they rapidly activate mitochondrial metabolism and presymbiotic growth of the fungal partner prior to root contact (Akiyama et al., 2005; Besserer et al., 2006, 2008). Recent data also suggest that SLs play a role in later stages of the symbiosis (Lauressergues et al., 2012; Yoshida et al., 2012). Symmetrically, AM fungi produce lipochitooligosaccharides (Myc-LCOs) that can stimulate root branching of *Medicago truncatula* by the symbiotic DMI signalling pathway. They can also stimulate mycorrhization in plant species of diverse families including non-leguminous plants (Maillet et al., 2011). Experiments based on biochemical and molecular approaches are being performed to examine whether SLs and Myc-LCOs influence the synthesis of each other and how the two signals could participate in the autoregulation of the AM symbiosis.

Reciprocal rewards help stabilize cooperation in the mycorrhizal symbiosis

Toby Kiers[1]*, Heike Bücking[2]

[1]Institute of Ecological Science, Vrije Universiteit; [2]Department of Biology and Microbioloy, South Dakota State University

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The maintenance of cooperation in the mycorrhizal partnership has long posed a problem for evolutionary theory. Cooperation between different species is hard to explain because selfish individuals can exploit mutualisms, reaping benefits while paying no costs. So, why cooperate at all? The problem is particularly puzzling in the mycorrhizal mutualism because both plant and fungi interact with multiple partners simultaneously: a single plant host is colonized by multiple fungal species and fungal 'individuals' interact with multiple plant hosts. This complex series of many-to-many interactions means that neither partner can be 'enslaved'. We manipulated the extent to which plants and fungal symbionts supply nutrients to their partners to show that plants can detect, discriminate, and reward the best fungal partners with more carbohydrates. In turn, their fungal partners enforce cooperation by increasing nutrient transfer only to those roots providing more carbohydrates. We concluded that cooperation between partners can be stabilized in a form analogous to a market economy, where there are competitive partners on both sides of the interaction and higher quality services are remunerated in both directions. Importantly, our work does not preclude the possibility that partners employ other mechanisms to control the growth/success of each other.

Multilateral belowground deals: foraging and nutrient dynamics amongst multiple mycorrhizal partners

Edith C. Hammer*

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Mycorrhizal fungi forage for mineral nutrients in a very heterogeneous soil environment. We found hyphal proliferation in and around different kinds of organic matter patches and even charcoal. Isotope labeling proves their ability to access mineral nutrients associated with the coal. An increased share of the plant carbon is allocated via the fungus to these nutrient patches. Recent findings indicate that the carbon-phosphorus (C-P) exchange between plant and fungus is functionally coupled and controlled. Does the tit-for-tat principle also hold when different species simultaneously are involved?

We assume that in nature a common mycorrhizal network (CMN) connects multiple plant individuals of one or more species by multiple fungal mycelia of one or more species. In a large and complex group of players, a bi-directional control is even more important to prevent CMN exploitation by members that don't contribute to the investments of the symbiosis. We found both in own pot experiments and in a meta analysis on existing literature that also in complex assemblies of plants and AM fungi, P seems to be directed to the largest C source. This has important implications for both con- and interspecific plant competition. Further, the importance of other nutrients than P, and other factors connected to the presence of a CMN will be discussed.

14C-photosynthate and 33P-nutrient translocation between extraradical mycelia of ectomycorrhizas via mycelial anastomosis

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Extraradical mycelia from different ectomycorrhizal (ECM) roots coexist and interact under the forest floor. According to their mycelial compatibility, mycorrhizal networks may connect with each other by mycelial anastomosis which is a ubiquitous phenomenon in filamentous fungi. Such anastomosis could serve important functions in ECM symbiosis. The objective of our research here was to investigate how the mycorrhizal network connection by mycelial anastomosis functionally extends the nutrient movement. Paired ECM *Pinus thunbergii* seedlings colonized by the same or different *Pisolithus* isolates were grown side by side in a rhizobox as their mycelia contacted each other. 14CO_2 or 33P-phosphoric acid was fed to leaves or a spot on the mycelium in one of the paired seedlings. Time-course distributions of 14C and 33P were visualized using a digital autoradiographic technique with imaging plates. Hyphal connections were observed between mycelia of the same *Pisolithus* isolate near the contact site, but hyphae did not connect between different isolates. 14C and 33P were translocated between mycelia of the same isolate. No 14C and 33P translocation occurred between mycelia of different isolates. These results provide direct evidence that contact and hyphal connection between mycelia of the same ECM isolate can result in an expanded extraradical mycelium network and enhanced nutrient translocation. Such contact can greatly enlarge the area and amount of nutrient exchange in the mycelial network, and also have an impact on the functions and nutrient cycling in the ECM symbiosis system.

Reprogramming of root development by the ectomycorrhizal fungus, *Laccaria bicolor*: involvement of auxin pathways

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During the formation of ectomycorrhiza, numerous short and swollen lateral roots form all over the root system. The presence of this type of roots during symbiosis illustrates the impact of the fungus on lateral root formation and on root growth. Auxin, which is one of the major regulators of root development, has been suggested to be at the cross point of fungus/plant signalling and the modification of root development during ectomycorrhizal establishment. In our transcriptome study, we detected in poplar roots an accumulation of transcripts related to auxin efflux carriers and to auxin homeostasis in response to *L. bicolor*. Auxin response in roots during contact with the fungus was assessed using the synthetic DR5 auxin inducible promoter fused to the GUS reporter gene. Auxin response in root apices is altered in the presence of *L. bicolor*, suggesting a modification of auxin homeostasis in this tissue. An auxin metabolome analysis performed on root apices showed an accumulation of IPA (indole-3-pyruvic acid), auxin conjugates, and 2-oxoindole-3-acetic acid, suggesting an impact of the fungus on auxin biosynthesis and auxin conjugation/degradation pathways. The implication of auxin transport is currently under investigation. We are focussing our studies on the analysis of one member of the PIN auxin efflux carrier family, PtaPIN9. Interestingly, interaction between transgenic poplars overexpressing or poplar silencing PtaPIN9 lines with *L. bicolor* did not lead to stimulate the formation of lateral root. Our results highlight an involvement of auxin transport and auxin biosynthesis in the development of the ectomycorrhiza.

Insights into the biochemical and molecular mechanisms involving PAHs impact on the AMF development and the establishment of a functional symbiosis

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Polycyclic Aromatic Hydrocarbons (PAHs), which are persistent organic contaminants in soils originating mainly from anthropogenic activities, are listed by the US-Environmental Protection Agency as priority pollutants and are of concern worldwide because of their carcinogenic and/ or mutagenic properties. Owing to the great persistence of these very stable molecules (half-life estimated to several years), the decrease of their contents in soil is limited. Among the emergent innovative remediation methods of PAHs polluted soils, the assisted phytoremediation by Arbuscular Mycorrhizal Fungi (AMF), living in symbiosis with most of the plants, could represent ecological and economic alternative. Unfortunately, little is known about the potential effect of PAHs on the mycorrhizal symbiosis. It is why our work aims to study the PAH impact on the two symbiotic partner development and the establishment of a functional symbiosis. Our findings pointed out a PAH ecotoxicity on the symbiosis caused by an alteration in the membrane lipid metabolism and an induction of an oxidative stress. Furthermore, our results suggested the involvement of the storage lipid metabolism to cope with this stress. Subsequently, PAH inhibition of the Phosphorus (P) transport from the AMF to the plant was highlighted using three complementary approaches: the 33P radiolabelling, the alkaline phosphatase activity assessment as well as the study of gene expression encoding a P transporter (GiPT), and an alkaline phosphatase enzyme (GiALP), demonstrating the alteration in the symbiosis functioning. Taken together, our data provide insight into the mechanisms of PAH impact on the AMF development and the establishment of functional symbiosis.

Communities of arbuscular mycorrhizal fungi: the DNA story

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There are increasing number of case studies published about Glomeromycota molecular diversity from various ecosystems worldwide. Commonly, some known, some unidentified ("environmental"), and some new species (molecular operational taxonomic units, MOTUs) are found. In order to compare such data and generalize the observed patterns, the sequence identity designation principles need to be unified. Data from case studies are collected and systematized in a public database MaarjAM (http://www.maarjam.botany.ut.ee), which applies unique MOTU nomenclature: 'virtual taxa' (VT). These are phylogenetically defined as groups of related sequences and roughly correspond to species level taxa. VT allow synonymisation of original MOTU designations by providing a short and easily usable name. Stability of VT nomenclature is created via type sequences, around which each VT evolves as new records become available. VT nomenclature, much alike binomial taxonomic nomenclature, allows comparability among data and consistent communication among scientists. VT delimitation principles can be further elaborated when more information about intra- vs interspecific nucleotide variation of Glomeromycota becomes available. Comparability of VT nomenclature with traditional taxonomy of Glomeromycota will improve with more morphospecies becoming sequenced and issues in the currently dynamic Glomeromycota taxonomy getting solved. As the registered number of VT already exceeds the number of Glomeromycota morphospecies, the DNA-only species would remain necessary in the near future. Application of VT is becoming more widespread and sequence data in the MaarjAM database are increasingly used as reference for environmental sequence identification. The current status, pros and cons, and future prospects of AMF DNA-based identification and community description are presented.

Spatial and temporal variation in the composition and structure of arbuscular mycorrhizal communities: do AM fungi show biogeographic patterns?

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The Arbuscular mycorrhizal (AM) fungi are a significant component of the belowground community. These fungi are globally ubiquitous, but distributions among host plants and sites suggest

complex patterns of interaction between host plant and local environment. A significant gap in our understanding of AM community structure is the extent to which AM species' distributions are regulated by the identity of and interactions with the host plant; by demographic processes (i.e., growth, local extinction, dispersal); or by their local environment (i.e., soil and climate properties such as temperature, pH, precipitation). The sampling resolution necessary to detect these relationships has only recently become possible because of DNA sequencing, including the most recent 'next generation' technologies. In this talk, I will present data based on systematic sampling in natural and agricultural environments analysed using sequencing that reveals the distribution of individual AM taxa and communities of AM fungi. These can be correlated to the physical environment in which they are found, including both soil and climate variables, independent of any interaction with the host plant. In this talk I will discuss how our understanding of the interaction between host plant and fungus in planta, is likely to be moderated by the environment in which the fungus lives' which may account for half of the fungal biomass.

The evolution of mycorrhizas and global biogeochemical cycles

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The co-evolution of plants and symbiotic soil fungi over 470 million years (Ma) ago set in chain a sequence of events that greened the earth's continents with terrestrial plants of increasing biomass and complexity, transforming the chemistry of the soil, oceans, and atmosphere. However, the contribution of the evolution of mycorrhizal fungi to global biogeochemical cycles, to date, has not been resolved. The rise of land plants enhanced silicate mineral weathering and the export of Ca, Mg, and P into the oceans, where the precipitation of carbonate sediments caused a 90% fall in atmospheric CO_2 from 410 to 340 Ma, coincident with the rise of forested ecosystems. We present evidence from experiments across a 1500–200 ppm gradient of atmospheric CO_2 on mycorrhiza functioning across key nodes of plant evolution from liverworts, through ferns to tree saplings, with either AM or EM. We reveal how falling atmospheric CO_2 provided a strong negative feedback mineral weathering rates, but favoured vascular plants over liverworts by increasing the functional efficiency of their mycorrhizas. Field experiments under tree taxa with crown diversification ages ranging from 25–160 Ma, reveal mycorrhiza-driven mineral weathering increased with advancement from AM to later, independently evolved, EM fungi, and from gymnosperm to angiosperm hosts with both fungal groups. Our process-based modelling corroborates the field and laboratory evidence that over geological time evolution has selected for vascular plant–mycorrhiza associations that have intensified weathering and increasingly driven global biogeochemical cycles.

Glomeromycota of the Serengeti: abundance and community composition are influenced by rainfall and soil properties

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There is growing interest in managing mycorrhizas in grasslands to increase belowground carbon sequestration. A better understanding of the factors controlling the dynamics of arbuscular mycorrhizal fungi (AMF) across environmental gradients is necessary before this goal can be achieved. Greenhouse studies indicate that mycorrhizal biomass is often inversely related to soil phosphorus availability. Taxa of AMF are known to vary in their habitat requirements; yet much remains unknown about the their distributions in natural ecosystems. The aim of our research was to study patterns in the abundance and community composition of AMF across precipitation and soil fertility gradients in the extensive natural grasslands within Serengeti National Park in Tanzania. We studied 48 long-term plots at 8 locations to assess responses of AMF to herbivory, rainfall, and soil properties. Densities of extramatrical hyphae and spores of AMF were highest at the driest sites, and (unexpectedly) at sites with the highest soil phosphorus content. Soil phosphorus levels are low in the Serengeti; consequently, a positive correlation between EMH and phosphorus could arise because it is a limiting resource for AMF. Alternatively, the extremely high densities of AMF in the soil could be a major reservoir of organic phosphorus in the soil. Herbivory reduced the density of Glomeraceae spores, but had no effect on spores from other families of AMF. Factorial experiments to uncouple the effects of rainfall and soil phosphorus on communities of AMF in the Serengeti are currently underway. These studies provide a better understanding of the ecology of Glomeromycota.

Coexistence and competition of arbuscular mycorrhizal fungal genotypes: lessons from inoculation experiments

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Introduction of propagules of arbuscular mycorrhizal fungi (AMF) into soils with native AMF background is becoming a common practice both in experimental and production conditions. However, there is an overall lack of knowledge on how inoculation affects intraradical AMF communities. More generally, little is known about the interactions of different AMF genotypes colonising plant roots. We have approached this topic in a series of cultivation experiments with a ruderal soil–AMF system and *Medicago sativa* as a model crop. Using real-time PCR with discriminating assays, we quantitatively followed the intraradical development of inoculated and native AMF genotypes to describe the response of the background AMF community to the propagule input. The results of the experiments indicate that both competition among AMF genotypes and regulation of intraradical colonization by the host plant contribute to the formation of specific AMF communities and determine their quantitative composition. The taxonomic level of discrimination among AMF seems crucial for the assessment of a community response to propagule input. Overall, the presented results contribute to the understanding of the assembly rules of AMF communities and their dynamics and indicate need of further research, which would link AMF community data with the functional aspects of AM symbiosis such as carbon and phosphorus fluxes.

Application of AMFs in global farming interventions: towards a full circle

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Since the knowledge about AMFs, its plant growth response activity is highlighted through large number of publications worldwide. The challenge has been that how to translate beneficial properties of AMF's in to plant production system as consistent response and effects. An account of how isolates were found those possessing desired traits and functions will be presented. These evaluation efforts subsequently helped to select isolates or consortia to attempt towards mass production technology development. Results from long-term and short term trials on various global locations do amply provided diverse learning's and indicated attenuation needs in delivery systems and formulations. Mass production technology developed have also undergone many desired changes. Many new genera and species were successfully introduced in *in vitro* culture first and then in commercially viable production processes. The newer knowledge and success towards seed coating would pave the way towards large-scale efficient delivery of AMF's in Global farming practices.

Commercial mycorrhizal inoculants as agricultural inputs: quality, efficacy, and adoption in Africa

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There are currently over 100 commercial mycorrhizal products widely marketed across continents. Mycorrhizal fungi are ubiquitous, hence assumed to work everywhere and on every crop. A study was conducted by Centro Internacional de Agricultura Tropical (CIAT) in collaboration with the International Institute of Tropical Agriculture (IITA) Nigeria, the Ethiopian Institute of Agriculture (EIAR), Chepkoilel Moi University (Kenya), and Rwanda Agricultural Board (RAB) to evaluate quality and efficacy of commercial and indigenous mycorrhizal products across soil types, regions, and crops. The mycorrhizal quality of products was evaluated prior to inoculation. The quality of most products was less than expected. Efficacy of products was dependent on crop, cultivar, soil type, and fertility level. Tissue

culture banana was more affected in Vertisols with a significant ($p<0.05$) increase of over 30% under nursery conditions and followed by subsequent increase in growth and yield under field conditions. The application of organic and inorganic fertilizer complimented the effect of commercial products. Indigenous inoculants significantly ($p<0.05$) increased growth and yield by over 100% in soils with P (mg kg^{-1}) levels of 50–80 and not 15.5–21.9. The yield of soybean was significantly increased by 39.9% when mycorrhizal product was combined with a rhizobial product legumefix, while maize was not affected by inoculation. The yield of groundnuts was significantly ($p<0.05$) increased by mycorrhizal inoculation. Effect of mycorrhizal products are variable across soil types and crops, knowledge on taxonomy, quality, and formulations of products is still limited and knowledge on interaction with biotic and abiotic rhizosphere environment is crucial appropriate recommendations.

Project ValueMicoTruf: prospecting, productivity, and add value to native ectomycorrhizal fungi and truffles supported by criteria of quality and indicators of sustainable production

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The native ectomycorrhizal fungi (EcMF), including truffles, represent an investment option growing worldwide, potentially benefiting the forest through mycorrhization whilst providing an attractive economical return. These are desirable traits given the threats for Mediterranean forests as result of poor management, fire, water scarcity, soil degradation, spread of invasive species, and diseases. In Portugal, the value associated with EcMF have been underestimated and the knowledge of native EcMF regarding life cycle, biology, ecology, and biotechnological applications is also insufficient. We present ValueMicoTruf, a project that aims to develop tools for sustainable production of native EcMF and truffles in the Centre Region of Portugal and provide successful solutions for a territory with a high density of forest (0.8 million ha). The project ValueMicoTruf seeks innovative technologies (mycorrhization and inoculum), processes (nutritional and organoleptic properties, and biomolecules), services (forest productivity and protection against biotic and abiotic stresses), and the criteria of quality and the indicators for sustainable management and production of native EcMF products and truffles. This project is part of a strategy to provide fundamental and applied research for understanding the biological and ecological functional diversity of fungi and add value to native EcMF connected to agro-forestry, restoration, food industries, innovative business initiatives, and national and international research institutions.

Glomus irregulare controls mycotoxin production in a virulent fungal pathogen on potato

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Trichothecenes are an important family of mycotoxins (fungal toxins) produced by many strains of the genus *Fusarium*. These mycotoxins cause a serious disease in infected plants and result in health problems for humans and animals that consume infected fruits or vegetables. Unfortunately, there are few methods for controlling mycotoxin production by fungal pathogens relying on chemicals. Here, we used two approaches to monitor the impact of the symbiotic arbuscular mycorrhizal fungus (AMF) *Glomus irregulare* on a trichothecene-producing *Fusarium sambucinum* strain T5 isolated from naturally infected potato plants. Using dual *in vitro* cultures, we showed that *G. irregulare* inhibited the growth of *F. sambucinum* and, more importantly, significantly reduced the production of trichothecene 4, 15-Diacetoxyscirpenol (4, 5-DAS). We tested the hypothesis that AMF may also control mycotoxin production in potato plants to protect against infection with *F. sambucinum*. We analysed the response of AMF-colonized potato plants infected by *F. sambucinum* by monitoring production of 4, 5-DAS. In response to *F. sambucinum* infection, we found that the AMF treatment efficiently inhibited the production of mycotoxin in roots and significantly reduced its production in tubers. In addition to the benefical effect of mycorrhizal symbiosis for plant growth, we documented that AMF efficiently control the growth of a virulent fungal pathogen and reduced mycotoxin production in tubers. This previously undescribed, non-conventional biological control of mycotoxin production by pathogen, has potential implications on potato crop production in agriculture and in the future of world food supply.

Promoting low-input agriculture through symbiosis

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Limiting agronomic inputs [i.e., macronutrients (in the form of fertilizers); water; management] is likely to be a primary concern for agriculture worldwide in the very near future. Further, the misuse and/or over-application of fertilizers have become major environmental issues. Transitioning from the high-input, high-output doctrine characterizing the Green Revolution, to an acceptable return, but with minimal investment, should be a focus for all agronomic sciences. We are studying the potential of a relatively recently described group of fungi, the Sebacinales, to facilitate low-input agriculture through mycorrhization. Our model system thus far has been the important bioenergy crop switchgrass (*Panicum virgatum*) and the orchid mycorrhizae, *Sebacina vermifera*. As biofuels derived from cellulosic biomass are still not cost competitive with petroleum, all agronomic inputs must be minimized to make this approach economically viable. Thus switchgrass production is a model for low-input agriculture. We have been able to enhance switchgrass biomass by 300% through colonization with *S. vermifera* and mycorrhized plants produce twice as much yield under severe drought conditions than non-colonized plants with normal watering. Preliminary studies have shown that the same strain of *S. vermifera* that we use to enhance switchgrass can also colonize numerous other plant species, including tobacco, tomato, alfalfa, and *Arabidopsis thaliana*. Our current work involves identifying native *S. vermifera* strains and expanding our studies to field trials in hopes that the beneficial effects we see in the greenhouse will translate to agronomic gains in the field.

WORKSHOPS

Looking for fungal molecular determinants of arbuscule differentiation

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Arbuscules are highly branched fungal structures with a life span of a few days which are continuously formed and dismantled inside root cortical cells. They provide an expanded contact surface between the symbionts and act as preferential sites of nutrients exchange. To get insights on the molecular determinants controlling arbuscule differentiation processes we investigated the involvement of NADPH oxidases (NOXs), the most-studied reactive oxygen species-producing enzymes, which were recently shown to play an important role in the control of fungal morphogenesis in many plant–fungus interactions. The AM fungus *Glomus intraradices* (Syn. *Rhizophagus irregularis*) possesses the main components, NoxA and NoxB catalytic subunits and NoxR and Rac regulatory proteins, of the NADPH oxidase complex. Gene expression profiles on whole roots and on laser microdissected samples suggested for Nox genes an enhanced expression in arbuscules. Complementation assay in heterologous systems (*Epichloe festucae* and *Botrytis cinerea*) are under way. The characterization of a fungal gene, with an arbuscule-specific expression pattern and no homologue in fungal databases, will also be presented.

Strigolactones as coordinators of am symbiosis and root development: comparative genomics and bioinformatics study

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Strigolactones (SLs), the newly defined plant hormones, are produced and secreted by plant roots and play multiple roles in the rhizosphere and in regulation of plant development. They were first identified as germination stimulants of the parasitic plants *Striga* and *Orobanche*, and later as stimulants of hyphal branching of arbuscular mycorrhizal fungi (AMF), suggesting a role for SLs as signals in these parasitic and symbiotic interactions. Later, a role for SLs was discovered in regulation of plant shoot architecture and in root development: SLs were shown to regulate lateral root formation and root hair elongation. Since roots are the site of arbuscular mycorrhiza (AM) symbiosis, and since a negative

feedback loop was found between colonization by AMF and SLs production in roots, a complex regulatory, SL-related network might be acting to coordinate root development and AM symbiosis. Here we present a genomics and bioinformatics study that focuses on possible components of coordination between root development and AM symbiosis, which unravels some of the putative components that act with SLs to regulate root development also in relation to AM symbiosis. Also, the intriguing question of the evolution of the biological role(s) of SLs will be discussed. On the one hand, SLs appear to have a pivotal role in determining plant architecture. On the other, the involvement of SLs in AM symbiosis may suggest an ancient and important role for SLs as signals in plant–fungus interactions.

Tit for tat: control of nutrient supply and exchange in arbuscular mycorrhiza

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Central feature of a mycorrhiza is the exchange of nutrients. In a functional symbiosis, plant and fungus integrate and allow integration to achieve this goal. Molecular-physiological analyses reveal how both partners realize integrative mycorrhizal functioning. Transporters at membranes of extraradical hyphae are the sites of mineral nutrient uptake in the soil. Gene expression analyses revealed that fungal transporters for phosphate, nitrate, and ammonium are differentially regulated depending on their contribution to plant nutrition. Mineral nutrients are transported through fungal hyphae from the soil to the root and released from the arbuscules into the apoplastic space between the two partners. The uptake across the periarbuscular membrane of the plant needs a proton gradient supplied by proton-pumping ATPases such as MtHA1 in *Medicago truncatula*. Functional analysis showed the importance of the gene mtha1 for mycorrhizal mineral nutrition of the plant and in turn for the formation of fungal structures inside the root. Mycorrhizal fungi colonizing the root represent an additional carbohydrate sink. Detailed time course measurements of photosynthetic parameters and respiration help to understand the carbon balance in the mycorrhizal system. Carbon exchange between source and sink organs in this system is considerably affected by the activity of sucrose transporters. Three corresponding genes were investigated in tomato and, analysis of LeSUT2 suggests that mycorrhizal function of the protein is not only based on transport activities, but rather on its molecular interaction with elements of phytohormone biosynthesis and signalling.

The mycorrhizal donor plant *in vitro* culture sytem: a key tool to study the mycorrhizal symbiosis establishment and plant defense mechanisms

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Root colonization by AM fungi is a dynamic process that causes major change in plants in term of nutrition and resistance to stresses. The mycorrhizal donor plant (MDP) *in vitro* culture system was recently developed by Voets et al. (2009) for the fast and homogeneous colonization of plant root at the seedling stage. This system appeared particularly relevant to study the early events of mycorrhiza establishment. Using this system we were able to investigate, *in vitro*, the gene expression changes during the 3 phases of the root colonization: the pre-colonization stage (before the contact with the root), the early stage (appressorium and first intraradical hyphae formation), and the late stage (intense root colonization). Using real-time QRT-PCR, we confirmed the efficiency of the system by tracking the expression of a selection of defense gene during these 3 phases. The results obtained opened the door for a wider investigation of genes associated to biotic stress, using a microarray approach. The expression profile obtained demonstrated a wide transcriptional change during the 3 phases and in particular suggested that the WRKY transcriptor factor genes could be involved in the mechanisms controlling arbuscular mycorrhizal establishment by the regulation of plant genes.

First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*

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Arbuscular mycorrhizal (AM) symbiosis is known to stimulate plant drought tolerance. However, the molecular basis for the direct involvement of AM fungi (AMF) in plant water relations has not been established. Two full-length aquaporin genes, namely GintAQPF1 and GintAQPF2, were cloned by

5′- and 3′-RACE from an AM fungus, *Glomus intraradices*. Heterologous expression of the two genes in *Pichia pastoris* indicated that GintAQPF1 was localized to plasma membrane; while GintAQPF2 was localized to both plasma and intracellular membranes. The aquaporins performed a strong water conductance as indicated by significant decrease of yeast cell volume upon hyper-osmotic shock and faster protoplast bursting upon hypo-osmotic shock. Growth of GintAQPF1- and GintAQPF2-transformed yeast cells was promoted by PEG, but inhibited by glycerol. Furthermore, under drought stresses expression of the two genes in arbuscule-containing cortical cells and extraradical mycelia of maize roots was significantly enhanced, and accordingly the hyphal length density and root relative water content were also increased by mycorrhizal colonization. As known to us, this is the first report on functional aquaporin genes from AM fungi. Our data strongly supported the potential water transport via AMF to host plants, and led to a better understanding of the key role of AMF in plant drought tolerance.

Functional analysis of the novel mycorrhiza-specific phosphate transporter AsPT1 and PHT1 family from *Astragalus sinicus* in the early period of the arbuscular mycorrhizal symbiosis

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Arbuscular mycorrhizas contribute significantly to inorganic phosphate (Pi) uptake in plants. Gene networks involved in the regulation and function of the Pht1 family transporters in legume species during AM symbiosis are not fully understood. Here we present six distinct phosphate transporters of the Pht1 family from mycorrhizal *Astragalus sinicus*. Mycorrhiza-specific induction for AsPT1 and AsPT4, AM enhancement for AsPT3, whereas AsPT2, AsPT5, and AsPT6 were down-regulated in AM roots. Pi starvation triggered the up-regulation of members of the Pht1 family. Cellular localization showed that AsPT1 and AsPT4 were localized in arbuscule-containing cells of the inner cortex. AsPT2 was detected in the tip and epidermis of roots. AsPT5 was expressed in the vascular tissue. The analysis of promoter sequences revealed conserved motifs in both AsPT1 and AsPT4. Overexpression of AsPT1 resulted in a significant change in arbuscular development in the transgenic plants was observed. In contrast, knockdown of AsPT1 by RNA interference lead to degenerating or dead arbuscule phenotypes, and a block in Pi transfer to the plant. These results suggest roles for Pht1 proteins in the acquisition and transport of inorganic phosphate during AM symbiosis, and AsPT1 and AsPT4 are required for AM development.

A positive-acting GATA-factor controls nitrate growth in *Laccaria bicolor*

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While the regulatory circuits of nitrogen utilization in saprotrophic ascomycetes are already well established, the control of nitrogen growth in basidiomycetes, and especially in ECM fungi, is poorly understood. The regulation of nitrogen usage in ECM species is of special interest as it is directly linked to the symbiotic success of such fungi and to the nitrogen cycling in forest ecosystems in general. *Laccaria bicolor* genome harbors five putative GATA-binding domain proteins. The lack of homologues of negative acting ascomycete-type GATA-factors suggests that the nitrogen utilization would be controlled dominantly via positive acting GATA-factors in this fungus. We have studied the participation of putative positive acting AreA/Nit2-type GATA-factor (LbGATA) in nitrogen utilization control in *Laccaria*. Expression of LbGATA is not under evident nitrogen source regulation. However, RNA silencing of this transcription factor results in strongly reduced fungal capacity to grow on nitrate. This growth phenotype correlates with minimal transcript levels of all the three nitrate utilization pathway genes (LbNrt, LbNr, and LbNir). While affecting nitrate growth, RNA silencing of LbGATA does not significantly alter the fungal growth on ammonium. Our results demonstrate the fundamental role of the GATA-factor specifically in nitrate utilization pathway activation in *Laccaria*. LbGATA is the first characterized positive-acting nitrogen growth regulator of ECM basidiomycetes.

Protein encoding genes as reliable tools for the molecular identification of *Glomus* species

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Molecular tools used to identify Glomeromycetes species primarily focus on ribosomal nuclear genes (SSU, LSU, ITS). Unfortunately, unlike other fungi, highly variable copies of these noncoding gene sequences (up to 16%) considerably reduce the precision of the molecular analyses especially for closely related species. Up to now, few studies on protein encoding genes have been performed mainly due to the presence of mobile and variable introns. New primers were designed to partially amplify elongation factor α± (765 bp), F0F1-ATP synthase subunit β (621 bp), H+-ATPase (1468

bp), glucosamine synthase Gln1 (909 bp) and PHO84 (*Saccharomyces cerevisiae analog*), a transmembrane phosphorus transporter PT (852 bp). All Glomeraceae strains sequenced were propagated at the Glomeromycetes in vivo and in vitro collection (GINCO-CAN). Intraspecific variation was not observed. At a threshold of 98%, all genes allowed segregation of morphologically different species. The sequences of H+ATPase, PT, and Gln1 genes were retained as the most efficient for species identification as a direct and proportional relationship was observed between molecular data and spore species morphologies. For species sharing very similar morphologies which made them difficult to separate, the molecular identification was performed with H+ATPase and PT gene sequences. With these protein encoding genes, closely related species are separated at 97% and 98% levels. With their single genome copy and the absence of mobile introns, the proposed protein encoding genes allow quick and accurate species identification.

Biogeography of ectomycorrhizal fungi

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Recent spread of molecular tools has greatly improved our understanding of microbial biogeography. Despite minute spore size and efficient wind-dispersal, distribution patterns of ectomycorrhizal (EcM) fungi are indicative of substantial dispersal limitation. EcM symbiosis involves a large number of plant and fungal taxa worldwide. EcM symbiosis has arisen independently and persisted at least 66 times in fungi. Herbarium records from 11 major biogeographic regions revealed three main patterns in distribution of EcM lineages: (i) Austral; (ii) Panglobal; (iii) Holarctic. The holarctic regions host the largest number of EcM lineages; none are restricted to a tropical distribution with Dipterocarpaceae and Caesalpiniaceae hosts. Unlike in macroorganisms, species richness of EcM fungi exhibits a unimodal relationship with latitude and temperature at the global scale, peaking in temperate and boreal ecosystems. Such a discrepant pattern could be ascribed to lower soil differentiation, relatively recent evolutionary emergence of host trees, greater autocorrelation range, and lower speciation rates in tropical regions. However, phylogenetic analyses and field observations suggest that many globally distributed EcM fungal lineages (inocybe, /clavulina, /russula-lactarius) have arisen in tropical ecosystems. Phylogenetic structure of EcM fungal communities is strongly determined by host family. To minimize host effect, global EcM fungal communities of *Alnus* were addressed, but these revealed no macroecological patterns, except a positive effect of soil calcium concentration on fungal richness. Within *Alnus*, phylogenetic relations and the confounding spatial effects strongly affect the global EcM fungal community. Disentangling the potential causes of these deviating macroecological patterns are the focus of further research.

Phylotaxonomy of glomeromycota (AM fungi): a consensus amongst leading scientists

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The publication of new scientific and formal names and taxonomic classifications should be undertaken with great care because its impact extends well beyond the circles of taxonomists and phylogeneticists. A widely usable taxonomy is best if founded on a scientifically sound phylogenetic classification which, by definition, must reflect evolutionary changes. For arbuscular mycorrhizal (AM)

fungi (Glomeromycota), only a classification based on universal evolutionary patterns and processes will provide a stable and operational framework for understanding these organisms. However, the recent publication of a large number of taxon-names within the AM fungi has resulted in conflicting systematic schemes and confusion amongst biologists working with these important plant-symbionts. Detailed evaluation of those nomenclatural changes showed several of them to be based on selective analysis (bias) or erroneous interpretation of partly poor quality data. This calls for resolution of such conflicts and for a provision of a synthesis to develop a consensus classification that is meaningful to a broad scientific community. After examination of the available morphological and molecular evidence within the framework of phylogenetic hypotheses, the classification of Glomeromycota should be revised by rejecting some new names on the grounds that they were founded in error, and by synonymizing others that, whilst validly published, were not evidence-based. A proposed 'consensus taxonomy'" should provide a robust, evidence based framework for additional original research aimed at clarifying the evolutionary history and systematics of this important group of symbiotic fungi.

Sporocarpia gigantea gen. nov., spec. nov., the most spectacular arbuscular mycorrhizal fungus

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Most species belonging to the Glomeromycota are known to form soil born spores solely or in light clusters. For some species such as *Glomus*, the ability to form dense cluster reaching up to few millimetres, have been mentioned. Only one species *Acaulospora sporocarpia* (Berch 1985) have been described from herbarium materiel with spore aggregates up to some centimetres. In the absence of any molecular data for this species, the position in the genus *Acaulospora* is doubtful (Schüessler & Walker, 2010). We give a complete description including DNA sequences of fruiting bodies and spores of an amazing arbuscular mycorrhizal species found in irrigated crop (millet, sorghum) in South Libya. This species formed sporocarps that crack the soil surface to merge above ground; sporocarps can measure up to 30 cm and weight up to 2 kg. The anatomical structure of these primitive fruiting bodies is described. Taking into account the great originality of this species within the Diversisporaceae family, we propose to create a new genus: Sporocarpia with the species 'gigantea': *Sporocarpia gigantea* Ducousso, Salah & De Lajudie.

Global patterns of mycorrhizal colonization intensity explained by temperature and soil conditions

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While possession of mycorrhiza by vascular plants is recognized as a paramount factor for ecosystem carbon and nutrient turnover, the intensity of root colonization by mycorrhizal fungi is an important, yet much less understood, trait that affects the magnitude of carbon and nutrient exchange between plants and fungi, and therefore within an ecosystem. We analysed the role of climate (temperature, precipitation, seasonality) and soil conditions (nitrogen mineralization rate, soil pH, soil carbon and nitrogen content, soil C/N, and phosphorus availability) as possible proxies for global patterns of intensity of vascular plant root colonization by arbuscular and ecto-mycorrhizal fungi. The analysis is based on a dataset compiled from published studies of colonization intensities of vascular plants by mycorrhiza available up to 2012 and our own data (in total 4887 vascular plant species in 230 sites, situated all over the globe). We demonstrate that site-level arbuscular mycorrhizal colonization intensity shows a unimodal response to mean annual temperature, with a maximum achieved at 2°C ($P<0.001$) and decline with increase of soil C/N level ($P<0.001$), $R^2=0.77$. Site-level ecto-mycorrhizal colonization intensity decreases with soil pH increase ($P<0.001$), although there is an interaction of soil pH and soil C/N ($P=0.002$), $R^2=0.42$. These findings suggest that at global scale, patterns of vascular plant colonization intensity by arbuscular and ecto-mycorrhiza are strongly controlled by soil fertility and climate. Sites with highest levels of plant root colonization by mycorrhizal fungi have conditions known as unfavorable for vascular plant growth: cold climate, infertile soils with high C/N and low pH.

Mycorrhizal fungi and *Eucalyptus gomphocephala* decline in southwestern Australia

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Eucalyptus gomphocephala, a woodland tree native to the Swan Coastal Plain of Western Australia, is in severe decline. It was hypothesized that mycorrhizal type and soil chemistry might be associated with *E. gomphocephala* decline. A bioassay pot experiment with *E. gomphocephala* as the trap plant was set up using intact soil cores collected from 12 sites with *E. gomphocephala* canopy condition ranging from

healthy to declining. Adjacent soil samples were collected for chemical analysis. The type (arbuscular or ectomycorrhizal) of mycorrhiza formed in the seedlings was related to the canopy condition of *E. gomphocephala* at the sites where the cores were taken. Ectomycorrhizal fungi colonization was higher in seedling roots in soil collected from healthy sites whereas arbuscular mycorrhizal fungi were dominant in roots in soil from declining sites. Furthermore, a number of soil chemical properties were related to both canopy condition and the type of mycorrhizal fungi that colonised roots. Tree health improved with increasing pH, exchangeable Ca and Na, Mn, Cu, and Zn. ECM fungi colonization increased with higher pH and Fe, whilst AM fungi were abundant with higher P, S and exchangeable K. It is suggested that factors involved in the reduced ECM colonization could include local distribution of host plants, the presence of damaging soil borne plant pathogens, adverse soil conditions, and the extent of labile carbon pools below ground.

Shift in functioning of the ectomycorrhizal fungal community along a natural nutrient gradient

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Plant communities in boreal forests vary along gradients in productivity, mineralogy, and hydrology according to predictable patterns. In contrast, separation of fungal species and functional groups into ecological niches is yet not well understood. Here we investigate whether also fungal communities reflect patterns of ecosystem fertility, aiming for a better understanding of the functional ecology of fungi and their interplay with the plant community. We analysed soil fungal communities in 26 pristine boreal forests in central Sweden by high throughput 454-sequencing of ITS2 amplicons. The forests were selected to represent a gradient of nitrogen availability as well as different vegetation types with respect to trees (*Picea abies, Pinus sylvestris*) and understory vegetation (herbs, *Vaccinium myrtillus, V. vitis-idaea, Calluna vulgaris*). We found that the fungal community composition varied strongly along the gradient and that composition of understory vegetation was an efficient predictor, probably due its integration of nutrient availability over time. We expected that plants would decrease their allocation of sugars to ectomycorrhizal fungi in response to high nutrient availability. In contrast, we found higher amounts of ectomycorrhizal DNA in nutrient rich forests. However, the fraction of cord-forming species decreased with increasing nutrient availability. Due to their dynamic growth and rapid mycelial turnover, dominance of cord-forming species leads to low-standing biomass. We conclude that the production in combination with the life-span of mycelia seems to be a decisive factor in determining standing fungal biomass (DNA). The results are discussed in relation to nutrient foraging strategies and mycelial morphology.

Effect of environmental gradient on community of arbuscular mycorrhizal fungi in coastal vegetation

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Plant species found in coastal vegetations are specifically adapted to extreme environmental conditions. Usually, a transition of the coastal plant species is found corresponding to the environmental gradient with distance from the sea, and the plants closest to the seaside usually face the most stressful conditions. In this study, the community structure of arbuscular mycorrhizal (AM) fungi associated with *Ixeris repens* (Asteraceae) was studied in coastal vegetation near the Tottori sand dunes in Japan. *I. repens* produces roots from a subterranean stem growing near the soil surface which provides an opportunity to examine the effects of an environmental gradient related to distance from the sea on AM fungal communities at a regular soil depth. We established a sampling plot of 20 X 50 m in the vegetation with the 20 m long side was on the seaward forefront of the vegetation, and root samples were collected at 37 points randomly chosen. Based on partial sequences of the nuclear large subunit ribosomal RNA gene, AM fungi in root samples were divided into 17 phylotypes. Among these, five AM fungal phylotypes in Glomus and Diversispora were dominant near the seaward forefront of the vegetation. Redundancy analysis of the AM fungal community showed significant relationships between the distribution of phylotypes and environmental variables such as distance from the sea, water-soluble sodium in soil, and some coexisting plant species. These results suggest that environmental gradients in the coastal vegetation can be determinants of the AM fungal community.

Ectomycorrhizal diversity enhances growth and nitrogen fixation of *Acacia mangium* seedlings

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The depletion of soil fertility became a great threat for tropical areas. To improve land fertility, chemical fertilizers are often used but these inputs are not ecofriendly for ecosystems. Alternately, strategies like biofertilizers and N_2 fixing trees can be used. Among these biofertilizers, ectomycorrhizal fungi

(EMF) which form a symbiotic relationship with species of the Legume family such as *Acacia mangium* an Australian tree widely introduces in arid areas. Inoculation with ECM improves *A. mangium* growth. However, the influence of ECM diversity on growth and on rhizobial symbiosis development of *A. mangium* remains unknown. The aim of this study was to investigate the influence of ECM diversity on *A. mangium* growth, on nodulation by a strain of *Bradyrhizobium* sp. on plant nutrient uptake by creating a gradient of ECM diversity (from one to six fungal). Acacia seedlings were individually grown in 0.25 L pots filled with a sterile sandy soil mixed with the required volume of fungal inoculum. After 4 months, plants were harvested and results showed that ectomycorrhizal inoculation with increased ectomycorrhizal rates significantly increased shoot, root, total biomasses, ectomycorrhizal colonization, total number of nodules per plant as well as phosphorus and nitrogen foliar contents. These results illustrate that ECM diversity influences positively plant growth and biological nitrogen-fixing symbiosis. They highlight the necessity to manage ectomycorrhizal symbiosis and/or to associate tree seedlings with multiple mycorrhizal symbionts in order to conserve an efficient abundance and diversity of ectomycorrhizal propagules in soils and optimize forest plantation performance for improving soil fertility.

Fungal functioning in a pine forest: evidence from a 15N-labelled global change experiment

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We used natural and tracer nitrogen isotopes in a *Pinus taeda* Free Air CO_2 Enrichment (FACE) experiment to investigate the functioning of saprotrophic and ectomycorrhizal fungi in nitrogen cycling for six years. Fungi were sampled in 2004 and 2010 and 15N patterns compared against other ecosystem pools. Ectomycorrhizal fungi with hydrophobic ectomycorrhizae (e.g., *Cortinarius*) acquired nitrogen from deeper in the soil profile than taxa with hydrophilic ectomycorrhizae (e.g., *Russula* and *Lactarius*) that acquired nitrogen from the Oi horizon. 15N enrichment patterns for *Cortinarius* and *Amanita* did not correspond to any measured bulk pool, suggesting that a persistent pool of active organic nitrogen supplied these two taxa. Saprotrophic fungi could be separated into those colonizing pine cones, wood, litter, and soil, with the nitrogen isotope signatures of the taxa colonizing these substrates reflecting these source preferences. 15N enrichments for six taxa ranged from 3–9% and these enrichments correlated analytically with both increased allocation by fungi to hyphal development and decreased nitrogen transfer to host plants. Natural abundance and 15N tracer labels in this FACE experiment proved useful for tracking nitrogen into different fungal taxa from nitrogen sources and provided new insights into interpreting natural abundance 15N patterns.

Mycorrhizal fungi drive long-term carbon sequestration in boreal forest

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Boreal forest soils play a major role in the global carbon cycle through functioning as both a large terrestrial source and sink. The prevailing dogma has been that soil organic matter primarily accumulates in the form of plant remnants. However, a large proportion of photosynthetically fixed carbon is directed belowground to roots and associated mycorrhizal fungi, potentially affecting carbon sequestration either positively or negatively. Here we present evidence from a fire-driven chronosequence of boreal forested islands in which forest age varies between 50 and 5000 years and where the humus layer exceeds 1 m in depth in the oldest forests. Based on a 14C bomb-carbon model

and stable isotope analyses, we show that 50–70% of the stored carbon is derived from mycorrhizal mycelium rather than from plant litter, and that the input of mycelial-derived organic matter increases with time since fire. We also reveal through biochemical markers and 454-pyrosequencing of molecular barcodes that carbon sequestration is regulated by functional and phylogenetic shifts in the mycorrhizal fungal community that are tightly linked to their growth-form and N mobilization strategy. Our results provide evidence for an alternative mechanistic basis for how carbon accumulates in boreal forest soils during succession in the long term absence of disturbance.

Arbuscular mycorrhizal fungal communities in grassland ecosystems are influenced by land use, geographic location, and vegetation cover

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Arbuscular mycorrhizal (AM) fungi are important soil organisms with multiple ecosystem functions. Their diversity and community structure is mainly influenced by factors affecting both soil and plants. In this study we assessed the influence of land use type and plant cover on the AM fungal community composition of three spatially separated grassland ecosystems. The study was carried out in three geographically separated regions known as the 'Schwäbische Alb' in the low mountain ranges of south-western Germany, the 'Hainich-Dün' in central Germany, and the 'Schorfheide-Chorin' in the lowlands of north-eastern Germany within the frame of the German Biodiversity Exploratories project (www.biodiversity-exploratories.de). Soil samples from a total of 27 study plots representing three land use types (3 plots of meadows, pastures and mown pastures each per study region) were collected in spring 2008. AM fungal communities were analyzed employing pyrotag sequencing of the NS31-AM1 fragment of the ribosomal 18S rDNA. We found a huge AM fungal diversity and the community structure was significantly influenced by soil pH, soil organic matter, available phosphorus, and plant cover. Nested permanova analysis revealed that the AM fungal diversity and community structure was significantly influenced by geographic location and land use type. Our results also indicated a significant concordance between AM fungal and plant community structure in these grassland ecosystems. The implication of these results will be discussed in line with the ecological network theory and the contribution of AM fungi in the functioning of the respective grassland ecosystems.

Shedding light into nutrient responses of AM plants: nutrient interactions may lead to unpredicted outcomes of the symbiosis

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Arbuscular mycorrhizal (AM) symbiosis is usually considered benefic to plants. However many plants, depending on growth conditions, present negative responses to AM. In this study we aimed at assessing if nitrogen (N) is determinant in mycorrhizal growth response (MGR) by answering the following questions: are AM beneficial for plant N nutrition, does it depend on N availability? Is MGR only determined by the mycorrhizal effect on N nutrition, or does C mediate this effect? How does C investment in fungal growth change with N availability? Is it connected to MGR? Rice (*Oryza sativa*) plants were inoculated with *Rhizophagus irregularis* or *Glomus mossae* and grown with a gradient of N supplies. C limitation was promoted by using low light. Plant biomass, photosynthesis, respiration, nutrient, and starch contents were measured. Mycorrhizal colonization was quantified and total C investment in fungal growth estimated. OsPT11 expression was used as indicator of symbiotic functionality. The results showed that MGR was never positive, it was negative under limiting N supplies, and was not related to plant C investment in mycorrhizae. Mycorrhization reduced N uptake at limiting N, leading to lower Zn uptake by the plant, which was mostly responsible for negative MGR. We obtained further evidence that mycorrhization and fungal C allocation are highly N dependent, as opposed to a general nutrient or P dependency, and that C:N acted as the signal for C investment in mycorrhizae. These findings indicate more complex nutrient effects of mycorrhization than previously considered, which could explain contradictory results obtained so far.

Influences of 4 year of shading and fertilization on plants and their associated arbuscular mycorrhizal fungi in an alpine meadow ecosystem

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Light intensity and soil fertility are important factors in biology, but relatively little is known about these two factors, especially their interaction, influencing the communities of plants and their associated arbuscular mycorrhizal fungi (AMF). Here, we conducted a 4 year field experiment with two levels of light intensity (natural light and 25% light) and two levels of soil fertility (ambient soil and fertilization with 6.4 g N and 7 g P m^{-2} yr^{-1}) treatments in an alpine meadow ecosystem to investigate the main and interactive effects of shading and fertilization on plant and AMF communities. Shading and fertilization dramatically reduced the plant species richness, with a loss of c. 70% species under shading plus fertilization (SF) treatment. Most AMF variables (including AMF colonization, extraradical hyphal length, spore richness, and phylotype richness in roots) did not respond significantly to the main and interactive effects of shading and fertilization, but the lowest values of these variables synchronously occurred in SF treatment, suggesting that shading and fertilization might have an additive effect on AM fungi. Moreover, we found that the AMF communities inside roots were significantly affected by both shading and fertilization, and this could be attributed to the changes of plant N and P concentrations by treatments. Our findings highlight the importance of soil fertility and light intensity in regulating both plant and AMF communities, and emphasize that increasing cloudiness and nutrient deposition in future can reduce the diversity of plants and AMF, and influence the sustainability of ecosystems.

Ectomycorrhizal fungi on Norway spruce: genotypic effects on fungal colonization and root characteristics in relation to host tree vitality and growth

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In boreal forests, the functional importance of mycorrhizal short roots of trees is high since they form the interface for water and nutrient exchange. Mycorrhizas use fungal specific enzymes in the mobilization of nutrients from litter and soil organic matter. Thus, mycorrhizal species composition has a major influence on the enzyme activity and nutrient foraging abilities of trees in nutrient poor boreal soils. We have accumulating evidence of intraspecific variation in the root associated ectomycorrhizal community structure among differentially growing Norway spruce (*Picea abies*) clones, and among spruce genotypes in general. Our recent results show that also the seedling short root formation is genetically controlled by trees, and that the short root formation seems to interconnect with later growth performance of spruce. The impact of host genotype on accompanying fungi is dependent of the association: regulation of the ectomycorrhizal community is stronger than that of the needle endophytes. The functional diversity of ectomycorrhizal symbionts appeared to be the driving mechanism behind the positive impact of taxonomical diversity of mycorrhizal fungi on vitality of spruce as estimated by production of chitinases and cellulolytic exoenzymes. Therefore, functional diversity of the associated ectomycorrhizal community also seem to be more important than taxonomical richness it self. Hence, in terms of belowground growth strategies of spruce, these findings imply that the genetically controlled variation in root tip formation and the ability to associate with symbiotic fungi have great influences on the potential of spruce to harvest beneficial interactions in forest soil.

Mycorrhizas in Europe's oaks: belowground indicators and their environmental drivers

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Oak forests are an extensive forest type in temperate regions worldwide. In Europe, forest ecosystems are threatened due to a loss of biodiversity caused by human and environmental factors among which increasing pollution is a key threat. Ectomycorrhizas are major ecological players in these ecosystems, but they are rarely used in measures of forest biodiversity status or condition because large-scale, high-resolution, standardized, and replicated data on mycorrhizal communities is lacking. Thus, we (1) assessed the mycorrhizal diversity of European forests creating baseline data against future forest changes; (2) tested for relationships between mycorrhizal community composition and forest condition; and (3) studied changes in mycorrhizal diversity and abundance across environmental gradients. We carried out a mycorrhizal analysis of 22 intensively monitored long-term oak plots of the ICP forests network across nine countries and covering strong natural and anthropogenic environmental gradients. We found that oak forests harbour from 23 ectomycorrhizal taxa in a Dutch plot up to 83 at a Romanian plot. Overall, we detected nearly 400 ectomycorrhizal fungi from which to identify a set of dominant fungi as belowground indicators and their environmental drivers.

Impacts of plant functional group removal on AMF diversity in plant roots in a grassland ecosystem

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Plant functional groups (PFGs) are expected to play an important role in determining communities of below-ground organisms. In the present study, we evaluated the effects of the experimental removal of different PFGs on arbuscular mycorrhizal fungi (AMF) communities in a grassland ecosystem. Plant species in the grassland ecosystem were divided into four plant functional groups, i.e., PR (perennial rhizome grass), PB (perennial bunchgrasses), PF (perennial forbs), and AB (annuals and biennials). Two removal ways, complete removal and equal disturbance removal, were carried out and AMF diversity in plant roots of 96 plots (2 removal ways X 16 PFG combinations X 3 replicates) was determined using T-RFLP. The results showed that, (1) Removal of PFGs could increase CV of AMF species richness; (2) five AMF species could act as indicator species for PFG complete removal, 10 AMF species were indicators for PFG equal disturbance removal; (3) Biomass of PR and PB were important determinants of AMF community composition; (4) non-linear relations between plant diversity and AMF diversity were found in complete removal treatments, but not in partial removal treatment, indicating intermediate disturbances will enhance the AMF diversity in grassland ecosystems. These results may be helpful in understanding the maintenance mechanism of AMF diversity and plant–AMF relationships in grassland ecosystems.

Investigating intra-specific genetic diversity in a population of *Rhizophagus irregularis* using high throughput sequencing techniques

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Within a species of arbuscular mycorrhizal fungi (AMF), genetically different AMF lines have either beneficial or detrimental effects on host plant productivity. For example, in a greenhouse study, non-mycorrhizal rice had a higher growth than rice inoculated with lines of the AMF *Rhizophagus irregularis* sampled in a Swiss agricultural field. The growth of rice, however, was much improved when rice was inoculated with single spore lines that were generated *in vitro* from the agricultural parental lines. Up to now, genetic markers used to distinguish different lines of *R. irregularis* were limited to amplified fragment length polymorphism (AFLP) or microsatellite markers, limiting fine-scale studies of AMF populations and AMF-plant interactions. Therefore, we are developing new genetic markers and investigating the genome-wide genetic diversity of a Swiss population of *R. irregularis* by using restriction-site associated DNA (RAD) sequencing. RAD sequencing is a method for detecting single nucleotide polymorphisms (SNPs) adjacent to restriction sites in whole genomes of multiple individuals by Illumina sequencing. The quantification of SNPs in *Rhizophagus irregularis* will allow us to estimate genetic diversity among lines and to design new markers to compare the genotypes of single spore lines to parental lines. Finally, RAD sequencing will allow us to link particular AMF genotypes to the phenotypes of the mycorrhizal fungus and its host plant.

A Field study of specificity in the arbuscular mycorrhizal symbiosis

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Host specificity in the arbuscular mycorrhizal (AM) symbiosis has been difficult to assess in the field. Here we report on a study designed to determine whether different plant species associate with unique communities of AM fungi, and how increasing plant diversity affects the composition of AM fungal communities? We sampled soil from a long-term common garden study — the biodiversity field experiment at the Cedar Creek Ecological Research Centre in Minnesota, USA. The soil samples were taken from plots with varying plant diversity (0, 1, 4, and 16 species) and AM fungal DNA were extracted from these samples for pyrosequencing to assess fungal community composition and host specificity in the AM symbiosis. Early results indicate that there is evidence for host specificity in the AM symbiosis and a significant relationship between plant and AM fungal diversity. The design of the study provides a unique opportunity to test for host specificity since long-standing plant monocultures have been growing in the field using a randomized design, thus removing confounding variables, such as environmental heterogeneity, which are typically problematic in field studies. Furthermore, though the effects of AM fungi as drivers of plant community structure have been previously investigated, there has been little research on how plant diversity can affect AM fungal diversity below ground. This study provides a better understanding of belowground/aboveground interactions and community scale dynamics of the AM symbiosis.

Molecular diversity of arbuscular mycorrhizal fungi and their influence on grapevine (*Vitis vinifera* cv. Cabernet Sauvignon)

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The main aim of the investigation was to study the diversity of native AM fungi from four different ecological zones of Northern Karnataka and their outstanding efficacy in P uptake and growth promoting potentials in *Vitis vinifera* cv. Cabernet Sauvignon. The diversity analysis revealed that the Shannon diversity index was the highest in Western Ghats (H'=2.85) followed by Northern dry zone (H'=2.74); Transitional zone (H'=2.19) and North eastern dry zone recorded poorer with respect to AMF diversity (H'=2.09). Further, the PCR based identification of nine efficient AM fungal isolates was done by the amplification of ITS region (375bp) from single spore of the tentatively identified *Glomus* sp. The BLAST results of ITS rDNA region showed that the native isolates AMF60, AMF528, AMF251, AMF135, AMF22 and AMF235 have fallen within the same cluster of *Glomus mosseae* (DQ 400160. The other two isolates viz., AMFG7and AMFG3 has also found to be closely related to *Glomus mosseae* (DQ 400141) in a separate group. Finally, the isolate AMFT25, which is morphologically identified as *Glomus geosporum* diverged separately in to a different clad. The polyhouse studies on response of grapevine to native AM fungal strains viz., AMFG3 and AMF528 recorded substantial increase in the plant growth parameters, physiological parameters, total dry matter production and soil enzyme activities. The study has brought out the diversity of AM fungi and their importance in the growth promotional ability in the grapevine.

Diversity of arbuscular mycorrhiza fungi in extreme petroleum hydrocarbon contaminated site

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Arbuscular Mycorrhizal Fungi (AMF) has been considered one of the most important microbes in plant rhizosphere and play an important role in phytoremediation of petroleum hydrocarbon (PH) contaminated soils. The aim of this study is to investigate AMF community structure in roots and rhizospere soils of three native plants *Persicaria maculosa, Eleocharis elliptica, Populus tremuloides* growing on sites extremely polluted by aliphatic hydrocarbons (AH), polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB). Cloning and sequencing method was employed to assess AMF biodiversity targeting 18S rRNA genes. The results showed that AMF community structure was modified by PH contamination and varied between different host plants. Glomaceae ribotypes were the most dominant AMF in contaminated sites while Acaulosporaceae ribotypes were the most abundant AMF detected in medium-contaminated sites. This study indicated host plant and PH pollutant can modify the AMF community structure, and provide valuable practice for phytoremediation of Petroleum Hydrocarbon pollutants.

Single molecule real-time (SMRT) sequencing of natural and manipulated Arbuscular Mycorrhizal Fungal assemblages in the field

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A survey at eight biogeographically separated grassland sites on slightly alkaline and acidic soils in Switzerland revealed highly site-specific arbuscular mycorrhizal fungal (AMF) assemblages. In an attempt to mimic mass immigration events after disturbance, topsoil samples were reciprocally transferred among all these sites and mixed into the respective local soils. By this experimental manipulation we confronted foreign potential immigrants, which were either edaphically pre-adapted or not, with site-adapted native AMF. AMF-specific environmental PCR amplicons (approx. 1800 bp) from roots and soil were analysed by Single Molecule Real-Time (SMRT) sequencing on the

PacBio RS platform (Pacific Bioscience). This third generation sequencing technology generates much longer sequence reads suited to cover the approx. 1800 bp AMF-specific amplicon, but with an lower accuracy as current pyrosequencing techniques. We used SMRT-sequencing to overcome the short read lengths generated by e.g. GS FLX+ amplicon sequencing (Roche) which makes reliable species-level resolution and molecular genetic tracing of AMF in the field challenging. The resulting SMRT-sequences showed sufficient phylogenetic resolution for analyses on the evolutionary and ecological forces driving AMF community assembly in the field. The compositional and structural data of the AMF assemblages from one of the eight experimental sites from before and after (re-)assembly will be presented and discussed.

Role of environment and geographical distance on the distribution of arbuscular mycorrhizal fungi at the landscape-scale

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In the past decade, there has been a considerable increase in the number of biogeographical studies conducted. These can be largely attributed to two reasons: increased availability of data and analytical tools, and the increasing awareness of threats to biodiversity which has led to numerous investigations into the status of biodiversity and potential impacts of environmental change. However, in contrast to animals and plants, the biogeography of microorganisms has been understudied. Based on the high abundance, small size and general biological characteristics of microbes it has long been thought that microbes are not dispersal limited, and that community compositions are a result of the current environment. This idea was exemplified in the Baas-Becking hypothesis which stated that everything is everywhere, but, the environment selects. To gain a better understanding of the distribution of arbuscular mycorrhizal fungi (AMF), this hypothesis was tested at the landscape-scale. AMF communities in plant roots were assessed at 40 geographically dispersed sites in Ireland using 18S rRNA terminal-restriction fragment length polymorphism. AMF community composition was influenced by abiotic variables (pH, rainfall and soil type), but not geographical distance. Co-occurring plant species supported contrasting communities of AMF, and the communities colonizing each plant species were consistent across pasture habitats and over distance. This is the largest and most comprehensive study that has investigated the landscape scale distribution of AMF. Our study supports the Baas-Becking hypothesis and demonstrates the strong influence that the local environment has on determining AMF community composition.

Large spatial-scale metagenomic analysis of arbuscular mycorrhizal fungi communities in western Switzerland

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High-throughput sequencing (HTS) techniques have recently been used to analyse AMF community structure in the roots of plants and in soil from both natural communities and from agro-ecosystems. So far, however, these efforts have been limited to sampling over small areas where there is little environmental variation in biotic and abiotic characteristics. In order to understand AMF diversity and its spatial structure on a landscape or regional scale, we have conducted HTS, using 454 pyrosequencing technology, on DNA extracted from soil at approximately 200 different sites in western Switzerland. The sampling has been conducted within the framework of a larger study where detailed information has been collected for each site regarding the plant community structure and a large number of abiotic variables. We will present a first analysis of this large dataset revealing at what scale the most AMF diversity is found and which biotic and abiotic factors are most closely associated with the observed AMF diversity. We will also compare the HTS dataset on AMF communities with another dataset generated by the same groups on diversity of fungal communities (comprising all fungal phyla) across the same region.

Molecular characterization of seven AMF hyphal fusion progenies and their eight parents using amplified fragment length polymorphism (AFLP)

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AFLP analysis was done as per the protocol of GibcoBRL AFLP Analysis System (Life Technologies Inc.) with total genomic DNA of seven AMF hyphal fusion progenies and their eight parents belonging to *Glomus* species. The information obtained was used for genetic mapping and population studies to find out the genetic variations/similarities among the seven hyphal fusion progenies and their eight parents. AFLP profiles obtained using three restriction enzymes- EcoR1, Mse1 and Pst1 were used

to construct dendrogram using NTSYS software (Rohlf 1994). A total of 156 AFLP loci were scored for presence or absence of alleles and the bands of same mobility for all the 15 accessions consisting of parents and progenies were scored as 1 (present) and 0 (absent) and the clustering of accessions was done based on UPGMA and Complete Linkage method of clustering. The similarity between each hyphal fusion progeny with its two parents and among all 15 AMF isolates was estimated using Jaccard's similarity coefficient. The results showed that all 15 accessions consisting of parents and progenies were clustered in two major groups. AFLP studies revealed that Progeny 9 and Progeny 12 share almost equal amount of genetic information with their parents; Progeny 7, Progeny 14, and Progeny 16 are more similar to one of their parents than the other; and Progeny 1 and Progeny 6 do not have much similarity with either of their parents.

Do bacterial communities associated with the black truffle contribute to the life cycle of the ectomycorrhizal fungus?

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Under natural conditions, ectomycorrhizal fungi are surrounded by diverse bacterial communities that can modulate physical, metabolic, and functional aspects of the fungal life cycle. In some cases, like the button mushroom *Agaricus bisporus*, interactions with bacteria are necessary for the production of fungal sporocarps. The cultivation of the black truffle *Tuber melanosporum*, globally known for its culinary interest, remains very erratic in truffle orchards. In this study, we analysed the bacterial diversity associated with ectomycorrhizae and ascocarps of *T. melanosporum* to decipher the potential role of truffle associated bacteria in the ecology of the fungus and in its ascocarp development. We used both culture dependent (bacterial collections) and independent (TTGE, 454-pyrosequencing, Geochips, in situ hybridization) methods to follow the dynamics of the composition of bacterial communities throughout the life cycle of the fungus and to characterize the functional properties of the bacterial communities. Our combined approaches revealed contrasted fingerprint patterns, and a higher abundance of functional genes related to nitrogen and sulphur in the ascocarp, suggesting a habitat effect. The composition of the bacterial communities evolved during the maturation of the ascocarps, the inner part of the truffle being mainly colonized by Rhizobiaceae and the outer part by Bacteroidetes. The potential role of these bacteria in the maturation of the ascocarp and in the biology of the fungus will be discussed.

Gene expression patterns and C- and N- allocation during combined mycorrhizal symbiosis and pathogen attack on *Quercus robur* L.

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Within the joint research project 'TrophinOak', we analysed multitrophic interactions with respect to the rhythmic growth of the common oak tree. Three partners, Quercus robur L. microcuttings DF159, the mycosymbiont *Piloderma croceum* strain F1598, and the root pathogen *Phytophthora quercina* isolate QUE6 interact in a microcosm system with sterile soil substrate under strictly controlled conditions (www.trophinoak.de). The respective impact of the symbiotic and pathogenic interactions on resource allocation pattern in relation to root and shoot flushes was studied using 15N- and 13C-stable-isotope labelling. The presented data show modifications in resource allocation partioning within lateral and principal roots, stems, and sink and source leaves during the endogenous rhythmic growth. The observed shifts in resource allocation are specifically affected by plant interactions with symbiotic or pathogenic microorganisms. 13C excess was significantly increased under the presence of *P. croceum* during both shoot and root flushes, indicating an increased CO_2-assimilation compared to the control plants. Under the influence of *Phytophthora quercina*, modifications in the 13C- and 15N-allocation partitioning were demonstrated and are presented. On the same plant material, transcriptomes of roots and leaves were investigated by Illumina RNA sequencing in order to bridge the C- and N- gene regulation pathways to the C- and N- allocation patterns. An insight into comparative expression patterns will be presented.

Plant responses to mycorrhization helper bacterium *Streptomyces* sp. AcH 505

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Formation of mycorrhizal symbiosis is stimulated by Mycorrhization Helper Bacteria (MHB), which either promote the growth of the mycorrhizal fungus or facilitate the colonisation of the plant root. Within the TrophinOak Project (www.trophinoak.ufz.de), we investigate in a Petri dish soil microcosm system how common oak (*Quercus robur* L.) coordinates its molecular responses during the interactions with the MHB *Streptomyces* sp. AcH 505, the ectomycorrhizal fungus *Piloderma croceum* and the oak powdery mildew *Microsphaera alphitoides*. Mycorrhiza formation with *P. croceum* was promoted by 30% by the MHB *Streptomyces* AcH 505. The symbiotic fungus *P. croceum* had a positive effect on the growth of the bacterium. Both microorganisms had a positive impact on root formation. We investigate the impact of a MHB on plant transcriptome by Illumina sequencing root and leaf RNA. We will present evidence for changes in oak gene expression levels during the interaction with the MHB and the ectomycorrhizal fungus. Powdery mildew infection of the leaves was suppressed in non-mycorrhizal plants treated with MHB AcH 505 on the roots. We will show how the defence related plant gene expression response, stimulated by powdery mildew infection, is modulated due to the MHB-oak interaction.

Atmospheric CO_2 and the functional symbiosis between basal land plants and Endogonales

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It has long been hypothesized that plants colonized the earth in partnership with arbuscular mycorrhizal fungi (AMF) to increase their access to growth-limiting mineral nutrients in exchange for photosynthetically-fixed carbon. This paradigm was supported by a growing body of evidence dating the origins of the symbiosis, but recent studies have revealed the most basal clades of liverworts (the earliest group of land plants) associate with Endogone, a more ancient group of endophytic fungi that predates the origin of the Glomeromycota. This suggests Endogonaceous fungi were the critical organisms initiating plant–fungal symbioses in soils. We present the first experimental evidence of the functional basis of the symbiosis between Endogone and basal liverwort taxa including comparative analyses with AMF functioning in more advanced groups of thalloid liverworts. This includes investigation of the effect of the dramatic fall in atmospheric CO_2 coincident with the rise of early land plants on the functioning of these symbioses to find clues as to why AMF rose to become the dominant kind of mycorrhiza on earth. We measured the carbon-for-nutrient exchanges between Endogone and the basal liverworts: *Haplomitrium gibbsiae, Treubia lacunosa, Allisonia cockaynii*, and *Neohodgsonia mirabilis*. By supplying 33P-orthophosphate to the fungal partner and 14C-CO_2 to the plant, we quantified the efficiency nutrient uptake and transfer via fungus into the plants under early Palaeozoic $[CO_2]$a (1500 ppm) and contemporary $[CO_2]$a (450 ppm). These results are compared to AM functional responses to the same drop in $[CO_2]$a to reveal the evolutionary significance of the enigmatic liverwort-Endogone symbiosis.

Host plant investment in multiple mutualists and the effect of herbivory

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Plants that host arbuscular mycorrhizal fungi often also invest photosynthates in other mutualists such as endophytic fungi. While mycorrhizae are important for nutrient acquisition, studies have shown that, among other things, endophytic fungi confer herbivory resistance to the host plant. It follows from this that the level of herbivory may influence the extent of investment of the plant in endophytic fungi. Further, though the endophytic and mycorrhizal fungi are spatially separated, with the endophytes primarily colonizing the shoot and mycorrhizae the root of the plant, competition for the same carbon pool suggests that they are likely to influence each other's success in colonizing the plant. Based on a field experiment involving the collection and microscopic examination of three species of grasses and a sedge from eight-year old herbivory exclosures across a herbivory gradient in the trans-Himalayas, this study focuses on two hypotheses to aid in better understanding the interactions of multiple mutualists with the host and with each other: (a) In regions with more herbivory, plants' investment in endophytic fungi may be more, and as a result, endophyte density in the shoots could be higher. (b) Since endophytes and mycorrhyzae depend on the same carbon pool, plants with high endophyte density may reduce their investment in mycorrhizae, and thus have a lower extent of mycorrhizal colonization in the roots. The findings of the field experiment and the consequences of plant decisions in balancing investment between multiple mutualists will be discussed.

Impact of arbuscular mycorrhizal fungi and earthworms on aggregate stability

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Earthworms and arbuscular mycorrhizal fungi (AMF) play important roles in modifying soil physical and chemical properties. Both may enhance soil aggregation, which in turn may affect nutrient and water use efficiency. However, little is known about their single and interactive effects on water-stable

aggregation, crop nutrition, and plant growth. A greenhouse experiment was run for 9.5 months to investigate single effects of earthworm (*Pontoscolex corethrurus* (endogeic) and *Dichogaster bolaui* (epigeic) and AMF species (*Glomus etunicatum* and *Scutellospora verrucosa*), and earthworm-AMF interactions on soil aggregate stability and growth and N and P uptake of maize (*Zea mays*) and pigeonpea (*Cajanus cajan*). The study used a humic nitisol, with grass residues placed on top. Water-stable macro-aggregates and micro-aggregates, AMF colonization, extraradical hyphal length, glomalin levels in various aggregates, and plant biomass, and P and N uptake were assessed. Crop and earthworm, but not AMF, were the most important factors influencing soil aggregation and glomalin pools in various aggregate size fractions. *Dichogaster* improved soil aggregation by increasing levels of macro-aggregates by 11% and reducing micro-aggregates by 19%. *Dichogaster* also increased glomalin pools in stable aggregates and improved biomass and nutrient uptake by 50% in pigeonpea. *Pontoscolex* had no effect. There was significant crop x AMF interaction on soil aggregation and glomalin. Interactions between AMF and earthworm were also observed on nutrient (P, N) uptake and biomass, but not on soil aggregation. This study highlights the importance of crops, soil macro-fauna (*Dichogaster*), and AMF on soil aggregation, crop nutrition and crop productivity.

Arbuscular mycorrhizas as inducers of crop defence in agriculture

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Mycorrhizal mycelial networks play a major role in nutrient cycling in natural ecosystems but this functioning can be impaired in agriculture where a number of factors combine to reduce the extent of mycorrhizal associations with crop plants. Soil tillage damages the integrity of mycorrhizal networks and high nutrient inputs diminish the tendency of plants to form associations with mycorrhizal fungi as the benefit to the plant of the symbiosis is proportionally lower. Commercial wheat varieties in Europe are selected to have very high yield potential and disease resistance when grown in modern high intensive, high nutrient, short rotation systems. There is increasing evidence that soil microorganisms, including mycorrhizal fungi, can induce broad spectrum resistance against pathogens (Systemic Induced Resistance). Therefore, such breeding and agronomic practices may have resulted in the benefits of this symbiosis being lost. We have investigated whether there are differential responses of wheat cultivars to mycorrhizal colonisation that could provide a new source of resistance to pathogens and more efficient nutrient utilisation by crops and found significant variation. Furthermore, we have evidence to suggest that there are differential responses to soil microflora in different cultivars that

trigger different levels of resistance to the wheat eyespot pathogen, which can reduce yield by up to 30% if severe. Further work is planned to determine the extent to which mycorrhization contributes to this effect.

Analysis of the bacteria colonizing the root endophyte *Piriformospora indica*

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Piriformospora indica is a well-known root endophyte, colonising the roots of a broad range of host plants including mono and dicotyledons. The fungus has been reported to provide enhanced plant growth and yield and protection against biotic and abiotic stresses. The association of the bacteria *Rhizobium radiobacter* with *P. indica* was recently discovered but the interaction between the two has been poorly studied. In the present study, we isolated the bacteria associated with different strains of *P. indica* (India, IGZ, and DSM 11827), and the results confirm the association of two different bacteria with *P. indica* (India and IGZ) whereas only one bacterium was found to be associated with *P. indica* DSM 11827. Barley plants inoculated with the isolated bacteria showed increase in plant growth parameters and early germination of barley seeds was observed if inoculated with Bac DSM (isolated from *P. indica* DSM11827). We also investigated the effect of different conditions on the symbiosis between the fungus and the bacteria, and the results indicated nutritional impact on the bacterial population in the hyphae of *P. indica* DSM 11827.

Pure culture establishment and mycorrhization of Asian chanterelles

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Cantharellaceae in Basidiomycota is one of the most popular edible ectomycorrhizal mushrooms in the world. However, cultivation study in this family is limited because of its difficulty to establish pure cultures from fruit bodies. The European yellow chanterelle, *Cantharellus cibarius* Fr., has been reported to harbor high density of bacteria and molds even in the young and fresh fruit bodies. We have recently established Japanese *C. cibarius* sensu lato isolates on nutrient agar media and tested for their ectomycorrhiza synthesis with Asian red pine (*Pinus densiflora*) seedlings *in vitro*. The established mycorrhizal pine seedlings grew well under acclimatized conditions and developed new mycorrhizal tips for over a year. Organic soil was better than the mineral one for both host and mycorrhizal growth under the acclimatization. To obtain diverse chanterelle cultures for their future commercialized applications, we have tested for fungal isolations from their mycorrhizal tips collected in forests. Three species, i.e., *C. cibarius*, *C. infundibuliformis*, and *Craterellus cornucopioides*, were successfully cultured from mycorrhizas on divers host trees, which was more efficient as compared with the conventional fruit body tissue isolation technique. Although phylogenetic sequence analyses of ITS region within the nrDNA cluster showed the validity of taxonomic positions of those cultures, Japanese *C. cibarius* clade had a short but distinct distance from European *C. cibarius* clade. These results suggest that Cantharellaceae mushrooms can be applied for their cultivation study by using suitable isolation techniques and mycorrhiza synthesis, which will allow their mycorrhizal tree plantations.

Reduced diversity of arbuscular mycorrhizal fungi in annually cropped fields of the Canadian prairie

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The absence of live plants during eight months or longer in cultivated soils of the Canadian Prairie each year, may impact the biotrophic arbuscular mycorrhizal fungi (AMF). We examined the diversity

of AMF in the soil of 302 wheat fields, adjacent roadsides, and native prairie remnant ecosystems. The use of primers AMV4.5NF/AMDGR in a tag-encoded 454-GS-FLX pyrosequencing protocol revealed the large diversity of Claroideoglomus (35% of the AMFOTU) in Canadian Prairie soils, the highest AMFOTU richness in roadsides (19 AMFOTU as compared to 13 in prairie remnant, and 12 in wheat fields), and different AMF communities in the three ecosystems examined. Sixty two percent of the 16 Diversisporaceae-related AMFOTU were only detected in wheat fields, whereas 6 of a cluster of 8 AMFOTU related to *Glomus macrocarpum* W5293, and 13 of 30 AMFOTU related to *Glomus iranicum* (ined.) were absent from wheat fields. One AMFOTU clustering with *G. iranicum* was a cultivated field specialist and rare in prairie remnants. The distribution of generalist AMFOTU had low relationship with environmental conditions, but the distribution of specialists was well correlated with soil properties. We conclude that annual cropping reduces the diversity and abundance of AMF in the Canadian Prairie.

Exploitation of native arbuscular-mycorrhizal (AM) resources under rainfed upland rice (*Oryza sativa* L.) based cropping systems

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Upland rice is grown in about 14.0 and 7.0 mha respectively in the world and in India which constitute 11 and 22% of total rice growing area. It is mostly grown under rainfed, drought prone, aerobic soil conditions. Upland soils in several eastern Indian states have acidic pH that impairs phosphorus (P) acquisition. The soil environment, however, favour arbuscular-mycorrhizal fungal (AMF) activities which, through its extended external mycelial network beyond the colonized roots, enhances acquisition of less mobile nutrient like P. Several possible avenues to exploit this ecosystem service by AMF were explored and AM-supportive crop management components integrated to develop a management package for enhancing P nutrition of upland rice. The management components contributing to enhance AM-aided P nutrition were (i) off-season tillage schedule, (ii) rice-based cropping system/rotation, and (iii) AMF inoculum of native source. The promising components were validated in farmers' fields that included (i) maintaining minimum space of 13 weeks between two consecutive off season tillage operations, (ii) two years crop rotation of maize (*Zea mays* L.) relay-cropped by horse gram (*Dolichos biflorus* L.) in the first year followed by rice in the second year, and (iii) application of on-farm produced soil-root based AMF inoculum (@1.5 t/ha). Integration of AM

fungal inoculation with the maize-horse gram rotation grown with optimum off season tillage had synergistic/additive effects in terms of AMF colonization (+22.7 to +42.7%), plant P acquisition (+11.2 to +23.7%), and grain yield (+25.7 to +34.3%) of rice variety Vandana.

Carbon expenditure and the economy of arbuscular mycorrhizal symbiosis

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The benefits of arbuscular mycorrhiza for plants and humans in terms of improving plant mineral nutrition, biomass production, abiotic and biotic stress tolerances, and diversity of vegetation have been intensively studied for decades. Yet, the plant's carbon (C) allocation to the mycosymbiont, its mechanisms, and the C costs of the symbiosis remain much less understood. Here, we briefly overview the different concepts to estimate symbiotic costs of the mycorrhiza, the quantitative data on C allocation to the mycorrhizal fungi, and we also show new information on the economy of the symbiosis, taking into account both C and phosphorus (P) fluxes as traced by radio- and stable isotopes. The experimental evidence shows that, depending on the identity of symbiotic partners and environmental conditions, the overall stimulation of plant´s growth by mycorrhizal fungi can be attributed to the proportion of symbiotic benefits and costs, and can vary from positive to negative. Yet a number of individual contributing parameters such as regulation of direct (root) and indirect (mycorrhizal-hyphae mediated) nutrient acquisition pathways, stimulation of photosynthesis or changes in plant's respiration rates remain poorly quantified. We propose tools and show results of preliminary attempts to measure some of these parameters. The ultimate goal of this research is to allow mechanistic understanding of mycorrhizal benefits and costs, eventually leading to establishment of mathematical models of the mycorrhizal physiology at the whole-plant level.

Management of mycorrhizal inoculant MycoUp applied through irrigation systems localized under intensive agriculture conditions

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The importance of the proper and efficient use of mycorrhizal fungi is well known. Currently, Symborg SL has patented a mass production system of a mycorrhizal inoculant from a new species of fungus forming mycorrhizae, *Rhizophagus* sp., currently in the process of characterization (being characterized). The specie was previously isolated from a sodium saline ecosystem, in the province of Murcia. In this paper we present the main results achieved with the use of this product, known as MycoUp. When applied at 3 kg/ha through the use of an irrigation system, it promotes increases in agricultural production between 10 and 20% in crops such as lettuce, peppers, tomatoes, melons, citrus, grapes, and stone fruits in general. Due to its nature, the mycorrhizal fungal specie used does not require any reductions of fertilizer in the intensive cultivation of these crops, it will not cause damages to the symbiosis and there is no need to remove the filters from irrigation systems, because the spores and propagules are less than 100 microns in size. The effectiveness of this product has been demonstrated in 100% of the cases by performing a control and monitoring of the interaction throughout the crop cycle. Its application not only promoted an increase in production, but caused increases in fruit brix, higher physiological activity and a marked precocity. These improvements are due to beneficial action of the symbiosis between plants and the specie of fungi forming mycorrhizae.

Cultivars as determinant of mycorrhizal dependency and nutrient uptake in soybean grown in vertisol

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Soybean productivity in marginal fields of Vertisols is often limited by recurring drought and high P-fixation. Ten different soybean cultivars were tested in unsterilized soil under microcosm conditions for their dependence to colonization with indigenous AM fungi (consortia of *Glomus intraradices, G. geosporum, and G. mosseae*) for improved nutrition, growth, yield, and maintaining soil quality parameters. Among the cultivars, NRC-7, Hardee, and JS 71-05 showed highest root colonization ranging from 32–42% and lowest range from 18–19% in SLS 525 and Bragg was recorded. Irrespective of cultivar, AM inoculation did enhance the growth, nodulation, soil enzyme activities (soil dehydrogenases, phosphatases, β-glucosidases), seed yield, and nutrient uptake. Mycorrhizal dependence (MD) calculated at native P level (4.58 mg kg-1) varied among the cultivars; JS-335 (32%) and JS 95-60 (23%) were found to be highly dependent whereas JS 71-05, Bragg, NRC-37, and Hardee were poorly dependent (7–13%) on mycorrhizal association for higher dry matter, seed yield, P, N, and micronutrients uptake and rest cultivars were observed to be marginally dependent (16–18%). Interestingly, mycorrhizal-JS 335 and -JS 95-60 cultivars showed higher root:shoot ratio than their non-mycorrhizal counterparts indicating the better candidates to thrive under drought conditions. Our results show that under uniform growth conditions soybean cultivars exhibited variation in AMF colonization and MD indicating the role of AMF-enhanced growth, yield and nutrient acquisition and can be selected further to optimize the higher responses towards AM inoculation at varied levels of P application.

Does *Tricholoma matsutake* live between symbiotic and saprophytic: a vertical view of Matsutake Shiro

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Tricholoma matsutake, a typical ectomycorrhizal fungus and a commercially valuable mushroom, is distributed patchily throughout Finland. However, improvement of sporocarp formation via mycorrhizal seedlings in nature and cultivation of this mushroom are still not promising. In nature complex ecosystem, organisms are individuals, but also members of a larger community that reflects interplay and communication between each individual within a population. Our studies have focused on the diversity of fungal and actinobacterial communities from organic layer to mineral layer, the soil chemical composition including organic carbon characteristics of both soil layers, and the litter cover and understorey vegetation in shiro area. Investigating of *T. matsutake* in this vertical direction has helped us to better understand the ecology of *T. matsutake*. Our results showed that *T. matsutake* is in symbiosis with pine and spruce trees; appears to be a specialized member of the soil microbial community that, except for its carbon source, does not require specific soil conditions; *T. matsutake* co-exists with a diversity of fungal and actinobacterial species. We also observed a higher enzyme activity involved in the degradation of organic carbon in the shiro and litter cover was somewhat higher above in shiro. In addition, *T. matsutake* can use certain organic carbon compounds and/or their degradation products of litter leached to the mineral layer from soil horizons above the litter cover. This flexible trophic ecology confers a considerable advantage to *T. matsutake*.

Mycorrhizae inoculated horticultural seedling production and transplantation models to field conditions

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The major plantation in the Mediterranean region is horticulture. Since many horticultural plants are mycorrhizal it is sound to use mycorrhizae inoculated horticultural seedlings. Seedlings of Eggplant aubergine (*Solanum melongena* L.), tomato (*Lycopersicon esculentum* Mill), pepper (*Capsicum annuum* L.), cucumber (*cucumis sativus*), watermelon (*Citrullus lanatus* Thunb.), and muskmelon (*Cucumis melo* L.) were inoculated with several selected and indigenous mycorrhizae applications under several inoculation and transplantation models in East Mediterranean region for several years. Mycorrhizae inoculated seedling production nursery experiments were carried out in greenhouse conditions. Seedlings were produced under glasshouse conditions using a nested tray and large containers. Also mycorrhizae inoculated seedlings were produced under field-scale conditions. Produced mycorrhizal and non-mycorrhizal seedlings were transplanted to the field conditions by using granular mycorrhiza inoculum or immersion of roots in a solution containing mycorrhizae inoculation. Two inoculation techniques were repeated several years under the field conditions. At harvest, several times fruits were collected and yield were determined. In the blossoming period, plant leaves and root samples were taken for nutrient content and mycorrhizal colonization respectively. The results revealed that under marginal soil conditions, mycorrhizal inoculation effectively infected plant roots and increased plant yield significantly. It has been made clear that pepper, cucumber, melon are highly responding to mycorrhizal application. Plants nutrient concentrations especially phosphorus (P) zinc (Zn) and copper (Cu) were also determined and mycorrhizae inoculation increased P, Zn, and Cu. After several years under field conditions, it has been concluded that for horticultural plants, mycorrhizae-inoculated seedlings production, and implementation to field is economic and ecologically appropriate and necessary.

Global Scenario on regulations and practices facilitating mycorrhizae application across borders and islands

Panel Discussion

"An Industrial Session and Webinar was organized during the Seventh International Conference on Mycorrhiza (ICOM7) at India Habitat Centre, New Delhi, India on 8 January, 2013; from 1530hrs - 1730hrs IST. The Webinar consisted of participants from mycorrhiza producing industries, seed industries and policy makers around the world via internet for the deliberation session titled "Global Scenario on Regulations and practices facilitating mycorrhizae application across borders and islands". Considering the nascent and evolving state of the mycorrhiza industry, it was felt that there is a need to understand the issues faced by the mycorrhiza industry from the production, distribution, usage, quality control and need for appropriate regulations' point of view. This session had enthused participants to be an active and dynamic part of ICOM7, even though they were not physically present at the venue. The Webinar took place via live webcast over the internet for those were located away from the ICOM 7 Venue. The session consisted of Panel Discussion for an hour followed by active Q&A discussion in the next hour in the presence of a moderator. The session covered pertinent issues related specifically to mycorrhiza industry like the growth and challenges for mycorrhiza production; regulation/no regulation impacts on growth of mycorrhiza use; quarantine, national standards and product specification; requirement of international association of mycorrhiza producing industries; should there be a mycorrhiza product testing association on the line of international seed testing association; need for globally acclaimed quality testing labs to verify the product quality in various parts of the world etc."

AM symbiosis and synthesis of terpenoids in aerial parts of plant

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Plants produce a wide variety of terpenoids, most of these are secondary metabolites and some have primary functions as hormones, sterol, parts of electron carrier moieties, and pigments. The biosynthesis of different isoprenoid compounds is influenced by AM-symbiosis. Though systemic effect of AM on the biochemistry and molecular biology of plant is now established, very little is understood regarding the relationship between mycorrhiza and terpenoids in the aerial parts of the plants. Root colonization by AM fungi favoured production of artemisinin, a sesquiterpene lactone in the aboveground parts of *Artemisia annua*. Mycorrhizal plants exhibited higher concentrations of artemisinin compared to non-mycorrhizal plants in spite of similar P-concentrations, suggesting involvement of mechanisms in addition to improved P-nutrition. Biosynthesis of artemisinin occurs in glandular trichome on the aerial parts of *A. annua* plants. The increase in concentration of artemisinin in M plants can be attributed to a higher glandular trichome density in the leaves. Artemisinin is biosynthesized through terpenoid pathway. The C5 building blocks from both the mevalonate pathway and methyl erythritol phosphate pathway are involved in its formation. The molecular analyses of leaf tissue revealed higher expression of four genes of artemisinin biosynthetic pathway in mycorrhizal plants. This increase in the transcript levels of key enzymes in mycorrhizal plants may be due to enhanced jasmonate levels. Exogenous supply of jasmonic acid to non-mycorrhizal *A. annua* plants displayed similar effect on artemisinin production, trichome density, and expression of these enzymes.

Expression of phosphate transporter genes in *Sorghum* and *Linum*: Developmental aspects and regulation in a common mycorrhizal network

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Arbuscular mycorrhizal fungi (AMF) are important plant symbionts, trading mineral nutrients beyond the reach of roots, in particular phosphate, in exchange to their hosts' photosynthetic products. Surprisingly, in a mixed culture between flax (*Linum usitatissimum*) and sorghum (*Sorghum bicolor*),

flax took up much more of the phosphate delivered by the common mycorrhizal network (CMN) than sorghum, although sorghum invested much more carbon into the CMN than flax. Is this difference in phosphorus uptake due to differential regulation of phosphate transporters in the two plants? To examine this question, a baseline was established by identifying all eleven phosphate transporters of the phosphate transporter family Pht1 in sorghum and studying their expression in different tissues. While some of them were not expressed in stems, leaves or flower organs, most of them were expressed in roots; two of them, SbPT10 and SbPT11, were expressed in the roots exclusively in the presence of AMF. We also identified two mycorrhiza-inducible Pht1 transporters in flax. When expression of the mycorrhiza-inducible phosphate transporters was studied in monocultures or mixed cultures of flax and sorghum, it turned out that the expression of AM-inducible Pht1 genes was only weakly related to mycorrhizal P uptake, but that it was differentially regulated depending on the fungal partner and the co-cultured plant. We conclude that in both sorghum and flax, expression of AM-inducible Pht1 transporters is initiated by arbuscule formation, but that the differential phosphate delivery by the CMN is not directly dependent on differential Pht1 gene expression in the two plants.

Functional plasticity of ectomycorrhizae: what have we learned from studies based on potential extracellular enzyme activities

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A considerable number of studies has been performed using enzyme activities as indicators for ECM functions in different environmental stress conditions (ozone, heavy metals, drought). It may now be time to discuss the results in a broader context: What can we learn from current findings? Which stress conditions showed clear effects which ones did not? What does the apparently great plasticity in ecosystems tell us? What kind of mechanisms have we detected? Are there critical functions we should especially focus on? Where should/could we go with further experiments? In order to stimulate discussion on approaches that could be useful in the field of ectomycorrhizal functional ecology, some selected studies using proteomic approaches and theoretical concepts related to macroecology of plants will be presented.

Localization and analysis of K^+ transport systems of the ectomycorrhizal model fungus *Hebeloma cylindrosporum*

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Ectomycorrhizal symbiosis established between woody plants and soil fungi is widespread in forest ecosystems. This mutual interaction is crucial for the vegetal partner to uptake 'hidden nutrients' efficiently and for the fungus to get access to carbon sources. We are analysing potassium (K^+) transport systems of the ectomycorrhizal fungus *Hebeloma cylindrosporum* aiming in understanding the functional differentiation of the fungal plasma membrane in specific sites for absorption and secretion. K^+ is used as model because of its importance as essential element for all organisms. Furthermore K^+ transport systems are well characterized in plants and animals. Few studies are dealing with the effect of ectomycorrhization on K^+ nutrition. Taking advantage from an EST library of *H. cylindrosporum* and more recently from the sequenced genome for in silico identification of candidate genes K^+ transport systems comprising Shaker-like and TOK-type channels as well as transporters from the Trk- and KUP/HAK-type have been identified. Functional properties of these systems are studied by heterologous expression in yeast and *Xenopus* oocytes. In parallel, we are developing cellular biology approaches (*in situ* hybridization transcriptional and translational fusions with a GFP marker) for fine localization of these transport systems within fungal tissues. Finally, over-expression and RNAi strategies are used to understand the exact role of these candidate proteins *in vivo* in pure fungal culture and ultimately in the mycorrhizal context. A model comprising the different putative fungal actors for K^+ uptake from the soil and its secretion towards the host plant *Pinus pinaster* will be proposed.

Arginine accumulation in the different parts of arbuscular mycorrhizal symbiosis after supplied with different forms of exogenous nitrogen and carbon sources

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Arginine (Arg) serves as important stored and transport carrier of nitrogen in hyphae of AM fungus. Thence, production of Arg by AM fungus colonized with the host plant (*Allium fistulosum*) in different exogenous nitrogen and carbon sources were studied. The results showed that when NH_4NO_3, urea, and Gln as exogenous nitrogen source was supplied separately to the ERM, both the Arg content in the ERM and the total nitrogen content in shoots are higher than those in other exogenous nitrogen sources; when NH_4NO_3, urea, Arg, and Gln as exogenous nitrogen sources separately, Arg contents in mycorrhizal roots were in higher levels. The results showed that the content of Arg both in the ERM and mycorrhizal roots were much higher than that in shoots. The different forms of carbon increased the dry weight of ERM and root infection rate but glucose reduced the Arg content in the ERM, mycorrhizal roots and shoots; sucrose and glycerol had no significant effect on the production of Arg. Both external Arg and glutamine (Gln) increased the Arg content in the ERM significantly. However, the different forms of carbon had no significant effect on total nitrogen content in mycorrhizal roots and shoots. Nevertheless, all the external carbon could promote the growth of AM fungus. Also, various light intensity influence N metabolism of AM fungus symbiosis. In 80%Lx light condition, Arg concentration is highest in ERM and lowest in mycorrhizal root, and Arg concentration in 60%Lx light condition is in between the other conditions.

Ectomycorrhizal fungi and SOM degradation

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In relation to the soil carbon balance, the flow of energy from the plant into the ectomycorrhizal (EcM) fungi has been traditionally considered as a contribution to the soil carbon pool via the production of fungal biomass. Additionally, it has been proposed that EcM fungi reduce the decomposition rate of soil organic matter (SOM) through the competition with saprotrophic fungi ('Gadgil' effect). On the other hand, there is growing evidence that this flux of easy metabolizable energy catalizes ('prime')

the decomposition rate. Furthermore, recent work has discovered new saprotrophic pathways how EcM fungi can degrade complex SOM. A recent study (Wallander et al., 2011) investigated the effect of fertilization on the growth of EcM fungi, using maize compost amended mesh bags in a series of experimental forests in Sweden. Here, we present complementary data on the alteration of the incubated maize compost from two sites with contrasting effect of fertilization of EcM fungal growth. We used pyrolysis GC/MS to reveal a detailed chemical view of the organic matter. Lignin degradation was strongly reduced in the fertilized plots that also experienced a strong decrease in EcM fungal growth. Also N-compounds, linked to the maize compost, showed the same trend. This fertilization experiment demonstrates the different roles of EcM fungi in the SOM dynamics: (a) the presence of EcM fungi has a positive effect on SOM degradation, (b) EcM fungi remove N from the SOM pool, and (c) their necromass contributes to the SOM pools1.

The role of carbon in fungal nutrient uptake and transport: implications for resource exchange in common mycelial networks

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Arbuscular mycorrhizal (AM) fungi are obligate biotrophs and depend almost exclusively on host derived carbon to complete their life cycle. However, AM fungi do not rely on a single host for their carbon supply, because they interact simultaneously within a common mycelial network (CMN) with multiple hosts from different plant species. Several hosts contribute to the carbon pool of the CMN, and plants interconnected through a CMN compete for limited fungal nutrient resources. It is currently not clear how fungal nutrient allocation is regulated in CMNs, but our recent results have demonstrated that carbon acts as an important trigger for fungal phosphate and nitrogen transport in the AM symbiosis, and that a reciprocal reward mechanism ensures fair trade between the symbiosis partners. We followed the uptake of phosphate and nitrogen by the extraradical mycelium (ERM), and studied fungal nutrient allocation in CMNs to host plants that differed in their photosynthetic capability. We found that the carbon availability had an effect on the phosphate and nitrogen allocation to hosts within a CMN, and that AM fungi preferentially allocated resources to hosts that were able to provide more benefit. However, the results also demonstrate that AM fungi adjust their phosphate and nitrogen allocation to hosts dependent on their carbon demand. Here, we discuss these recent research findings in relation to strategies that both partners may use to regulate and maximize their nutritional benefit from the AM symbiosis.

Ecophysiological properties of mycorrhizal symbiosis link C and N availability with organic matter turn-over in boreal ecosystems

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Boreal and sub-arctic ecosystems contain a major global store of terrestrial carbon, but the mechanisms that regulate the dynamics of this C in relation to ecosystem productivity and nutrient availability are not yet well understood. Mycorrhizal mycelium represents a primary input of C for long-term storage in soils and humus layers. Here, three distinct types of mycorrhizal communities are identified in ecosystems with large differences in photosynthesis rates and nutrient availability. Ascomycetes, primarily forming ericoid mycorrhiza (e.g., Helotiales), dominate in the most low-productive ecosystems. Their often melanised hyphae in combination with a lower capacity for enzymatic organic matter oxidation lead to high C sequestration in spite of low ecosystem productivity. With increasing photosynthesis rates, intensified N limitation favours ectomycorrhizal fungi with extensive and differentiated, cord-forming extraradical mycelia (e.g., *Cortinarius, Suillus, Leccinum*). Their potent peroxidase enzymes and dynamic mycelial development make these fungi efficient in foraging for organic nutrients in complex polymeric substrates while maximizing relative allocation of nutrients to the host. In more nutrient rich forests, other ectomycorrhizal species (e.g., Atheliaceae) dominate, which build more diffuse, non-aggregated, but yet persistent, mycelia. Efficient C utilization and a large exposed hyphal surface area makes these fungi well adapted to maximize retention of soluble, inorganic nutrients. It is hypothesized that ecophysiological differences at the interface of the fungal cell wall and the surrounding micro-environment are decisive in regulating how these different mycorrhizal communities act to regulate long-term carbon sequestration at the ecosystem level.

Beech roots are simultaneously colonized by multiple genets of the ectomycorrhizal fungus *Laccaria amethystina* clustered in two genetic groups

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In this study, we characterize and compare the genetic structure of aboveground and belowground populations of the ectomycorrhizal fungus *Laccaria amethystina* in an unmanaged mixed beech forest. Fruiting bodies and mycorrhizas of L. amethystina were mapped and collected in four plots in the Świętokrzyskie Mountains (Poland). A total of 563 fruiting bodies and 394 mycorrhizas were successfully genotyped using the rDNA IGS1 (intergenic spacer) and seven SSR (simple sequence repeat) markers. We identified two different genetic clusters of *L. amethystina* in all of the plots, suggesting that a process of sympatric isolation may be occurring at a local scale. The proportion of individuals belonging to each cluster was similar among plots aboveground while it significantly differed belowground. Predominance of a given cluster could be explained by distinct host preferences or by priority effects and competition among genets. Both aboveground and belowground populations consisted of many intermingling small genets. Consequently, host trees were simultaneously colonized by many *L. amethystina* genets that may show different ecophysiological abilities. Our data showed that several genets may last for at least one year belowground and sustain into the next season. Ectomycorrhizal species reproducing by means of spores can form highly diverse and persistent belowground genets that may provide the host tree with higher resilience in a changing environment and enhance ecosystem performance.

Ectomycorrhizal fungal communities in Japanese Douglas-fir forests

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The genus *Pseudotsuga* has a typical continentally disjunct distribution. Douglas-fir (*Pseudotsuga menziesii*) is widely distributed in western North America and is associated with many ectomycorrhizal (ECM) fungi, including Pseudotsuga-specific fungi (*Rhizopogon* spp. or *Suillus* spp., etc.) as major components. In Asia, *Pseudotsuga* is only found as small endemic populations in Japan, Taiwan, and

China. Although the disjunct distribution over a long geological time may provide us an interesting opportunity to address the effect of host-fungus coevolution on ECM fungal communities, we know nothing about ECM fungi on Asian Douglas-fir. In this study, we examined belowground ECM communities in four remaining Japanese Douglas-fir stands. In total, 100 soil samples were collected, and fungal and host identity of each ECM tip was determined by molecular analyses. Observed ECM fungal richness amounted to 136, from which about 360 ECM fungal species were estimated to exist in the forests. Japanese Douglas-fir shared many ECM fungi with coexisting *Tsuga* and *Abies* trees, while ECM communities on coexisting broadleaf trees were conspicuously different from those of the conifers. ECM fungi on Japanese Douglas-fir included Cenococcum, Russulaceae, Boletaceae, Cortinariaceae, Thelephoraceae, and Amanitaceae species, while the known Douglas-fir specific lineages were not found on mature trees (but a Pseudotsuga-specific *Rhizopogon* species was found in dormant spore communities in our bioassay experiment). Our results indicate that coevolutionary niche conservatism is not a major determinant in structuring ECM fungal communities, and frequent host switching and subsequent regional speciation would be prevailing in ECM fungal communities over geological time scales.

Responses of arbuscular mycorrhizal fungal communities to soil acidity: acid-tolerant fungi are the generalists that occur in a wide range of soil pH

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Arbuscular mycorrhizal (AM) fungi play a significant role in the establishment of pioneer vegetation in acidic soil. There is increasing evidence that soil pH is a major driver for AM fungal communities, but how soil acidity acts as selection pressure against the communities has yet to be elucidated. Six field sites with different soil pH were chosen from subarctic, temperate, and subtropical Japan, and 12–15 rhizosphere soil samples of the common pioneer grass *Miscanthus sinensis* were collected from each site. Trap culture was conducted with *M. sinensis* seedlings using the soils, and community compositions of AM fungal phylotypes in the roots were determined based on fungal LSU rDNA sequences. Ordination analysis confirmed that pH is a strong driver for the communities, and AM fungal richness and diversity decreased with increases in soil acidity. A plot of AM fungal phylotypes versus pH at which they occurred indicated that those occurred at acidic pH generally occurred at neutral pH, but not vice versa. To confirm these observations, *M. sinensis* seedlings were grown on the soils at pH 3.4, 4.0, and 5.5 in the presence of the indigenous AM fungi either from a neutral or an acidic soil. Diversity of the neutral soil community decreased with increased soil acidity, whereas that

of the acidic soil community was consistent irrespective of pH. These observations suggest that soil acidity is strong selection pressure against AM fungal communities, and the fungi in acidic soil are the pH-generalists that occur in a wide range of pH.

Inter and intraspecies spatial interactions of *Rhizopogon vesiculosis* and *R. vinicolor*: sister species share space, but siblings have boundaries

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The architecture and spatial distribution of the symbionts comprising common mycorrhizal networks are critical to the understanding of belowground ecosystems. This study focused on two different ectomycorrhizal fungal (EMF) sister species, *Rhizopogon vesiculosis* and *R. vinicolor,* which are dominant in North American Douglas-fir ecosystems. We examined whether there was spatial overlap of the two sister species and whether the different genets of each species overlapped. We collected and mapped samples of *R. vesiculosis* and *R. vinicolor* in twelve 78m^2 plots in Douglas-fir stands of British Columbia over two sampling seasons. The individual genets were identified using nine microsatellite markers. The sample location was plotted and grouped into convex hull shapes using R-software with the package Vegan. We found spatial overlap of different species of *Rhizopogon*, but there was no overlap between different genets of the same species. This data will be put into context with recent findings from other EMF population studies.

Spatial structure of ectomyorrhizal fungal communities across different scales

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Understanding diversity patterns and the underlying processes is the main goal of ecology and biogeography. Research in biogeography has mostly been carried out on macroorganisms, but little is known about microorganisms. Ectomycorrhizal (EcM) fungi are one of the key microbial groups in many forest ecosystems, supplying nutrients to their host plants and contributing to nutrient cycling. We examined the species richness and community structure of EcM fungi across different spatial scales. Our results show that spatial processes play an important role in structuring

EcM fungal communities at local, regional, and global scales; the range of spatial autocorrelation in fungal communities is greater than reported previously. The range of spatial autocorrelation of communities at the local scale varies remarkably across different ecosystems, being mainly related to host distribution, disturbance, and climate. Insights into the intraspecific variations, functional traits (e.g., enzyme activity), and interactions with other soil organisms can shed more light on underlying mechanisms of spatial distribution of EcM fungi at fine scales.

Facilitation and suppression by mycorrhizal networks

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Many mycorrhizal fungi are not host specific and one fungal individual can colonize and interconnect a considerable number of plants. The existence of these so-called mycorrhizal networks implies that fungi have the potential to facilitate growth of other plants and distribute resources among plants irrespective of size, status or identity. Here we present novel data and demonstrate that the presence of mycorrhizal 'mother' plants facilitate seedling establishment of 7 other plant species in experimental dune grassland communities, while 3 plant species did not benefit and showed a neutral response. Facilitation only occurred when fungal hyphal networks were established by the 'mother' plants and when mycorrhizal plant species acted as network providers. Moreover, mycorrhizal networks are not only beneficial. In four complementary experiments, we show that a number of mycorrhizal and non-mycorrhizal plant species (mainly ruderals and weeds, including *Arabidopsis thaliana*, the model plant for molecular biology and genetics) are suppressed by the presence of mycorrhizal networks. Overall this work shows that mycorrhizal networks play a key role in plant communities by facilitating and influencing seedling establishment and by changing plant–plant interactions.

120,000 years of change: the response of arbuscular mycorrhizal fungal communities to post-glacial ecosystem development and retrogression

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Despite the important role of arbuscular mycorrhizal (AM) fungi in ecosystems, knowledge of how AM fungi respond to ecosystem development and retrogression is scarce. Here, we present the results of a study characterizing AM fungal communities across a 120,000 year-old soil chronosequence that includes all stages of ecosystem development; from the glacial forefront through primary succession, through peak biomass, and into ecosystem retrogression. This system presents strong, contrasting gradients of soil nitrogen and phosphorus, providing an ideal system to understand how ecosystem nutrient status influences mycorrhizal communities. Fifty roots were collected along two transects of 50 meters each at 10 different sites across the chronosequence. AM colonizing each root were characterized using T-RFLP, Sanger, and 454 sequencing. The AM fungal community was dominated by a few operational taxonomic units (OTUs) that matched with species from the genera Glomus and Acualospora. Presence–absence and relative abundance of OTUs suggested strong niche partitioning, with some OTUs occurring early in succession, others in peak biomass forests, and others in retrogressive stages. Additionally, AM fungal richness increased from the forefront to peak biomass stages and dropped-off sharply at the retrogression stages. Changes in soil chemistry across the chronosequence such as the decline in soil phosphorus throughout and the increase in soil nitrogen during the succession and peak biomass stages have shaped the structure of the AM fungal community. Both plant and AM fungal communities follow a similar richness pattern, pointing to soil chemistry as a critical driver of community structure during ecosystem development.

Impact of long-term soil hypoxia on arbuscular mycorrhizal fungal communities in mofette areas (natural CO_2 springs)

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Natural CO_2 springs (mofettes) are specific and extreme ecosystems, characterized by high soil CO_2 and reduced soil O_2 concentrations. This leads to a locally hypoxic environment that is similar to those found in waterlogged or flooded soils. In mofettes, geological CO_2 exhalations result in a long-term abiotic selection pressure affecting local soil biota. The quantification of arbuscular mycorrhizal (AM) fungi from plant roots sampled in the Stavešinci mofette area (Slovenia), confirmed the presence of apparently unique AM fungal assemblages across a range of soil CO_2 concentrations. We examined whether AM fungal phylotypes abundant in locations with high soil CO_2 concentrations are local specialists or widespread generalists able to tolerate a range of different soil conditions. To answer this, a high resolution metagenetic approach (454 pyrosequencing) was used to see if those phylotypes present in areas with high soil CO_2 concentrations are also found in low abundance among AM fungal communities from soils with ambient CO_2 concentrations. To get a wider overview, mofette areas in Italy and Czech Republic were also included into our study. In addition to hypoxia, we investigated other abiotic soil factors driving changes in AM fungal communities under the extreme environmental conditions found in mofette sites. Results show that some fungi are more strongly associated with local variations in the soil environment, particularly hypoxia, than with other abiotic soil characteristics or distributions of their host plants.

Biodiversity, taxonomy, conservation, and ecology of arbuscular mycorrhizal fungi: Indian scenario

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Plant-microbe interactions are the interesting events that contribute for the sustainable agriculture and forestry. Among microbial communities, mycorrhizae are symbiotic associations that form between the roots of most plants and fungi. Bidirectional movement of nutrients characterizes these symbionts where carbon flows to the fungus and inorganic nutrients get transported through mycorrhizal network to the plant. Nomenclature, classification, and systematics of AM fungi are still problematic and based on molecular approach along with morphotaxonomy, AM fungi are grouped under Glomeromycota. AM fungi are conditioned by soil factors. Acidic to neutral soils with low pH have a large number of AM fungi. It has been recorded that red sandy loam soils, soils supporting forest vegetation with low moisture and less nutrients have supported more AM fungi. AM fungal communities have also varied in relation to soil type, soil factors, and vegetation/crop type. Soil pot culture, aeroponic culture, root organ culture, and use of carrier materials are of much significance in the multiplication cum conservation of AM fungi. AM fungi are known to get disseminated inter-continentally prior to continental drift. Further, their effective dispersal is carried out through rodents, earthworms, ants, wasps, wind, water, etc. Agricultural practices are known to play important role. The present paper discusses the aspects of biodiversity, taxonomy, conservation, and ecology of AM fungi from the Indian context.

Soil solution phosphorus level as a basis for determining VAM dependency or VAM responsiveness of plant species

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Among several soil factors, soil phosphorus status is known to exert significant influence on the magnitude of dependency of plants species on VAM fungi or responsiveness of plant species to VAM fungal colonization. The determination of VAM dependency or VAM responsiveness is essential for utilization of these fungi in practical agriculture in a predictable manner. The information on VAM dependency or VAM responsiveness will enable us to match plant species to soils depending upon phosphorus requirement of plant species, soil solution phosphorus status and VAM inoculum potential of soil. Further, it will assist us to decide whether to inoculate a soil with an effective strain of VAM fungus and /or increase the soil solution phosphorus level to obtain maximum benefit from VAM symbiosis. The magnitude of VAM dependency or VAM responsiveness determined, on the basis of

established soil solution phosphorus status rather than applied phosphorus or available phosphorus level, will permit soil to soil comparisons since phosphorus adsorbing capacities of soils is taken into consideration during the development of phosphorus sorption curves.

Anti-oxidative compounds from edible ectomycorrhizal fungi and their ability to protect cells from oxidative damage

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Ectomycorrhizae are an integral part of the forest ecosystem. Establishing medicinal properties of edible ectomycorrhizae would increase their economic value. Our study is an attempt to isolate bioactive molecules with anti-oxidative properties from edible ectomycorrhizal fungi (*Lactarius deliciosus, Boletus edulis*) and test their ability to offer protection against oxidative stress associated cellular damage in *Saccharomyces* yeast. The anti-oxidative potential of ectomycorrhizal fungi was compared with *Ganoderma lucidum* (medicinal mushroom) and *Agaricus bisporus* (non-mycorrhizal mushroom). Phenols, flavonoids, and polysaccharides were the non-enzymatic antioxidants studied while superoxide dismutase (SOD), catalase, and peroxidises are the anti-oxidative enzymes studied. Based on biochemical tests, *Boletus* sp. exhibited better anti-oxidative potential, showing highest: SOD activity (1.2 units/mg protein), phenolic content (8.1mg of gallic acid equivalent (GAEs) per gm dry weight), and flavonoid concentrations (41.7 mg qeurcetin equivalent per gram of dry weight). *Saccharomyces cereviceae* were subjected to oxidative stress (0–100mM acetic acid) and cellular damage was recorded by studying DNA fragmentation, and cell viability. Pre-treatment with *Boletus* sp extracts gave enhanced cell protection. At a concentration of 17mg/ml there was a 39% increase in cell viability. The presence of catalase enzyme also added to the antioxidative activity of the mycorrhizal mycelial extracts. The DNA fragmentation assay showed protection against DNA damage in pre-treated yeast cells. The study is indicative that edible ectomycorrhizal fungal metabolites can provide protection against oxidative damage in *S.cerevisiae.* The study can be further extended by checking the protective ability of fungal metabolites against oxidative damage in mammalian cell lines.

Activation of defence in tea and mandarin plants against fungal pathogens triggered by AMF, PGPR, and PGPF

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Root colonization was found to be dominant in tea by *Glomus mosseae* and *G. fasciculatum*, while in mandarin it was *Glomus fasciculatum* and *Gigaspora gigantea*. *Bacillus pumilus* (PGPR) and *Trichoderma asperellum* (PGPF) isolated from tea and mandarin rhizosphere respectively, both showing *in vitro* antagonistic activities against *Sclerotium rolfsii*, *Fusarium solani* causing sclerotial blight of tea and root rot of mandarin respectively were selected for dual application following root inoculation with AMF. 18S rDNA sequence based molecular detection of *S. rolfsii*, *F. solani*, and *T. asperellum* while 16S rDNA sequence of *B. pumilus* were done. Besides, IgG were purified from PAbs separately raised against root pathogens, PGPF, PGPR, and AMF (*G. mosseae*, *G. fasciculatum*, and *Gi. gigantea*) and packaged into serological formats. Successful colonizations of tea and mandarin roots with AMF were confirmed by their cellular localization in root tissues following FITC labelled immunofluorescence assay. Enhanced growth of the saplings in AMF inoculated plants wase evident as compared with untreated healthy plants. Application of *G. mosseae* in tea saplings while *G. fasciculatum*, *Gi. gigantea* in mandarin saplings singly or jointly suppressed the disease markedly. However, significant disease reduction was noticed in both plants after successful colonization with AMF and following dual application of PGPR and PGPF. Induction of defence enzymes (chitinase, β, 1-3 glucanase and peroxidase) by treatment with AMF, PGPR, and PGPF in both plants with concomitant increase following inoculation separately with pathogen, was correlated with the observed resistance.

Direct and indirect influences of arbuscular mycorrhizal fungi on phosphorus uptake by two root hemiparasitic *Pedicularis* species: do the fungal partners matter?

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Because most parasitic plants do not form mycorrhizal associations, nutritional roles of AM fungi in them have never been tested. High AM colonization in wild individuals of root hemiparasitic *Pedicularis* species makes this genus ideal for testing both direct and indirect effects of AM fungi on nutrient acquisition. We used 32P labelling in compartmented pots to test direct and indirect influence of AM inoculation on P uptake by two *Pedicularis* species, *P. rex* and *P. tricolor*. Contributions of the AM pathway via *Glomus*

intraradices and *G. mosseae* were tested in the absence of host plant for *Pedicularis*. The influence of AM inoculation on P uptake from a host plant into the root hemiparasites was tested with barley (*Hordeum vulgare*) as host plant and *Glomus intraradices* as AM fungus. The results showed that AM colonization levels were very low in both *Pedicularis* species, despite heavy colonization in barley roots. Contribution of the AM pathway via external hyphae to P acquisition by both *Pedicularis* species was also very low (generally less than 1% of total P uptake). However, a significant increase in shoot P content was observed in the *G. mosseae-P. rex* symbiosis. For both *Pedicularis* species, inoculation with *G. intraradices* strongly suppressed haustorium formation and hence host-derived P. Roles of AM fungi in nutrient acquisition by root parasitic plants were quantitatively demonstrated for the first time. The ecological significance of the interference by AM fungi on nutrient acquisition from hosts by root hemiparasites needs to be tested under field conditions.

Pre-symbiotic interactions between *Terfezia boudieri* and *Helianthemum sessiliflorum* reveal a novel role for auxin: redirection of roots growth by intervention in gravitropism

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The desert truffle *Terfezia boudieri* establishes mycorrhizae with *Helianthemum sessiliflorum* (Cistaceae) during the short wet season in desert habitats. Establishment of this association could be problematic due to low numbers of penetration sites available in the main roots. Moreover, the host root growth rate is ten-fold higher than the mycelia and since mycelia are present only in the upper soil layers, the fast growing root tip may readily cross the mycelia layers evading inoculation. We report here on mechanisms that the fungus has acquired to assure efficient inoculation. The first mechanism is host sensing by chemoattraction. We developed a bioassay to test chemoattraction and have partially characterized the active molecule. A second mechanism is secretion of the plant hormone indole-3-acetic acid (IAA) by the fungus. Secreted fungal IAA induces proliferation of lateral roots and inhibits root growth resulting in higher number of penetration sites and coordinated growth rates between partners. We also show, in *Arabidopsis*, that roots respond to the secreted IAA prior to the physical contact, as evidenced by analyses using the DR5-GUS, IAA sensitive promoter. The third novel mechanism is IAA interference with the gravitropic response of the root. In dual cultures, roots deviate from the gravity axes in response to the secreted IAA. In view of the above findings, we propose that fungal IAA redirect root growth retaining the penetration sites in roots in the upper soil layers colonized by the fungus.

Secretion of thermostable proteins is induced in ectomycorrhizal fungi and inoculation pine by excessive copper and cadmium

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Heavy metal pollution in soils is a worldwide environmental problem, and it causes ecosystems to deteriorate. Cadmium and copper are studied because of their severe pollution status in the soil of China and their impairment of human health. This research tested the hypothesis that several thermostable proteins also exist in the exudates of ectomycorrhizal fungi (ECMF) mycelia and inoculation pine under heavy metal stress. *Xerocomus chrysenteron* is a typical ectomycorrhizal fungus. The proteins secreted by *X. chrysenteron* were characterized in pure and cadmium or copper-containing media. The proteins were different from glomalin which is a thermostable hydrophobic glycoprotein with strong sequestration of heavy metals and is produced by arbuscular mycorrhizal fungi. The results showed that the thermostable proteins contained a protein of molecular weight 31 kDa. Excessive cadmium and copper induced an ectomycorrhizal fungus and inoculation pine to produce several thermostable proteins. After 4 weeks of cultivation, proteins secreted by ectomycorrhizal fungi and inoculation pine are gi | 113307, gi | 4501883 and gi | 288300146. The proteins contain two main amino acid sequences 'K.SYELPDGQVITIGNER.F' and 'K.QEYDESGPSIVHR.K' and their unique amino acid sequence is 'K.QEYDESGPSIVHR.K'. Proteins secreted by ectomycorrhizal fungi and inoculation pine are gi | 113307 andgi | 288300146 under copper 50 ppm. The proteins contain two main amino acid sequences 'K.SYELPDGQVITIGNER.F' and 'K.QEYDESGPSIVHR.K' and their unique amino acid sequence is 'K.QEYDESGPSIVHR.K'. Proteins secreted by ectomycorrhizal fungi and inoculation pine are gi | 115386236, gi | 116198005 and gi | 288300146 under cadmium3 ppm and 0.75 ppm. The proteins contain two main amino acid sequences 'K.SYELPDGQVITIGNER.F' and 'K.QEYDESGPSIVHR.K' and their unique amino acid sequence is 'K.QEYDESGPSIVHR.K'. This is the first evidence that ECM fungi and inoculation pine can exude a thermostable protein homologous to actin, and the first evidence that Cd and Cu induced an ECM fungi and inoculation pine to producing thermostable proteins which may be an important factor in improving the resistance ECM to heavy metals.

Functional analysis of a phosphate transporter gene GigmPT in arbuscular mycorrhizal fungus *Gigaspora margarita*

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The majority terrestrial plants are able to form symbiotic associations with arbuscular mycorrhizal fungi. The symbiosis, termed arbuscular mycorrhiza, is a reciprocal symbiosis, improving plant uptake of phosphate. The first step of the fungus-mediated uptake is carried out by fungal membrane Pi transporters (PT) that transfer Pi from the soil into the extraradical hyphae. Here we report the cloning and the functional analysis of a gene encoding a phosphate transporter (GigmPT) from the arbuscular mycorrhizal fungus *Gigaspora margarita* during mycorrhizal association with *Astragalua sinicus* roots. The GigmPT polypeptide belongs to the major facilitator superfamily (MFS). Homology modeling reveals that GigmPT exhibits twelve transmembrane helices divided into two halves connected by a large hydrophilic loop in the middle. These analyses show that GigmPT expression is regulated in response to external Pi concentrations. Phosphate concentrations, typical of those found in the soil solution, result in expression of GigmPT. The function of the GigmPT was confirmed by complementation of a yeast phosphate transporter mutant. The kinetic analysis of GigmPT reveals that it belongs to high affinity phosphate transporter family (Pht1). Expression of GigmPT was localized to the arbuscule and external hyphae of *G. margarita* colonized with A. sinicus roots which suggest that external hyphae are the initial site of phosphate uptake from the soil. These data imply that GigmPT is a high-affinity PT involved in the phosphate acquisition and transportation and in turn *G. margarita* helping in improvements of the nutritional status of the host plant.

On the evolutionary meta-stability of mixotrophy in orchids

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Mycoheterotrophs exploiting carbon from their mycorrhizal fungi arose repeatedly in plant evolution. Intermediate evolutionarily steps exist where the plant is green and photosynthetic, but uses carbon from its fungal associates. The so-called mixotrophy or partial mycoheterotrophy is at least described for temperate orchids and Monotropoideae. In some mixotrophic orchids, the rare survival of achlorophyllous plants (albinos) further supports their use of fungal carbon. To understand the reasons for their rarity, we compared albinos with co-occurring green individuals of the mixotrophic, perennial orchid *Cephalanthera damasonium*. We investigated vegetative traits, namely shoot phenology, dormancy, CO_2 and H_2O exchanges, mycorrhizal colonization, degree of heterotrophy (using ^{13}C abundance as a proxy) and susceptibility to pathogens and herbivores. We monitored seed production and germination. Albinos displayed (i) more frequent shoot drying at fruiting, possibly due to stomatal dysfunctions; (ii) lower basal metabolism; (iii) increased sensitivity to pathogens and herbivores; (iv) higher dormancy and maladapted sprouting; and probably due to the previous differences; (v) fewer seeds, with lower germination. Over the shoot growing season, green shoots shifted from using fungal carbon to an increasingly efficient photosynthesis at fruiting, when fungal colonization reached its minimum. Conversely, achlorophylly in fruiting albinos may contribute to carbon limitation, explaining the above-mentioned trends. With a 10^3x fitness reduction, albinos failed a successful transition to mycoheterotrophy because of maladaptive traits inherited from their recent green ancestors. *A contrario*, successful transition to mycoheterotrophy requires joint evolution of several traits. The required evolution of multiple loci likely explains the evolutionary meta-stability of mixotrophy.

Is it a ghost or a thief? Evidence for nitrogen theft in the epiphytic orchid *Dendrophylax lindenii*

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Dendrophylax lindenii, or the ghost orchid, is an enigmatic, endangered leafless epiphytic orchid found in southern Florida and the Caribbean. As with all orchids, *D. lindenii* likely relies upon mycorrhizal fungi for nutrient acquisition. However, nothing is known concerning its mycorrhizal associations. Furthermore, no leafless epiphytic species have been studied using molecular or isotopic methods, to

our knowledge. We characterized the stable carbon and nitrogen isotopic composition of *D. lindenii* samples from two sites to investigate nutrient dynamics. We also characterized the mycorrhizal status of these orchids using microscopy, DNA sequencing and phylogenetic analysis. Other co-occurring orchid species, non-orchid epiphytes, host bark and host leaves were collected in order to reveal patterns of nutrient uptake across the entire epiphyte community. *D. lindenii* displayed 13C patterns characteristic of C4/CAM photosynthesis, with no evidence for carbon mixotrophy. Most epiphytes had depleted 15N values consistent with uptake following atmospheric deposition. However, orchids were the least depleted, and 15N of *D. lindenii* was close to zero at one site and above zero in the second site. The N signature of *D. lindenii* closely matched that of host tree leaves and bark, leading us to speculate that this orchid may be 'stealing' nitrogen from host trees via mycorrhizal fungi. Molecular analyses reveal that *D. lindenii* is specialized toward a narrow, previously unknown clade in the Ceratobasidiaceae. Other co-occurring orchids displayed less specificity and were only rarely associated with the same fungi as *D. lindenii*.

Shifts in mycorrhizal fungi during the evolution of autotrophy to mycoheterotrophy in *Cymbidium* (Orchidaceae)

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Mycoheterotrophic plants, which completely depend upon mycorrhizal fungi for their nutrient supply, show unusual associations with fungal partners. The processes involved in shifting fungal associations during cladogenesis of plant partners from autotrophy to mycoheterotrophy have not been demonstrated based on a robust phylogenetic framework. Consequences of mycorrhizal shift were examined in *Cymbidium* (Orchidaceae) using achlorophyllous and sister chlorophyllous species. Fungal associates of the two achlorophyllous mycoheterotrophs (*C. macrorhizon* and *C. aberrans*), their close relatives, the chlorophyllous mixotrophs (*C. goeringii* and *C. lancifolium*) and an outgroup, the chlorophyllous autotroph *C. dayanum*, were identified by internal transcribed spacers of the nuclear ribosomal DNA sequences. Molecular identification of mycorrhizal fungi revealed: (1) the outgroup autotroph is predominantly dependent on saprobic Tulasnellaceae; (2) the mixotrophs associate with the Tulasnellaceae and ectomycorrhizal groups including the Sebacinales, Russulaceae, Thelephoraceae, and Clavulinaceae; and (3) the two mycoheterotrophs are mostly specialized with ectomycorrhizal Sebacinales. Fungal partners in *Cymbidium* have shifted from saprobic to ectomycorrhizal fungi via a phase of co-existence of both nutritional types of fungi. These three phases correspond to the evolution from autotrophy to mycoheterotrophy via mixotrophy in *Cymbidium*. We demonstrate that shifts in mycorrhizal fungi correlate with the evolution of nutritional modes in plants. Furthermore, gradual shifts in fungal partners through a phase of co-existence of different types of mycobionts may play a crucial role in the evolution of mycoheterotrophic plants.

Endophytic and mycorrhizal communities associated with roots of endangered native orchids from the Atlantic forest

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In this study, we characterized the fungal communities associated with three tropical orchid species, *Hadrolaelia jongheana, Hoffmannseggella caulescens*, and *Hoffmannseggella cinnabarina* found in different vegetation formations of the Atlantic Forest. All three species are included in the Brazilian Flora Species Threatened List. The composition and diversity of fungal communities were determined by constructing clone libraries and by applying diversity and richness indices. The influence of ecological aspects was also evaluated. Our results demonstrated the presence of Basidiomycetes and Ascomycetes fungi associated with the roots of these plants. Among the Basidiomycetes found, Sebacinales (81.61%) and Cantharellales (12.10%) were the dominant orders. The Ascomycetes included the Helotiales (29.31%), Capnodiales (18.10%), and Sordariales (10.34%), among others. A Shannon-Wiener diversity index (H`) analysis showed higher fungal community diversity associated with *H. caulescens* and *H. cinnabarina* roots in ferruginous formations, which differed from the H` values of *H. cinnabarina* from granitic inselbergs, suggesting that local factors influence this diversity. The epiphytic *H. jongheana* orchid also showed high fungal community diversity. The Simpson diversity index values showed that all of the libraries presented dominant species, and LIBSHUFF analysis showed that the fungal communities were structurally different from each other. We can conclude that the three orchid species studied present a high diversity of fungal communities. This study generates important information for the development of conservation strategies for threatened and endemic Brazilian flora species in an important and threatened hotspot.

How do plants parasitise fungi? Resolving the biochemical basis for mycoheterotrophy

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The seeds of 10 percent of plant species, including orchids, are so small that seedling development requires carbon and nutrients to be supplied by a fungal partner, but the mechanisms of this mycoheterotrophic nutrition are unknown. We investigated the effect on carbon and nitrogen isotope fractionation in two orchid species, of the switch from mycoheterotrophy in seedlings to autotrophy in green-leaved adults. Juveniles of both species had unprecedented 15N enrichments, extreme tissue nitrogen concentrations, and enhanced 13C abundance, but these were lost in the autotrophic adults. Cellular-scale mapping of metabolites in seedlings by imaging MALDI-MS provides evidence of a novel 15N concentrating mechanism in heterotrophic orchid seedlings based on their utilisation of fungal glutamine as a carbon source, further supported by laboratory growth experiments. The findings shed light on two longstanding questions: why many mycoheterotrophic plants are strongly enriched in 15N, and which biochemical pathways are exploited by these plants to obtain carbon from their fungal partners.

Mycorrhiza on green roofs: spontaneous establisment of native orchids on roofs in Switzerland

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In 2009, data were collected from 31 orchid roofs in Switzerland. On these roofs, a total of 11 different species of orchid were found, all of them on the RED LIST of endangered species in Switzerland. On some roofs, the abundance attained up to 30 000 flowering individuals. All these orchids established spontaneous and all of them are obligatory dependent on mycorrhiza fungi. Since the completion of the building, thus the green roof, it took more than 10 years and longer to recognize first blooming individuals of the Family Orchidaceae. Deducing these results and noticing the facts that the orchid seeds can cover hundreds of kilometres and that one single orchid produces thousands of seeds, the roofs, inaccessible for humans, represent ideal conditions for the protection of species. The

disappeared and half dry meadows can be imitated by inoculation and settlement of orchids on the roofs. The aim should be enhancing species richness and protection of flora and fauna.

Mutualism and parasitism between *Allium fistulosum* and arbuscular mycorrhizal fungi at different soil phosphate availability

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Changes in growth of plants with arbuscular mycorrhizal (AM) colonization differ among cultivars and soil phosphorus (P) levels. Purpose of the present study was to clarify dependency of different cultivars of *Allium fistulosum* at different soil P levels. Twenty cultivars of *A. fistulosum* were grown in pot with or without (control) AM fungus Glomus R-10. Mycorrhizal dependent and independent cultivars were selected and were grown in soils with four (307, 644, 1654, and 3339 mg P_2O_5/kg) phosphate concentrations under glasshouse condition and field condition. AM colonization, root length, shoot dry weight, and shoot P concentration were determined. All cultivars were well colonized with Glomus R-10. Shoot P uptake and shoot dry weight increased with the AM colonization. Mycorrhizal dependency (MD) was different among cultivars. Three types of cultivars with different MD — Ohtone, Motoharubansei and Mogamigawa (high), Kujoufuto and Koshizu (middle), Kannon hosonegi and Shounan (low) — were screened. Shoot dry weight of inoculated mycorrhizal dependent cultivars were higher than control plants at all P levels. Shoot dry weight of inoculated independent cultivars were higher than control plants only at low P levels and lower at high P levels. Shoot dry weight of inoculated mycorrhizal dependent cultivars were also higher than control plants under field conditions. These results suggest that selection of highly AM dependent cultivars is important to improve plant growth and to reduce P fertilizer application with inoculation of AM fungi.

Cellular acquisition and allocation of carbon and nitrogen in orchid symbiotic protocorm unveiled by isotope microscopy

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Members of the Orchidaceae establish a unique symbiosis with fungi during seed germination and seedling development. The growing embryo (protocorm) relies on a fungal symbiont for nutrients and is therefore an example of a mycoheterotroph. This system is an excellent model to study allocation of elements during the establishment of the symbiosis. Isotope cellular imaging based on secondary ion mass spectroscopy (SIMS) is, to date, only a methodology to distinguish added elements as nutrients from those of original cell components using stable isotopes. Using the isotope microscope, here we show for the first time, cellular allocation of C and N during the establishment of symbiosis between an orchid species and a fungal symbiont by tracking isotopes labelled in compounds that were added only to fungal mycelium external to developing protocorms. The major findings of fungal-host transfer were a direct transfer of C and N from live hyphae, and significant inflows of the elements within hyphae accompanied with fungal senescence. The fungal origin of C and N was then primarily allocated towards the protocorm meristem region where neighboring uncolonized parenchyma cells functioned as storage. Elevation patterns of 13C/12C in host cell wall regions and amyloplasts showed that sugar export/import status of host cell was switched reversibly triggered by fungal colonization/senescence. The new methodology revealed cell–cell communication of the symbiosis and new insights into dynamic allocation events of elements in cells.

Plant growth response to mycorrhizal fungi is stronger under extreme abiotic conditions

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Plant growth response to colonization by arbuscular mycorrhizal (AM) fungi can range from positive to negative. However, most studies that evaluate plant growth response do not consider the environmental context that the plants are growing in. Here, I report on an experiment where seedlings of three different plant species were grown in the presence/absence of AM fungi along three environmental gradients (soil nutrient content, soil pH, and soil moisture). In all three gradients, I found that plants were able to survive and grow at the extreme ends of the gradients when colonized by mycorrhizal fungi, but not when the fungi were absent. At optimal conditions, however, the plants either did not respond to mycorrhizal fungi or showed signs of growth depression. This data indicates that the major benefit of the symbiosis to plants is that they broaden their niche breadth, and are thus more generalists in their interactions with their abiotic environment. These results will be discussed in context of ecological stability and the maintenance of species diversity in a fluctuating environment.

Bio-reforestation: mycorrhizal fungi promoted plant growth in degraded tropical peat swamp forests and post mining lands

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Degraded tropical peat swamp forests (TPSF) and post mining lands (PML) reforestation activities are underway but large-scale, cost-effective, and transferable methods to support reforestation programmes are urgently needed. Low-cost forest management using highly effective mycorrhizas to promote seedling and tree growth is an important issue in reforestation programmes. Some mycorrhizal fungi have been proven efficient for improving growth of TPSF and PML plant species in nursery and field scale. The research revealed that local mycorrhizal fungi improved plant growth and increase plant vigor under extreme growth conditions, such as low pH, poor aeration, and deficient nutrition. Limitedness of planting materials of TPSF and PML native tree species had become one of the major obstacles in setting up experiment using these species. Availability of these planting materials has become scarce due to difficulties of finding mother trees in their natural habitat and

disturbance on these habitats. Appropriate strategy is, therefore, required to manage TPSF and PML including preparation of highly qualified seedlings and this can be achieved through interference of comprehensive technology. Mycorrhizal inoculation technology required for mass production of highly qualified seedlings include selected mycorrhizal fungi, peat and compost media, shoot cuttings, charcoal, and slow release fertilizer. Further management of planting and maintenance activities is also crucial in order to help the saplings to grow well in the field. Bio-reforestation is a significant effort to manage carbon and sustain environment for global benefits.

Superiority of *Glomus intraradices* in enhancing proline biosynthesis and imparting tolerance to pigeonpea plants under salt stress

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Soil salinity is an increasing problem worldwide, particularly in arid areas, but research findings show that AM fungi have the potential to ameliorate effects of salinity. However, plant-growth responses to AMF are found to vary with the host plant, endophyte, and soil. Proline is an important osmoprotectant accumulated as a result of enhanced biosynthesis and repressed degradation under stress. Pigeonpea (*Cajanus cajan*) is a widely grown pulse-crop in India but little information regarding its salt tolerance is available. Aim of the present study was to investigate differential responsiveness of AMF species (Indigenous mycorrhiza, *Glomus mosseae, Glomus intraradices, G.mosseae+G. intraradices*) on growth and proline metabolism in two pigeonpea genotypes (Paras, Pusa 2002) under salt stress (0, 40, 60, 80 mM NaCl). Investigations revealed that while salinity reduced plant biomass in both genotypes, mycorrhiza enhanced growth in all inoculations. Pusa 2002 was more salt tolerant and ensured higher stress protection through increased proline synthesis compared to Paras. Mycorrhizal symbiosis boosted proline biosynthesis by significantly increasing pyrroline-5-carboxlate synthetase (P-5-CS) and glutamate dehydrogenase (GDH) activities with a concomitant decline in proline dehydrogenase (ProDH) activity. This indicated a strong role of AM symbiosis in enhancing stress tolerance in pigeonpea by significantly modulating proline metabolism. In our study, *G. intraradices* was the most efficient AM fungus compared with the other two fungi in terms of root colonization, plant growth, and enzyme activity under salt stress. At 80mM NaCl, maximum colonization was shown by *G.intraradices*, indicating its insensitivity to saline environment and its potential use in agriculture.

Effects of arsenic and arbuscular mycorrhizal fungi on the fern *Pteris vittata*: from proteome analyses and molecular characterization to field experiments

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Arsenic (As) is a dangerous soil and water contaminant, seriously threatening human health. It is also phytotoxic, often resulting in plant death. The fern *Pteris vittata* can tolerate and rapidly accumulate As in its fronds, even at high concentrations. Arbuscular Mycorrhizal Fungi (AMF) are known to induce tolerance to various stresses, but little information exists on the effects of AMF on As-hyperaccumulating plants. We investigated the morphological, biochemical, and molecular effects of As treatment and AMF inoculation on *P. vittata* grown under hydroponic conditions. Particular attention was paid to the modulations induced in the *P. vittata* leaf and root proteome, and to the localization and expression of two arsenate reductases. Symptoms of As stress, concerning photosynthesis, carbon metabolism, and cellular homeostasis were recorded, and a protective effect of the AMF was shown. In both leaves and roots of mycorrhizal plants, the over-expression of glycolytic enzymes indicated their possible involvement in arsenate reduction. In the fronds, the up-regulation of Met synthase and AdoMet synthetase could correlate with As(III) methylation. In the roots, the over-expression of porins suggests their involvement in arsenite sequestration into vacuoles, as the last detoxification step in both mycorrhizal and non mycorrhizal ferns. The presence of arsenate reductase was shown at the transcript and protein level in roots and shoots. Finally, a field experiment was performed in a site highly polluted by arsenic and by a number of heavy metals: *P. vittata* plants survived and accumulated large arsenic concentrations. The results on this 'case story' will be presented.

Arbuscular mycorrhizae for green and sustainable remediation of polluted soils: a feedback from laboratory to field-scale experiments

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To restore polluted soils, phytoremediation was found to be a feasible approach for *in situ* clean up of surface soils. It offers the great advantage of being inexpensive, environmentally friendly, and not altering the soil matrix. However, phytoremediation efficiency might firmly rely on the settlement of appropriate vegetation which closely depends on the pollutant tolerance of plants. It is within this context that arbuscular mycorrhizal symbiosis represents a challenge for sustainable development. Our findings showed that this issue is based not only on the exploitation of its potential to remove pollutants (Verdin et al., 2006) but also on its ability to protect plants against pollution (Debiane et al., 2008, 2009, 2011, 2012). The relevance of arbuscular mycorrhizae in phytoremediation of polluted soils by different harmful pollutants (polycyclic aromatic hydrocarbons (PAHs), Dioxins/furans and metallic trace element (MTE)) will be discussed thanks to a feedback from laboratory to field scale experiments. The benefits of arbuscular mycorrhizal inoculation (by *Glomus irregulare* or by a commercial inoculum) in many plant species (*Chicorium intybus* L, *Miscanthus giganteus*, *Trifolium repens* L, *Lolium perenne* L and *Medicago sativa* L) on the growth, the tolerance and the removal of PAHs, dioxins/furans and MTE were studied. In addition, our data contribute, at the mechanistic level, to a better understanding of the mycorrhizal colonization role in plant protection against the pollutant toxicity. They demonstrated that the mycorrhizal plant tolerance resulted from oxidative stress alleviation and lipid regulation which lead to the limitation of membrane and genome damages.

Mycorrhizal fungi as modulators of secondary metabolite and antioxidant production under extreme conditions

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The effect of inoculation was studied on model plants: *Allium cepa* and *Cichorium intybus* which are widely cultivated for their high content of anioxidative substances and commercial applications in pharmacology and food industry. Mycorrhizal (*Glomus intraradices* or *G. mosseae*) and non-mycorrhizal plants were cultivated in commercial non-polluted and heavy metal-rich substrata. Differences in plant reaction depended on plant cultivar and fungal strain. The greatest differences between mycorrhizal and nonmycorrhizal plants were observed in quercetine, kaempferol, 3,5-DCQA, and Crepidiaside B. AM fungi have repeatedly been demonstrated to alleviate heavy-metal stress on plants. In presence of arbuscular mycorrhiza new antioxidant homeostasis is established. In chicory, decrease of SOD activity and increase of catalase and peroxidase activity were observed indicating reduction of superoxide production. There were, however, less differences in concentration of medically important compounds in chicory on polluted soil between mycorhizal and nonmycorrhizal roots. Although, mycorrhizae formation is an important biotechnological tool in plant cultivation, the use of some strains can decrease concentration of some compounds of therapeutic value.

Rapid colonization of soils and mining wastes by roots and mycorrhizas in landforms restored after mining

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The abilities of roots and their mycorrhizal symbionts to colonize often unfavourable materials are critical to successful ecosystem restoration. In this study, we examined the capacities of tree and grass roots, and there mycorrhizal partners, to colonize a range of soils and hostile waste materials following restoration of landforms constructed after mining. Remarkably, few major barriers to root penetration were apparent in any of the minesoils examined and roots had colonized a range of materials including some substrates putatively hostile to plant growth. Furthermore, significant mycorrhizal activity was measured in all landforms assessed, despite their lack of inoculation with ecto- or arbuscular mycorrhizal propagules. Arbuscular mycorrhizal spore concentrations were remarkably high in some surface soils but declined markedly with depth in the profile. The consistent colonization by roots and mycorrhizas of an eclectic range of post-mining soils and wastes demonstrated the innate capacity of (at least) the gross components of the plant–soil system to develop rapidly and extensively in favourable and unfavourable post-mining substrates. Of particular interest was the comprehensive and relatively-rapid natural colonization of AM fungi in the biologically sterile, and sometimes chemically-hostile mine wastes.

Mycorrhiza-assisted reclamation of abandoned ash pond and its carbon sequestration potential

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Coal contributes to about 40% of electricity generation in the world and India is strongly dependent on coal for electricity generation (about 200 billion tonnes of reserves in India). This has created around 26,300 hectares of fly ash dumps from some 70 thermal power plants in India. The Energy and Resources Institute (TERI) has successfully reclaimed abandoned ash ponds of coal-based thermal power plants using selected mycorrhiza and plant species. This has lowered the risk of contamination to the surrounding environment. It has innumerable advantages such as prevention of dust emission, checking soil erosion, stabilizing the surface areas of ash, preventing potential ground water contamination, and finally, adding native vegetation cover, which is very vital in the long term. In the present study, one abandoned ash pond at Korba, located in India, which was reclaimed (in 2000) using mycorrhiza was compared with the non-reclaimed ash pond. The reclaimed site constituted 30 acres. Both reclaimed and non-reclaimed ponds were studied for their nutritional status, microbial population using DGGE, microbial activities, FAME profiling, mycorrhizal and fungal diversity using partial sequences of rDNA and carbon sequestration potential using Modified Comprehensive Mitigation Assessment Process (PROCOMAP) model. Establishment of different plant species not only brought about an aesthetical green look to barren grey lands, it also resulted in six-fold increase in organic carbon, four-fold increase in available nitrogen, and about three-fold increase in microbial biomass in reclaimed ash. Bacterial, fungal, and mycorrhizal DNA was detected in the reclaimed ash ponds with a variation in the FAME profile as compared to the control. In the mycorrhiza-reclaimed fly ash overburdens of Korba, the highest carbon storage was observed in Melia (595tC/ha), followed by Eucalyptus (286tC/ha), Gmelina (168 tC/ha) and lowest in Albizzia (116tC/ha). Carbon stock was found to stabilize after 50–60 years in regeneration model. Mitigation scenario with wood products was always found to be higher than the mitigation scenario without wood products.

Does resource partitioning influence community assembly by ectomycorrhizal fungi in clearcuts and forests?

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When conifer seedlings re-establish in clearcuts after harvest, they are colonized by a different group of ectomycorrhizal fungi (EMF) than seedlings in nearby forests. One hypothesis to explain this observation is that altered soil abiotic properties in clearcuts interact with unique EMF physiologies, thereby affecting the host-fungal or fungal–fungal interactions that determine which fungi are successful colonizers. This presentation will summarize two studies that tested for physiological differences between clearcut-dominant and forest-dominant EMF in subalpine forests. One study found that *Picea engelmannii* seedlings planted into clearcuts differed from those planted into forests, both in EMF symbionts, and in activities of four exoenzymes at the mycorrhizoplane. Surprisingly, however, within both forests and clearcuts, neither EMF communities, nor their enzyme activities partitioned by microsite type (decayed wood vs mineral soil), even though soil chemical properties differed substantially. In a second study, one-year-old, naturally regenerating *Abies lasiocarpa* seedlings were reciprocally transplanted between and within clearcuts and adjacent forests. Activities of four extracellular enzymes were higher for ectomycorrhizas of seedlings transplanted into forests, regardless of whether they originated in clearcuts or forests. This suggests a high degree of phenotypic plasticity with respect to exoenzyme activity in both forest and clearcut EMF. For four enzymes, activities were higher for seedlings transplanted from clearcuts to forests than for other seedlings. Taken together, these two studies suggest that physiological differences associated with resource partitioning are not structuring EMF communities on young seedlings in subalpine forests and clearcuts.

Can agricultural practices affect AM fungal population and diversity?

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Modern agricultural practices to enhance food production to meet the needs of increasing human population are posing problems to AM fungi. Agricultural practices like application of fertilizers, herbicides, pesticides, and cropping systems affect these fungi. Monocropping with a particular crop results in the development of a predominant AM fungus in soil. Mixed cropping stimulates the proliferation of AM fungi, compared with monocropping. Cultivating a non-mycorrhizal host or leaving the land fallow will reduce the propagules of AM fungi in soil. Solarization of soil reduces the AM fungal population. Alley cropping also influences AM fungal population depending on the tree and annual crop involved. In tropical soils, application of organic matter either in the form of compost or organic amendments stimulates proliferation of AM fungi. Application of heavy doses of fertilizers, especially phosphorus, have a negative effect on AM population. Most pesticides inhibit colonization and development of AM fungi in plants, though some have no effect and a few even increase mycorrhizal colonization. Diversity of AM fungi decrease as the land-use intensity increases. Minimum tillage and non-weeding enhance AM fungal population than intensive tillage. Modern intensive agricultural practices are evidently a threat for AM fungi. Studies related to the effect of agricultural practices on AM fungi give an idea of managing these fungi for improving crop productivity.

Arbuscular mycorrhizal fungi for reforestation in south Ecuador

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Arbuscular mycorrhiza (AM) is formed by approximately 80 percent of all land plants with AM fungi (AMF), which deliver nutrients (e.g., nitrogen and phosphor) and water to the plant and receive carbohydrates in exchange. Regarding trees, this symbiosis is very widespread especially in the tropics; former studies in our research area in South Ecuador showed that >98% of the investigated tree species are associated with AMF. Our approach was to improve reforestation attempts with native tropical trees by applying native AMF inoculum in the tree nursery. A former nursery experiment had shown improved growth performance of tree seedlings when an undefined mycorrhizal root inoculum was applied (Urgiles et al., 2009). We now aimed to apply defined AMF isolates to find out which AMF persist in and have positive impact on native Ecuadorian tree species. Native Ecuadorian AMF were isolated and characterized on the morphological and molecular level (SSU-ITS-LSUpartial rDNA region, Krueger et al., 2012), produced individually and then mixed and applied as a 'cocktail' to inoculate three native tree species in an Ecuadorian tree nursery. To address the question whether inoculation improved performance of the seedlings, plant growth was measured and AMF were detected in roots through the nursery and field phase by 454 GS-FLX titanium sequencing of the LSU rRNA gene D2 region. We studied, which of the applied AMF persisted in roots and which non-inoculated native AMF colonized the roots after out-planting in the field. This provides information to improve AMF inoculum for more efficient future reforestation attempts.

Agricultural management practices affect ecosystem services and plant productivity by changing arbuscular mycorrhizal fungal communities

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It is now well established that agricultural practices alter the composition and diversity of arbuscular mycorrhizal (AM) fungal communities. However, the impact of changing AM fungal communities on the functioning of the agro-ecosystems is still poorly understood. In earlier work we observed that soil tillage drastically altered AM fungal community composition. Here we tested, using experimental grassland as model system, whether AM fungal communities from tilled and non-tilled soils, differently influence plant productivity, nutrient acquisition, and nutrient leaching after simulated rain. AM fungal communities from tilled and non-tilled soils varied greatly in their effects on the grassland communities. AM fungal communities from tilled soil tended to increase overall biomass production compared to AM fungi from no-till soil. This effect was mainly due to a significant growth promotion of *Trifolium* by tillage-AM fungi. In contrast, *Plantago* biomass was higher with non-till AM fungi, while *Lolium* was not affected by different AM fungal communities. Moreover, AM fungal communities from tilled soil reduced nitrate, ammonia, and phosphate leaching losses from the grassland communities compared to the no-till communities. Differences in hyphal length, arbuscular colonization and AM fungal community composition are the most likely explanations for the differences between tillage and no-tillage AM fungal communities. The obtained results demonstrate that agricultural management practices affect ecosystem services and plant community structure by changing the belowground microbial community.

Root-associated fungi in a changing climate: impact of elevated CO_2, drought, and warming

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Arbuscular mycorrhiza, ericoid mycorrhiza, dark septate endophytes, and fine endophytes may play an important role in plant nutrient uptake, soil organic matter turnover, and carbon cycling. Global change factors could possibly affect abundance of and community composition among root-associated fungal endophytes thereby altering ecosystem functioning. This project aims to investigate effects of elevated CO_2, drought, and warming and all interactions in the heathland ecosystem experiment CLIMAITE dominated by *Deschampsia flexuosa* and *Calluna vulgaris*. By microscopy we detected significantly increased colonization of *D. flexuosa* by arbuscular mycorrhiza in response to elevated CO_2 after 5 years of treatment and in summer and autumn after 6 years. Dark septate endophytes responded positively to warming and negatively to drought. In *C. vulgaris* dark septate endophytes responded negatively to warming and ericoid mycorrhiza responded with a significant three-way interaction. Hence, future climate may alter root associated fungal abundance. Currently, we are analysing the seasonal variation in fungal colonization of the roots by microscopy and fatty acid analysis, and investigate effects of the climate change treatments on fungal community composition by pyrosequencing. As arbuscular mycorrhiza may increase soil aggregation, soil structure and protein (glomalin) content are investigated. Finally, the functional role of fungal isolates on *C. vulgaris* nutrient uptake will be assessed by a microcosm experiment. Together these studies will improve our knowledge on the influence of root endophytes on dominant heathland plants in NW Europe, and on the effects of climate change on the abundance of and the community composition among root-associated fungi.

Impacts of above-ground grazing on ectomycorrhizal communities associated with birch trees are minimized by mycelial networks linking grazed and ungrazed trees

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Changes in carbon allocation below-ground by ectomycorrhizal (ECM) host plants have been associated with changes in the composition of the ECM fungi on the roots. Growing plants in elevated carbon dioxide levels can result in fungi which produce large mycelial structures becoming more prevalent on the root systems. Conversely, above-ground grazing or clipping of foliage has been shown to reduce the abundance of the same fungi. These community shifts have been attributed to increases and reductions in C availability, respectively. However, clipping experiments are usually carried out using isolated hosts or at a plot scale with all hosts receiving the same treatment. This will rarely mimic field conditions because grazing or browsing is patchy which can, where plant density is high, result in grazed and ungrazed plants growing in close proximity. We used pyrosequencing to determine the ECM community associated with birch (*Betula* sp.) saplings growing in a dense naturally regenerating stand, which had been subjected to different simulated browsing treatments for 10 years. Surprisingly, we found no significant treatment effects on the ECM fungal community composition even though some of the clipping treatments resulted in severe growth reductions, even death, of the birch trees. We propose that the most plausible explanation for this finding is that ECM mycelial networks connecting clipped and unclipped birch trees supported the C demands of the ECM fungi associated with the clipped trees thereby reducing the impact of the clipping treatments.

Native arbuscular mycorrhizal fungi as prospective biotechnological tool for sustainable agriculture and conservation in the Arabian Peninsula

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Agriculture in the Arabian Peninsula has to cope with drought, heat, soil salinity, and low soil fertility, particularly due to low phosphorus (P) availability. Under such environmental conditions, the beneficial symbiosis between plants and arbuscular mycorrhizal fungi (AMF) is a key factor to promote sustainable agriculture: AMF play a crucial role in the P acquisition by plants and for soil aggregate stability. Furthermore, under arid conditions, mycorrhized plants are more drought tolerant and give better yields than non-mycorrhizal ones. It is obvious that in the Arabian Peninsula, the AMF themselves are also exposed to drought, heat, and soil salinity. AMF strains that can cope with this may have particularly interesting physiological properties allowing them to function under these conditions. Therefore, native AMF strains represent a promising biotechnological tool to improve agriculture in arid regions of the world. Here we present a pioneering international collaborative programme aiming to isolate, identify, and functionally assess the AMF from Omani agricultural and natural habitats. We have recovered taxonomically unique communities of AMF and obtained strains in pure cultures forming a core of an expanding Gene Bank. Preliminary experiments have been conducted to evaluate the ability of some of the strains in promoting seedling growth of agricultural and ecological keystone plants in Arabia namely *Phoenix dactylifera* (Nakhal) and *Prosopis cineraria* (Ghaf), respectively. Currently we are developing methods to exploit the selected AMF strains on a larger scale to contribute to sustainable agriculture and conservation efforts in the Arabian Peninsula.

POSTER SESSION

Cloning and expression analysis of phosphate transporter genes from finger millet *Eleusine coracana* (L.) in response to phosphate and to colonization by arbuscular mycorrhizal (AM) fungi.

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Phosphate is one of the essential mineral nutrients for plant growth and development. Most of the plants acquire phosphate from the soil either directly by roots, or indirectly via arbuscular mycorrhizal (AM) symbiosis. Plant phosphate transporters have been reported to be involved in both of the process. Although the phosphate transport in plants like Rice, Medicago; is well-documented at the physiological level, information regarding the Finger millet (*Elucine coracana* L.) phosphate transport and its genetic regulation is lacking. In a previous study, we have found that some of genotypes of finger millet showed differential response in term of growth and yield in presence of AM symbiosis. This differential response may be due the genetic factors, mainly the transporter genes from root. With the objective for better understanding of mechanism of phosphate uptake in finger millet, we have cloned four putative phosphate transporter (EcPT1-4) genes. These genes have been cloned using the primers based on the conserved region of rice PT genes. These putative EcPT genes show high degree of amino acid sequence similarity with high-affinity phosphate transporters from Arabidopsis, and rice. The transcript level of these four genes in the seedlings of finger millet was studied under different levels of phosphorous and in the presence of association with AM fungus, *Glomus intraradices*. The expression patterns of the EcPT genes were consistent with a role in the acquisition of phosphate from the environment and suggest that they may be involved in phosphate uptake at the symbiotic interface in mycorrhizal roots.

Quantification of *Amanita caesarea* and *A. ponderosa* extraradical mycelium in soils with variable sporocarp productivity

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Amanita caesarea and *A. ponderosa* are well appreciated edible ectomycorrhizal fungi distributed in temperate Mediterranean regions. Since variability in the annual sporocarp production is very high,

the approaches based on detecting extraradical mycelium in the soil may be a sound alternative for studying the dynamics of these fungal species. Molecular techniques based on Taqman® real-time PCR have been adapted for quantifying DNA from both fungal species in the soil. Soil cylinders were collected in both sporocarp-productive and non-productive areas in spring 2011 and 2012 in the Natural Park of Sierra de Aracena y Picos de Aroche (Huelva, Spain) within the natural distribution area of both fungal species. Total DNA was extracted from each soil cylinder to perform real-time PCR with previously designed specific Taqman probes and primers. DNA from pure cultures of both species was used for standards. Although mycelium of both species were detected in both, productive and non-productive areas, soil mycelium biomass was significantly higher in productive than in non-productive stands for both fungal species. However, mycelium biomass also varied significantly between the collection years and the location. This technique allowed to detect amounts of mycelium over 0.4 µg mycelium / g soil. The results obtained are a preliminary approach to the study of spatial and temporal dynamics of the extraradical mycelium of *A. caesarea* and *A. ponderosa*, providing a stable parameter to complement sporocarp observation for production models.

Ectomycorrhizal communities in managed oak forests in central Poland: effect of chronosequence and host decline

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Pedunculate oak (*Quercus robur* L.) and sessile oak (*Q. petraea* [Matt.] Liebl.) are a widespread species of ecological and economic importance in Europe. Both species are obligatorily associated with ectomycorrhizal fungi (EMF). Until now several papers describing ECM communities of pedunculate and/or sessile oaks in different ecosystems and site conditions has been published, but none of the previous studies has focused on comprehensive description of EMF communities from one distinctive region. Therefore the aim of this study is to present extensive data describing the fungal species richness and composition of oak forests. Study sites were located in Krotoszynskie Forests considered as the largest oak forests in Poland. Altogether 225 soil samples were taken from 19 study sites: stands in different age, various declining tree stage and nursery. Morphological assessment and molecular approach based on PCR and sequencing of the fungal ITS rDNA were performed to identify ectomycorrhizas. One hundred and ten EMF taxa associated with oaks have been recorded. Generally *Cenococcum geophilum* and *Lactarius quietus* were the most frequent species, excluding nursery and regeneration stage sites. In nursery experiment first mycorrhizas of Scleroderma verrucosum were observed 40 days after acorns outplanting. Altogether 8 EMF taxa has been recorded during the first year of seedlings grown. Chronosequence study shown correlation between relative abundance of some group of fungi (*Russula* spp, *Lactarius* spp.) and age of trees. In declining stands lowest number of EMF species richness and highest abundance of *L. quietus* were noted for oaks with high defoliation level.

Effects of warming, grazing and their interaction on arbuscular mycorrhizal fungi in an alpine meadow on the Qinghai-Tibet Plateau

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Understanding the response of arbuscular mycorrhizal fungi (AMF) to warming and grazing is critical to assess the impact of disturbance on AMF community under future global warming. In this study AMF in soils and plant roots were analyzed in a manipulated warming-grazing experimental system in an alpine meadow on the Qinghai-Tibetan Plateau. The content of easily extracted glomalin related soil protein was significantly increased by warming with grazing. A total of 65 operational taxonomic units (OTUs) of AMF were identified using molecular techniques, in which 54 OTUs were recovered from soil, 34 from plant roots, and 21 shared both in soil and plant roots. Glomerales was dominant in soil and plant roots, and warming significantly increased its abundance in soil ($P = 0.000$), but not in plant roots. Warming significantly decreased the abundance of Diversisporales with no-grazing in soil ($P = 0.002$), but significantly increased with no-grazing in roots ($P = 0.004$). Warming significantly increased OTU numbers in plant roots with grazing (warming ï,´ grazing, $P = 0.017$), but not in soil regardless of grazing or not. Nonmetric multidimensional scaling analysis revealed that AMF community composition was significantly affected by warming in soil ($r^2 = 0.1762$, $P = 0.049$) but by grazing in plant roots ($r^2 = 0.1979$, $P = 0.041$). Our results suggest that AMF respond differently to warming and grazing in soil compared to plant roots in alpine meadow ecosystem on the Qinghai-Tibetan Plateau.

Cloning and functional characterization of a novel H^+-dependent phosphate transporter gene from an ectomycorrhizal fungus *Boletus edulis* s.l. Isolate in Southwest China

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Boletus edulis s.l., a large and highly valuable species of delicious edible mushroom, is one of the most frequently encountered ectomycorrhizal fungi in Yunnan Province, southwestern China. It is well-known that trade-off in exchange of photosynthetic carbon for soil-derived nutrients, particularly phosphorus in ectomycorrhizal association. In present study, a phosphate transporter gene was first cloned, identified, and functionally characterized from an ectomycorrhizal fungus *B. edulis* s.l. isolate in southwest China. The full-length phosphate transporter gene from *B. edulis* s.l. (named BePT) was 1632 bp and expected to encode a polypeptide of 543 amino acid residues. Evidence from

bioinformatics showed that the BePT protein consisted of 12 transmembrane domains. The yeast mutant complementation analysis suggested that BePT was functional Pi transporter in yeast cells mediating Pi uptake. When expressed in a Pi-uptake deficient yeast mutant at different pH, BePT protein mediated the highest Pi uptake at pH 6.0, and the activity of BePT was dependent on the electrochemical membrane potential mediated by the yeast P-type H^+-ATPase (H^+/Pi). When expressed in yeast cells by BePT-GFP proteins, GFP fluorescence was observed at the plasma membrane. Our results demonstrated that BePT had a phosphate transporter in yeast *Saccharomyces cerevisiae* and played an important role in Pi acquisition.

The Kalahari truffle and host relations

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Truffles are seasonal hypogeous fruiting bodies of mycorrhizal fungi. They have been used as food sources for centuries and are highly praised for their nutritional value and flavour. The desert truffles, *Kalaharituber pfeilii* (Kalahari truffle), *Eremiomyces echinulatus* and *Mattirolomyces austroafricanus* are the only species known to occur in the Kalahari region of southern Africa, extending into Botswana and Namibia. Several desert truffle species have been found to associate with *Helianthemum* (rockrose), a plant which does not occur in southern Africa. Previous investigations have suggested associations between *K. pfeilii* and plant species such as *Acacia eriloba* (camel thorn), *A. haematoxylon* (grey camel thorn), *A. hebeclada* (candle pod), *Cynodon dactylon* (kweek) and *Stipagrostis* sp. (bushman grass) but the relationships have been based on presence of truffles located near these potential host species. The actual relationships have not been proven. Fruiting bodies and surrounding roots of a suspect host, *Stipagrostic ciliata* (tall bushman grass) were collected from the Haakdoring Duin, Upington, South Africa. The fruiting bodies were identified by observing their morphological characteristics and by molecular methods. Isolations were made from fruiting bodies and roots on modified Fontana medium. DNA was extracted from all isolates, roots and fruiting bodies and PCR amplification using the *K. pfeilii* primers TPR1 and TPF3. Sequences were submitted to GenBANK for comparative identification. The fruiting bodies, roots, and all isolates from fruiting bodies and roots were identified as *K. pfeilii* confirming that *S.ciliata* is a host plant of the Kalahari truffle.

Tracing arbuscular mycorrhizal diversity in maize (*Zea mays* L.) roots at different depths in soil

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Root growth is a continuous phenomenon, which keeps on growing in soil and therefore, reaches at different depths during the course of plant growth. However, Arbuscular mycorrhizal fungal spores are not motile and move only by displacement of soil. Root architecture also differs with the depth of soil as lateral roots become lesser going down in soil. Therefore, an effort was made to find out if the AMF also differs at the species level in different sections of roots. Maize seedlings were grown in soil and vermiculite mixed in 2:4 ratio in GI pipe column. Indigenous AMF spore population in soil was 50/g. Mycorrhiza helping *Pseudomonas* spp.-R62 and R81 (PGPR) were used either as sole or combined with AMF as treatment. Sterilized soil was used for PGPR and control treatments. Each treatment was replicated four times, and plants were grown in glasshouse. After 50 days, the columns were split in two, and the roots were washed and divided into four portions each of 15cm. Thin and thick roots from each portion were separated. Some of the roots were used for mycorrhizal infection rating and rest were used for DNA extraction. ITS region of AMF was amplified using AML1 and AML2 primers, cloned, and sequenced. Shoot fresh and dry weight were measured as a growth parameter. Results will be presented in light of AMF species distribution in roots at different depths of soil and the effect of *Pseudomonas* spp on the infection of AMF.

Mycorrhizal status (Glomeromycota) of *Hedysarum flexuosum* in Morocco

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Arbuscular mycorrhizal AM fungi form a symbiotic association with most terrestrial plants. They promote their growth and their resistance to many biotic and abiotic stresses. In order to evaluate the interactions between these rhizospheric microorganisms and (*Hedysarum flexuosum* /Tangier ecotype), three sites were retained and sampled in the Tangérois. The counting of the spores of AM fungi in the rhizospheric soil of these sites allowed identifying 5 morphotypes. The highest density of spores (5893 per 100g of soil) was observed in the second site (Pont Anasser) but without being significantly different from others sites. Among the 5 morphotypes, 3 genuses of AM fungi were able

to be identified as *Scutellospora, Glomus, and Acaulospora*. At site 1, the determination of the rate of mycorhization of *Hedysarum flexuosum* roots showed a frequency of mycorhization 97.78%, an intensity of mycorhization of 45.01% and an arbuscular intensity of 28.84%, it appears that *Hedysarum flexuosum* is a very mycorrhizal plant.

Hidden fungal community inside ectomycorrhizas at *Castanopsis*-dominated forest

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Fungal root endophytes have been reported as co-associated ascomycetes detected in ectomycorrhizal root tips, but its interaction with ectomycorrhizal fungi remains largely unknown. We investigated the community structure of ectomycorrhizal and co-associated endophytic fungi to improve understanding of their biology. Isolation of root endophytic fungi from the surface-sterilized root tips were attempted after random selection of ectomycorrhizas from the soil core samples, which were taken from a Japanese chinquapin (*Castanopsis cuspidata*)- dominated forest in Kyoto, Japan. From 782 ectomycorrhizal root tips, 160 isolates were obtained, categorized into over 15 types by PCR-RFLP. The nucleotide sequence analysis showed that the root endophytes most frequently found belong to the ascomycetous order Helotiales, which includes undescribed root-associated fungi and species forming ericoid mycorrhiz. The many other fungi isolated such as *Oidiodendron* sp. or *Robillarda sessilis* were also thought to be root endophytes. Furthermore, we identified their putative fungal partner, i.e., the symbiotic fungi that form these ectomycorrhiza to investigate the interaction between the root endophytes and ectomycorrhizal fungi. DNA extracted from single root tips served molecular identification with nucleotide sequencing. Based on these results, the community structure and the possible interaction of these two types of fungi, namely, ectomycorrhizal fungi and fungal root endophytes will be discussed.

Patterns of soil fungal communities in subtropical Chinese forests in relation to plant diversity

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Soil fungi play a key role in terrestrial ecosystems. A vast diversity of saprotrophic and mycorrhizal fungi contribute to plant development through efficient decomposition and nutrient uptake, respectively. However, knowledge on the impact of plant diversity on the fungal community composition of subtropical forest ecosystems is limited. The study was carried out within the frame of the world's largest biodiversity and ecosystem functioning experiment in forest ecosystems, located in southeast China. In 2009/2010, two study sites were planted with 42 tree species. These comprise equal proportion ectomycorrhizal or arbuscular mycorrhizal tree species following tree diversity gradient levels of 1, 2, 4, 8, and 16 plants per plot. This is an ideal field experimental platform to study the role of plant diversity on soil fungal community in a subtropical forest ecosystem. In October 2011, soil cores from a subset of 4 and 8 tree species diversity levels were sampled from one of the study sites. The fungal community was analysed employing pyrotag sequencing of the fungal ITS rDNA region, which will provide a deep-resolution of the fungal community. In this poster, we will present results on the fungal diversity and the influence of plant diversity, soil and plant related parameters on the fungal community composition. The main drivers for fungal community composition with the extent of mycorrhizal fungi will be identified for this early stage of forest development and its implication on the functioning of this particular forest ecosystem will be discussed.

Diversity of fungal mycorrhiza in *Eucalyptus globulus* forests in the region of Panafiel, North Portugal

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Eucalyptus (*Eucalyptus globulus*) is one the plant species known to form both types of mycorrhizal symbiosis (ECMF and AMF) and that may be one of the causes of its high nutrient and water use efficiencies. Eucalyptus is original from Australia, but grows very well in Portugal where it is cultivated for the paper pulp industry. The growth of eucalyptus raises concerns on its impact on biodiversity – since it is planted as intensive and dense forests – and on soil fertility. Taking the perspective that soil is the biggest reservoir of biodiversity, the objective of this study was to assess the diversity of AMF in a forest planted with *E. globulus* in the region of Penafiel, northern Portugal, fertilized or not with nitrogen at the start of planting. AMF diversity was assessed using nested PCR-DGGE. Soil samples were collected in spring and autumn and subjected to DNA extraction, nested PCR-DGGE, sequencing, and analysis of the sequences. The results showed that nitrogen fertilization or season had no influence on the AMF community of *E. globulus*, where there was a predominance of the FMA species *Scutellospora heterogama* (synonymous: *Endogone heterogama* T.H. Nicolson & Gerd, 1968, *Gigaspora heterogama* (T.H. Nicolson & Gerd.) Gerd. & Trappe, 1974). These results are very interesting since it is known that this fungi is very efficient in taking up nitrogen and phosphorus, which may contribute to the observed efficiency of the culture in using nutrients, even in poor soils.

Comparative transcriptome revealed activation of the pathways involved in phosphate/cation uptake and arginine biosynthesis during polyphosphate-overplus in an arbuscular mycorrhizal fungus

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Arbuscular mycorrhizal (AM) fungi are capable of accumulating a massive amount of polyphosphate in the vacuoles, which reaches 60-70% of total phosphorus in the cell and has been known as polyphosphate-overplus. Our previous studies demonstrated that ATP was used as direct substrate for polyphosphate biosynthesis and monovalent and divalent cations were accumulated synchronously during polyphosphate synthesis. The regulatory mechanism of polyphosphate-overplus at the gene expression level, however, has yet to be elucidated. *Rhizophagus* sp. HR1 was grown with *Lotus japonicus* in the mesh bag-separated two-compartment culture system under P-deficient conditions for 8 weeks. Extraradical mycelia were collected from the hyphal compartment prior to and 4 h after phosphate (Pi) application, and total RNA was extracted. mRNA was purified, reverse-transcribed, and sequenced firstly with Roche FLX system to obtain long cDNA sequences (contigs) and then with Illumina HiSeq2000 to assess read abundance. The reads obtained by HiSeq2000 were mapped on the contigs obtained by FLX, which enabled us to detect changes in expression levels of 10,759 genes during the initial phase of polyphosphate-overplus. Transcripts of the genes encoding H^+/Pi symporters, Na^+/Pi symporters, plasmamembrane- and vacuolar-type cation transporters, polyphosphate polymerase (vacuolar transporter chaperons), and the enzymes involved in arginine biosynthesis were increased during polyphosphate-overplus, but those of the genes responsible for energy (ATP) generation i.e. glycolysis, pentose-phosphate pathway, oxidation, and electron transport chain were not. The present study provides a global picture of dynamic cellular responses during polyphosphate-overplus in the fungi.

Colonisation patterns of root tissues by *Phytophthora* taxon *Agathis* in relation to mycorrhizal root nodules of kauri *Agathis australis*

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Kauri *Agathis australis* plants pre-colonised by the arbuscular mycorrhizal fungus *Glomus intraradices* were studied using fluorescence microscopy for possible spatial partitioning of tissue interactions between mycorrhizae and Phytophthora taxon *Agathis* (PTA). The first challenge was to discriminate between the hyphae of the two organisms within root tissues. We report on a fluorescent in situ hybridization protocol that utilizes a fluorescent probe attached to a real-time PCR-specific primer for PTA. This tool can assist in distinguishing hyphae of PTA from those of *G. intraradices* inside roots and root nodules. We report on the development of the protocol and specificity assay. Cross sections of infected kauri roots and nodules showed that inter- or intra-cellular hyphae developed mainly in the cortex. The presence and induction of necrosis of host cells is described with respect to differential-levels of fluorescence. The number of PTA hyphae growing in the root cortex was compared to that in mycorrhizal root systems, and specifically in and around mycorrhizal tissues infected by the pathogen and arbuscule-containing cells. Results are discussed in relation to possible processes involved in the phenomenon of bio-protection in arbuscular mycorrhizal plants.

Plant root associated fungal community structure at small spatial scale in an Arctic ecosystem

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Many important plant groups in the Arctic lack Mycorrhiza, therefore the importance of mycorrhizal fungal communities in the Arctic have long been neglected. Recent investigations suggest that root-associated fungal diversity is an important component in arctic ecosystems. Considering the rapidly changing arctic climate, the need to understand the diversity and dynamic of root-associated fungi in the Arctic are urgent. In this study, we aimed to relate the fine scale spatial structure of mycorrhizal communities associated with the *Bistorta vivipara* to a set of biotic and abiotic variables. Mycorrhizal

communities were determined using 454 pyrosequencing of ITS1 amplicons. A total of 79 plant root systems were sampled from 81 equal-sized subplots in a 9 m^2 area at the Midtre Lovénbreen glacier foreland (Ny Ålesund, Svalbard archipelago). Soil characteristics and intensive aboveground vegetation cover analyses were performed to assess eventual relationships with the distribution of fungi. Filtering and subsequent clustering (97% cut-off) into operational taxonomic units (OTUs) were performed using the CLOTU pipeline, applying strict parameters to avoid overestimating the fungal species richness. Fungi with taxonomic affinity to Agaricales, Sebacinales from phylum Basidiomycota, and Helotiales from phylum Ascomycota were frequent in plant roots. Fungal community structure and spatial distribution was assessed using parallel DCA and GMNDS ordinations, followed by semi-variance analyses. Our preliminary results showed weak compositional gradients for the root-associated fungi, with no spatial structure on the spatial scales addressed (down to distances of 30 cm). The results indicate a more fine-grained patchiness in arctic ecosystems than in alpine areas previously studied.

Comparison of AM fungal root colonisation measurement techniques

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Arbuscular mycorrhizal (AM) fungi are an important plant mutualist that contribute greatly to plant community dynamics and could improve food security worldwide. However, the study of the relationship between AM fungi and plants is currently limited because we cannot compare between the different methods used by different research groups to measure the relationship. While there are currently multiple methods for measuring AM fungal colonization of host plants, we have very little understanding of how different measurements correlate with each other. The most common method involves clearing, staining, and scoring colonization with a microscope; however, other non-morphometric methods have also been used. Higher throughput methods such as qPCR should enable larger and more diverse experiments. Here we compared four different methods (root staining, PLFA, qPCR, chitin quantification) used to quantify AM fungal colonization. In this study, we focused on the AM fungus *Glomus mossae* colonizing *Plantago lanceolata*. In a controlled glasshouse experiment, we grew 168 *P. lanceolata* in an autoclaved sterile sand: soil (2:1) mixture inoculated with *G. mossae*. Replicated samples were harvested at 8 time intervals to test the efficacy of colonization measurements at different growth stages. The results show differences between the different colonization measurements. For example, microscopy measurements are more accurate than PFLA, but are more time intensive. These results provide insight into when each measurement will provide the most information, and allow us to compare the measurements of AM fungal host plant colonization between different studies.

LJPAP3 encoding purple acid phosphatase involved in P-transfer in the plant-fungal interface of arbuscular mycorrhiza

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Molecular mechanism underlying phosphate transfer from arbuscular mycorrhizal fungi to the host plants is of great interest but poorly understood, so far. Arbuscules are thought to be the main site of nutrient exchange between them. We have identified LjPAP3 that encodes purple acid phosphatase is expressed in arbuscule-containing cortical cells of *Lotus japonicus*, and the product is localized in the periarbuscular space. In order to elucidate a role of LjPAP3 in arbuscular mycorrhizal symbiosis, we constructed LjPAP3-RNAi lines of *L. japonicus* and characterized their phenotypes with respect to growth and P nutrition. *L. japonicas* B-129 (wild type) and its RNAi lines of the LjPAP3 were inoculated with Rhizophagus irregularis DAOM197198. Dry weight and P content of the shoots were measured at 4 weeks after inoculation. The roots were subjected to assessment of fungal colonization. Levels of LjPAP3 expression were analysed by semiquantitative RT-PCR. Two LjPAP3-RNAi lines in which the levels of LjPAP3 transcript were specifically reduced to those in the non-mycorrhizal roots were constructed. Arbuscular colonization was slightly reduced in the LjPAP3-RNAi lines, but the morphological characteristics of arbuscules in the lines were similar to those in the wild type. Shoot dry weight and P concentration were reduced in the RNAi lines compared with those in the wild type. These results suggest that LjPAP3 may involve in P nutrition of arbuscular mycorrhizal plants.

Viruses in arbuscular mycorrhizal fungi: a new player in the symbiosis

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Fungal viruses (mycoviruses) that possess a double-stranded (ds) or single-stranded (ss) RNA genome often have a significant impact not only on phenotypic expression of the host fungus but also on higher order biological interactions. Recently, we demonstrated for the first time that aglomeromycotan fungus harbours mycoviruses. Four isolates of arbuscular mycorrhizal fungi were grown in the two-compartment culture system in association with marigold. About 0.5-1.0 g of extraradical hyphae was collected from more than 70 pots for each fungus, and dsRNA was extracted, purified, and electrophoresed. For global analysis, the dsRNAs were reverse-transcribed with the 6N-anchored primer, amplified with the anchor primer, and then sequenced with Roche FLX system. Three out

the four isolates, *Rhizophagus* sp. RF1, *Acaulospora colombiana* HB1, and *Claroideoglomus etunicatum* H1-1, possessed four, three, and two dsRNA segments, respectively, but *Rhizophagus* sp. HR1 did not. Eight contigs obtained from *Rhizophagus* sp. RF1 by FLX pyrosequencing showed similarities to four different dsRNA and ssRNA viruses, consistent with the electrophoretic observations. Among the four viruses, GRF1V-M possesses a 4,557 bp dsRNA genome and was phylogenetically distinct. A GRF1V-M-free culture line obtained by single spore isolation produced twice as many spores as RF1V-M-positive lines. GRF1V-S possesses RNA, probably an ssRNA genome of 2,895 nt, and phylogenetic analysis suggested the virus belongs to the genus *Mitovirus* and closely related to that found in the ectomycorrhizal fungus *Tuber excavatum*. These observations suggest that the fungi harbour diverse and evolutionary distinct mycoviruses that are a potential player in the symbiosis.

Phylogenetic diversity of Asian Caesar's mushrooms

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The Caesar's mushroom, *Amanita caesarea*, is one of the most well-known gourmet mushrooms in the world. In Asia, several relatives are known, e.g. *A. caesareoides*, *A. hemibapha*, *A. javanica*, *A. similis*, and *A.esculenta*, all of which are delicious mushrooms. The morphological identification of these mushrooms is, however, difficult due to its colour variation of basidiomata, which may cause the misidentification of the species, and vague interpretations of its fungal ecology and biogeography. In this study, we aim to estimate the phylogenetic diversity of Asian Caesar's mushrooms for the safety of their biological and commercial uses. Samples from forty Asian Caesar's mushrooms were collected from Japan and Thailand, inspected microscopically, and their rDNA ITS regions – amplified by PCR – were phylogenetically analysed. Japanese *A. caesareoides* samples constructed a single clade with a Chinese *A. hemibapha* sample, which positioned as the sister clade of *A. caesarea*. However, a Thai *A. hemibapha* sample externally similar to *A. caesareoides* constructed a distant clade from *A. caesareoides*. A Japanese *A. javanica* sampled constructed two sister clades, both of which were distant from Thai *A. javanica*. In addition, a Japanese yellow cap specimen externally identifiable as *A. javanica* was included in the *A. caesareoides* clade. Japanese *A. similis* clade was regarded as the sister constructed with Thai *A. javanica*. Japanese *A. esculenta* specimens possessed more distant positions than all the other Caesar's mushrooms. These results suggested that phylogenetically quite diverse Caesar's mushrooms are present in Asia, but their Japanese and Thai populations are genetically rather distant.

Community structure of arbuscular mycorrhizal fungi (AMF) in relation to their functions in agro-ecosystems

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Arbuscular mycorrhizal associations are multi-functional, assisting the plants in nutrient acquisition, water uptake, mediating carbon transfer, and protecting roots from pathogens. Mycorrhizal diversity is important for agro-ecosystem functioning but techniques are lacking for determining composition of mixed communities within plant roots. Main project aims were to develop a targeted quantitative molecular tool, qPCR, to observe relative abundance of AM fungal species within the root system and to investigate the effect of changes in this relative abundance on plant health, in relation to water stress. Water stress experiments, undertaken with strawberry, show a reduction in plant development when subjected to drought stress. Inoculation of strawberry with mixtures of AMF is beneficial to plants under water stress when compared with un-inoculated control treatments. Different mixtures of AMF applied as treatments showed little difference in plant response but the final composition of AMF within the roots was not known. The effects of drought on the relative abundance of AMF colonisation are being further examined by quantification of AMF presence in the root using newly developed q-PCR methods. When these results are analysed, will we understand how different initial treatments with mixtures of AMF relate to final colonisation by the individual components within the mixtures.

Mitochondrial intergenic regions as molecular marker: a reliable tool to distinguish closely related AMF species and assess their intra-specific diversity

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Arbuscular Mycorrhizal Fungi (AMF) are vital components of the microbial soil community, forming the most commonly occurring symbiotic association with roots of more than 80% of terrestrial plant species. AMF are members of the Glomeromycota phylum. They are agriculturally most important symbionts that help plants in the uptake of several nutrients, such as phosphorus, in exchange of plant's carbohydrates. Many studies have shown the unusually high polymorphism that occurs within AMF individuals at the nuclear DNA level, which was evident through analysis of rRNA and later in protein coding genes, such as β tubulin, H^+ ATPase, and P-type II ATPase. Since this phenomenon

hinders the development of reliable molecular markers to distinguish between isolates of the same AMF species. However, it has been shown that mitochondrial DNA is homogeneous within isolates of the same species. Further, some mitochondrial intergenic regions vary substantially among isolates of *Rhizophagus irregularis* and closely related species with the presence of numerous insertions/deletions and eroded mobile elements. Based on three different partial or complete mitochondrial genomes sequences available in the GenBank database, *Glomus irregulare* strain DAOM-197198, *Glomus intraradices* isolate FACE#494, and *Rhizophagus intraradices* clone JGIBTPH-5L11, we have designed primers to amplify defined intergenic regions and compared them for a few isolates available in the Centre for Mycorrhizal Culture Collection (CMCC) TERI. These results suggest that mitochondrial intergenic sequences do provide useful information to distinguish between closely related *Glomus* species and provide an assessment of intra-specific diversity.

Screening of potent ectomycorrhizal isolates towards understanding molecular mechanism of gold nanoparticle biosynthesis

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Molecular mechanism of gold nanoparticles biosynthesis (GNPs) could be important for development of commercial production of desired size, shape, and monodispersity controlled GNPs. A few ectomycorrhizal fungi (*Laccaria fraterna, Laccaria laccata, Paxillus involutus*, and *Amanita muscaria*) are selected. These ectomycorrhizal fungi showed high reduction property evaluated by potassium ferricyanide test in extra cellular and cell free extract (*in vitro*) conditions. The total reduction ability of each ectomycorrhizal fungi is directly correlated with their corresponding gold nanoparticle biosynthesis potential (by absorbance analysis). The gold nanoparticles synthesized by *Laccaria fraterna* showed wide size range of 15-60±10 nm by zeta particle size analyser. Similarly, *Laccaria laccata* reveals 10-65±8 nm, *Paxillus involutus* reveals 5-50±8 nm and *Amanita muscaria* reveal 15-45±6nm size range of GNPs. Among them *Laccaria fraterna* (highly potent gold nanoparticle biosynthesizers), mediated GNPs biosynthesis reveals 15-50±8 nm and 20-65±6 nm GNPs in extra cellular and cell free extract (*in vitro*) respectively. *Laccaria fraterna* will be further used for the molecular mechanism evaluation of GNPs biosynthesis by identifying the cellular localization, involvement of macro and micro biomolecule using TEM imaging, confocal optical slicing, protein depletion, micro and macro molecular separation, and combinatorial *in vitro* solution studies.

Genetic variability within n-rDNA region of ectomycorrhizal isolates originating from temperate ecosystems

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Identification of ectomycorrhiza (ECM) fungi based on morphological characters has been laborious and time consuming, especially sample collected from environment. Additionally, due to its microscopic nature and limited morphological characters it is difficult to detect intraspecies variation. With this view aim of present study was to confirm the earlier identification of these fungi, which was based on morphological characters, and also to find suitable molecular restriction fragment length polymorphism (RFLP) markers for the identification of ECM fungi up to the level of species or isolates as part of an expandable database of RFLP patterns of the ITS region of ECM fungi. Mycelia of fourteen species of ectomycorrhizal fungi representing five genera were isolated in pure culture and characterized by morphological and molecular methods. Molecular identification was performed by analysis the small subunit and internal transcribed spacers (SSU-ITS) of the nuclear encoded ribosomal RNA (n-RNA) gene region using RFLP. The region was first amplified by polymerase chain reaction (PCR) with specific primers and then cleaved with different restriction enzymes. The degree of polymorphism, although extensive, proved inadequate for proper identification of most of the isolates. Depending on the restriction enzymes used, the genera or species could be grouped on the basis of common fragment patterns, thereby confirming the potential of the SSU-ITS region in PCR-RFLP in molecular characterization and identification of ectomycorrhizal fungi.

Morphological and molecular characterization of *Diversispora spurca* grown as monosporal culture with *Trifolium alexandrinum* as a suitable host plant

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Monosporal culture of *Diversispora spurca* AMF is successfully raised and maintained in a controlled greenhouse condition originating from the soils of Northern India. *Trifolium alexandrinum* is used as a host plant. After six cycles of raising in greenhouses, spores were isolated for morphological and molecular analysis. The analysis reveals abundance of loose sporocaps, which was not reported earlier. Spores carries double wall and globoid in shape of 25-90 μm in diameter: Thin hyaline outer

wall and laminated, flexible inner wall. Subtending hypha hyaline to light yellow-straight, cylindrical. The total genomic DNA was extracted from these spores using Qiagen extraction Kit for molecular characterisation. A DNA sequence of the rDNA locus, which contains partial sequences of 18S rDNA (SSU)-ITSI-5.8S rDNA-ITS II and 28S rDNA (LSU), were obtained using nested PCR and cloning. The primers used for the first PCR were SSUmCf-LSUmBr and ITS1 FP-ITS4 RP for first nested PCR and LR FP-FLR RP for second nested PCR. These PCR products were individually cloned using pGEM-T Easy vector cloning system and sequenced using Sanger di-deoxy method. The sequences were analysed using BLAST-N program and were compared with the available sequences in the database to construct a phylogenetic tree. This pure culture is maintained with an aim to provide starter AMF cultures for research activities and for inoculum production to evaluate the agricultural productivity enhancement potential.

Progress towards characterization of different genera of arbuscular mycorrhiza fungi from diverse habitats of India

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Spores of Arbuscular Mycorrhiza Fungi (AMF) from different habitats of India are collected, trapped, and multiplied in a greenhouse and investigated with an aim to assess the biodiversity. Since most AMF species does not sporulate in all seasons under natural conditions, they are grown as trap cultures in green house from where Monosporals are raised. Single spore are morphologically scrutinized and monosporals are initiated with different host plants including *Sorghum vulgare*, *Allium cepa*, and *Trifolium alexandrinum*. The successful cultures are then identified using conventional methods (voucher specimen of spores).Ribosomal DNA locus, which contains partial sequences of 18S rDNA (SSU)-ITS I-5.8S rDNA-ITS II and 28S rDNA (LSU), was cloned and sequenced. The sequences were analysed using BLAST-N program and compared with the available sequences in the database. A Phylogenetic tree was constructed to show clear clade separation. Characterization of these Monosporals has enumerated a vast diversity of AMF, which include species such as *Funneliformis mosseae, Glomus claroideum, Glomus etunicatum, Glomus hoi, Rhizophagus irregularis, Diversispora spurca, Paraglomus majewskii* and many unculturable *Glomus* spp. Among all these cultures the widespread occurrence of *Glomus etunicatum* indicates that it is a highly dominant species among all and probably easily culturable. Determination of AMF biodiversity through above methods mainly depends on the ability of extracting the spores from the soil and raising fungi in Monosporal culture conditions. Basic methodology has been developed but lot more is to be innovated to scavenge out the unculturable genus concealed in Indian and Global soils.

Morphological and molecular characterization of a new mycorrhizal *Glomus* species

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A new species of mycorrhizal fungus, belonging to the genus *Glomus*, has been obtained in pure pot culture in greenhouse with *Plantago* and *Sorghum* as host plants. The morphological characters of the species are described based on parameters studied from in situ pot cultures. This unidentified *Glomus* spp. was studied for various characters viz. spore size, wall layers, colour, hyphal attachment, reaction in PVLG and PVLG:Melzer's reagent(1:1), arbuscules, vesicles, intraradical hyphae. The two features that need to be highlighted are that chlamydospores are formed in loose aggregation in bunches hypogeously with 10-100 spores in soil as well as in roots. These spores are never found singly. This species produces spores that are globose, subglobose, and sometimes irregular in shape ranging from 11 µm-30 µm in size. The spore contains 3 wall layers and turns orange-red on reaction with Melzer's reagent. The total genomic DNA was extracted from these spores using Qiagen DNasy plant minikit. A DNA sequence of the ribosomal DNA locus, which contains partial sequences of 18S rDNA (SSU)-ITS I-5.8S rDNA-ITS II and 28S rDNA (LSU), were sequenced using nested PCR and cloning. The sequences were analysed using BLAST-N program and the results were compared with the available sequences in the database. A phylogeny tree was then constructed to show a clear clade separation with the available sequences in the database.

Morphotaxonomy and molecular characterization of a new strain of *Rhizophagus* spp. from Andhra Pradesh, India, and its establishment as monosporal culture

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The new strain of *Rhizophagus* spp.monosporal pot culture has been established successfully with *Sorghum vulgare* and *Allium cepa*. This *Rhizophagus* spp. forms bunches of hyaline juvenile spores. The mature spores are light yellow to dark orange, globose, subglobose, 55-98µm in diameter. Spores have three wall layers, in total (3.4-4.3µm thick). The innermost layer usually closes the pore at the spore base and forms the hypha with septa. The subtending hypha of each spore is straight with narrow funnel shaped opening (7-8µm). Innermost layer becomes conspicuous in PVLG: Melzer"s (1:1). Additionally it's a high sporulating species. A DNA sequence of the ribosomal DNA locus, which contains partial sequences of 18S rDNA (SSU)-ITS I-5.8S rDNA-ITS II and 28S rDNA (LSU), were obtained using nested PCR and cloning. The primers used for the first PCR was SSUmCf-LSUmBr and for first nested PCR we used, ITS1 FR-ITS4 RP while for second nested PCR we used, LR FP-FLR RP. These PCR products were individually cloned using pGEM-T Easy vector cloning system and sequenced using Sanger di-deoxy method. The sequences were analysed using BLAST-N program and the results were compared with the available sequences in the database. A phylogeny tree was then constructed to show that this culture grouped together with *Rhizophagus* spp.

Pure culture synthesis of ericoid mycorrhizal fungi their morphology and molecular analysis in association with rhododendron plant roots

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Ericaceous host plant exhibit intracellular penetration by the fungus, mycorrhizal infection occurs only in outer tier of cortical cells because fungal hyphae never penetrate stele. Fungus loosely grows over the root surface and formation a weft of hyphae and penetration of root cells without appressorium formation is reported. Some of the isolated fungal species from Rhododendron plant roots induced to form ERM in pure culture. Association of Ascomycetous fungi with Mycorrhizal fungi having Rhododendron hairy roots as determined by the pure culture Mycorrhiza synthesis technique. The ecological relationship between ERM fungi and Rhododendron plants are examined towards morphology and development of the association with Rhododendron plants in axenic and in vivo experiments. Transmission, Scanning, light electron, laser scanning, & confocal microscopy revealed interesting features towards colonization pattern of epidermal cell and hyphal complex morphology under axenic petri dish culture and non-sterile pot cultures. These fungal strains isolated from the feeder roots of Rhododendron forest range in Indian Himalayan Mountains. Characterization of ERM fungal isolates from culture collection was done using rDNA ribosomal internal transcribed spacer (ITS1-5.8-ITS2) region and Universal primer ITS1F, Ascomycetous specific primer ITS4 A and sequence analysis. We tested their ability to form ericoid mycorrhiza. Fungal specific primers for ITS regions are used to amplify DNA from pure fungal cultures. Molecular approach has revealed genetic diversity and molecular ecology of ERM fungi, exhibits that the ericaceous plants are very promiscuous, with multiple occupancy of their fine roots, and gives insight of the species level diversity of ericoid fungi.

Molecular analysis of oil-polluted sediments coupled with greenhouse and *in vitro* trap-cultures revealed dominance of Glomeraceae species over other taxa.

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Land reclamation of abandoned polluted-sites by the use of plant-microbe interactions are considered to be important processes driving the efficacy of such practices. Nowadays, increasing attention is devoted to the rhizospheric and endophytic microorganisms. Among them, arbuscular mycorrhizal (AM) associations are of particular importance since they play a central role promoting plant growth and health and soil quality. Despite their largely known positive impact for the restoration of perturbed environments, relatively little is known about their physiology, mainly due to the difficulties to growth them in pure cultures, hampering also details about their biochemistry and genetics. In this experimental study, we examined the molecular diversity of AM fungi associated to naturally occurring *Eleocharis* sp. plants, by trapping environmental rhizospheric samples in leek (*Allium porrum L.*) (mesocosm approach) and subsequently isolating them under *in vitro* conditions in association with Ri T-DNA-transformed chicory roots (*Cichorium intybus L.*) (microcosm approach). The molecular and morphological analyses under both conditions showed a dominance of Glomeraceae species over other taxa and provide useful information about the interaction of AM fungi with plants in polluted environments a key step to successful implement bioremediation strategies.

Genetic diversity and population structure of *Cenococcum geophilum* at coastal pine forests in japan

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In Asian coastal pine forests, one of ectomycorrhizal fungus, *Cenococcum geophilum* was suggested to be distributed ubiquitously and dominantly. To clarify the genetic diversity and population structure of *C. geophilum*, we analysed the fungus associated with coastal *Pinus thunbergii* at regional, local and soil-core scales. *P. thunbergii* roots were sampled at 8 geographically distant sites, and 14 to 26 samples were excavated as soil cores within 1-5 ha. Using *C. geophilum*-like mycorrhizae, the ITS region was amplified with *C. geophilum* specific primers and, for positive samples; the genetic diversity was evaluated by analysing the gpd gene, and by applying microsatellite markers. Genotypes

generated from the microsatellite analysis were used to assess spatial autocorrelation at local scales and to evaluate genetic distances and their clusters at regional scales. For the gpd gene sequence, positive samples of the ITS amplification was phylogenetically discriminated into 4 different clades, but most samples were nested with one of the clades. The microsatellite analyses showed that a total of 105 genotypes were confirmed with 8-19 types per site, and 1-3 types per core. Although significant spatial autocorrelation was not found at any sites within local scales, genetic distances were positively correlated with physical distances at regional scale. Individual samples were assigned into either one of 2 populations with reflecting map location. These results suggest high genetic diversity of *C. geophilum* at both local and soil-core

Mycorrhizal fungus stimulates the growth of mycorrhization helper bacterium in plant-soil-microcosms

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Host plant roots and mycorrhizosphere microbes are important and potentially interacting factors shaping the performance of mycorrhization helper bacteria (MHB). Both in the presence and in the absence of pedunculate oak seedlings, we investigated the effect of soil sterilization on the interactions between the MHB *Streptomyces* sp. AcH 505 and the extraradical mycelium of the widely distributed ectomycorrhizal fungus *Piloderma croceum*. Specific primers were designed to perform real-time PCR with DNA extracted from soil samples, based on the intergenic region between gyrA and gyrB genes of AcH 505 and the internal transcribed spacer of *P.croceum*. The real-time PCR assays allowed precise and specific quantification of DNA from AcH 505 and from *P. croceum* in pure cultures and in soil, respectively. In soil microcosms, the PCR signal of AcH 505 increased in the presence of *P. croceum*, but this increase was not significant in sterile microcosms with the plant. The fungal PCR signal was not affected by AcH 505 in the absence, but the fungal signal decreased in the presence of the plant. This decrease took place in non-sterile microcosms only. In conclusion, the provided tools enable us to directly detect and quantify a MHB and a mycorrhizal fungus in soil substrate. Our study indicates that both the presence of microorganisms and the presence of plant roots influence the outcome of MHB-fungus interactions, and that mycorrhizal fungi may enhance MHB growth.

Metal tolerance of *Exophiala pisciphila* H93 (ACCC32496), a dark septate endophyte from an ancient lead and zinc slagheap in southwest China

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Metal tolerance of *Exophiala pisciphila* (ACCC32496, H93), a dark septate endophyte (DSE), isolated from the roots of *Arundinella bengalensis* naturally regenerating in an abandoned lead-zinc mine in southwest China, was determined. Median effect concentration (EC50) on growth of H93 shown that H93 has relatively high tolerances for Pb with EC50 of 800mg/L, 1010mg/L for Zn, and 111mg/L for Cd, respectively. Morphology of H93 diversified a lot, including hyphal swollen and yeast-like growth and large amounts of acetic acid secreted in the culture solution supplied with the excessive metals. TEM- and SEM- EDS analysis suggested that Cd was mainly distributed in its cell wall. The activity of SOD and CAT, and the content of GSH increased with the elevated Cd concentration in the culture solution. Based on the above research, a natural resistance-associated macrophage protein gene, EpNramp was cloned first from H93 using degenerate primers and DNA-walking technique. EpNramp was 1716 bp, expected to encode a polypeptide of 371 amino acid residues, and shared a high degree of homology with nramps from other fungi. Phylogenetic analysis showed that the NRAMP protein clustered together with *Aspergillus* and Coccidioides. When expressed in yeast cells, EpNramp significant restored normal growth of fet3fet4 mutant on the iron-depleted media, which suggested that EpNramp entirely or partly mediated the transport of divalent metal cations, such as Fe^{2+} in *Sacchromyces cerevisiae*. Subsequently, the potential role of the EpNramp protein in the tolerance of *E. pisciphila* is also discussed by real-time PCR.

Community of arbuscular mycorrhizal fungi (AMF) in *Pyrus pyrifolia* var. culta and co-occurrence of the AMF with *Plantago asiatica* in pear orchard

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We examined 1) Colonization rate and communities of arbuscular mycorrhizal fungi (AMF) in the roots of *Pyrus pyrifolia* var. culta (Japanese pear) to investigate the effect of phosphorus (P) fertilization, and

2) AMF community in the roots of Japanese pear and *Plantago asiatica* to determine the presence of shared AMF. In the first study, soil cores containing the roots of Japanese pear were collected from 13 orchards in Tottori Prefecture, Japan. Result of soil analysis showed extreme accumulations of soil available P in many of them. The AMF colonization rate was negatively correlated with soil available P. The AMF communities were examined on the basis of the partial fungal DNA sequences (SSU rDNA) amplified by AMF-specific primers AML1 and AML2, and the obtained AMF sequences were divided into 14 phylotypes. The number of AMF phylotypes (species richness) was also negatively correlated with soil available P, which suggested the lower AMF diversity in P accumulated soil conditions. In the second study, soil cores were collected from 3 orchards, and the AMF sequences from both host plants were divided into 23 phylotypes. The same AMF sequences (100% sequence identity) were detected from both plants in the same soil cores in 8/12 samples. Redundancy analysis showed that the effect of host plant species on the distribution of AMF phylotypes was not significant. This result showed that AMF communities were not differentiated between the two host plants. Thus, it was suggested understory herbaceous plants could be functional as AMF inoculum sources for orchard trees.

Metals and arbuscular mycorrhiza affect the leaf proteome of a selected poplar clone in a long-term experiment.

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The arbuscular mycorrhizal (AM) symbiosis, while improving the mineral nutrition of the plant, can also increase its tolerance towards some pollutants, like metals. Therefore, the use of mycorrhizal plants has been proposed in order to increase the efficiency of phytoremediation processes. Although the symbiosis exclusively concerns the plant roots, some plant responses can be systemic. Therefore, in this work a clone of *Populus alba L.*, previously selected for its high tolerance to copper and zinc, was used to investigate the effects of the symbiosis with the AM fungus *Glomus intraradices* (now *Rhizophagus intraradices*) on the leaf protein expression. Poplar leaf samples were collected from plants maintained in a glasshouse on polluted (copper and zinc contaminated) or unpolluted soil, after four, six and sixteen months of growth. About 450 proteins were reproducibly separated on 2DE maps for each harvest. At the first harvest, the most relevant effect on protein modulation was exerted by the AM fungi, at the second one by the metals, and at the last one by both treatments. This work demonstrates how importantly the time of sampling affects the proteome responses in perennial plants. In addition, it underlines the ability of a proteomic approach, targeted on protein identification, to depict changes in a specific pattern of protein expression.

Mechanisms of Zn detoxification in metallicolous and non-metallicolous *Suillus luteus* populations

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Zinc is an essential micronutrient but can become toxic when present in excess. Elevated concentrations of Zn in the environment may cause a selective pressure on exposed biota and can lead to the evolution of tolerant ecotypes. Adaptive tolerance to Zn has developed in populations of the ectomycorrhizal fungus *Suillus luteus*. A wide range of Zn detoxification mechanisms including exclusion, chelation, compartmentalisation, anti-oxidative response, and repair mechanisms is known and, may be involved. Gene expression and enzyme activities of key players in different detoxification pathways were measured and Zn, Fe, and Mn content were determined in several Zn-tolerant and sensitive isolates (i.e. genotypes) upon Zn exposure. All Zn-tolerant isolates show an excluder profile. Selection for a constitutive high expression of a Zn CDF-family transporter occurred in these isolates. Anti-oxidative and repair mechanisms may be important in detoxification for individual Zn-tolerant and sensitive isolates. Each isolate tends to have its own preferred approach to handle Zn related oxidative stress and damage. A high intraspecific variation was detected in both populations and may contribute to the success of this species in adapting to disturbed sites.

Arbuscular mycorrhizal fungal community analysis in arable soil of different agro-climatic zones of Assam (India) using denatured gradient gel electrophoresis (DGGE) fingerprinting technique

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We investigated partial 5.8S-ITS2 and LSU-D2 rDNA fragments based-DGGE fingerprints of AMF community from soil of 16 arable fields of six different agro-climatic zones of Assam. Enzyme assays (phosphatase, dehydrogenase and urease) and physico-chemical analysis of the soil were carried out. UPGMA (unweighted pair group mean average)-based cluster analysis revealed three prominent clusters of AMF community in each of 5.8S-ITS2 and LSU-D2 rDNA fingerprints of which one cluster representing two zones was common to both. Further, considerable similarity in fingerprints of cluster 2 was observed. However, the fingerprints of cluster 3 were quite dissimilar suggesting the

complexity of AMF community structure of soil. Analysis of DGGE banding pattern also suggested that studying partial 5.8S-ITS2 and LSU-D2 rDNA fragments together can give more comprehensive information for understanding complex AMF community structure of field soil. Enzyme assays revealed that phosphatase and urease activity was highest in hill zone (162 µg g^{-1} dwt h^{-1} and 29080.3 µg NH_4-N g^{-1} dwt $2h^{-1}$ respectively) and lowest (46.63 µg g^{-1} dwt h^{-1} and 10691 µg NH_4-N g^{-1} dwt $2h^{-1}$ respectively) in upper Brahmaputra valley zone (UBVZ). Moreover, dehydrogenase activity was also highest in hill zone (357.44 TPF (µg/dwt) and the lowest in Barak valley zone (12.711 TPF (µg/dwt). Soil of the studied region was mostly acidic and low in available phosphorus having maximum recorded value of phosphorus 12 Kg/ha for hill zone signifying the importance of AMF for improving soil fertility.

Genomic insights into two plant growth-promoting rhizobacteria, R62, and R81

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The use of "biofertilizers" in the form of beneficial soil microbes such as the plant growth promoting rhizobacteria (PGPRs) shows great promise in sustainable agriculture. Prominent among the PGPRs are some fluorescent pseudomonads associated with the plant rhizosphere as well as the mycorrhizosphere - a region surrounding the hyphae of so-called arbuscular mycorrhizal fungi (AMF). Under the auspices of the "Indo-Swiss Collaboration in Biotechnology" (ISCB), a consortium of Indian and Swiss research groups has recently shown that the combined application of AMF and two strains of such fluorescent pseudomonads - named R62 and R81 - strongly improve the yield and quality of wheat in marginal farms of India. These two PGPR strains had been selected after screening more than 3000 strains of bacteria isolated from roots of wheat grown in marginal soils of Bhawanipur District Budaun (Uttar Pradesh India). We sequenced the genomes of R62 and R81 using an 8-kb paired-end 454 approach with about 40-fold coverage per genome. To obtain glimpses of the genomic architecture we took advantage of the "rapid annotation using subsystem analysis" and found that the R62 genome contained 5354 protein-coding sequences (CDS) whereas the R81 genome had 5602 CDS. The closest neighbours among completely sequenced pseudomonads were *Pseudomonas fluorescens* strain Pf0-1 for R62 and *P. fluorescens* strain SBW25 for R81 each with a genome size of about 6 Mb. Through comparative genomics, we were able to identify some key features thought to be important for plant growth promotion.

Discovering native Sebacinales: the road not taken

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Sebacinales are basal hymenomycetes with diverse mycorrhizal abilities, ranging from ectomycorrhizae to ericoid and orchid mycorrhizae. No other fungal group shows a diversity of mycorrhizal types comparable to that found in the order Sebacinales. *Sebacina vermifera* (Sebacinales) colonization stimulates plant growth, seed germination, and enhances plant fitness. There are about 500 Sebacinales entries currently deposited in GenBank®, mostly from environmental root samples, demonstrating the fascinating diversity and commonness of this order, mainly as a mycorrhizal partner of plants. However, there are only 15 cultivable isolates of *Sebacina vermifera* (all Australian) currently available worldwide. To address this, we are engaged in a search for additional Sebacinales fungi by employing a high throughput PCR based screen to identify native strains inhabiting switch grass (*Panicum virgatum*) adapted to specific agro-climatic conditions of the United States.

DNA was extracted from 63 switch grass root samples collected from the Tallgrass Prairie Preserve in northern Oklahoma. Using primers specific for Sebacinales rDNA, the desired amplicon was obtained from three samples. These positive samples were sequenced and BLASTed against the NCBI database, confirming them as derived from *Sebacina vermifera*. Subsequently, formulation and standardization of selective media for isolation of *Sebacina* from roots was performed. Roots were collected from positive plants, washed, dried, and plated onto selective media for fungal isolation. So far, 126 pure fungal isolates were subjected to PCR screening using Sebacina specific primers. 12 isolates were found to be positive. Sequences from nine samples were putatively matched with *Sebacina vermifera*. Further validation is currently on going.

Early succession of ectomycorrhizal fungi on the Mt. Kamieńsk spoil heap (poland) and its relationships with tree stand development

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Tree stand development and afforestation success in degraded or post-industrial habitats depends largely on the establishment of proper symbiotic relationships between outplanted trees and pioneer ectomycorrhizal fungi (EMF), especially in initial and early phases of stand development. Spoil heaps are an example of difficult habitat where usually high efforts are needed for successful forest reclamation. Patterns of fast and effective succession of EMF accompanying a chronosequence of Scots pine (*Pinus sylvestris*) plantations (1-20 years old) on Mt Kamieńsk, an outer spoil heap of the "Bełchatów" opencast brown coal mine, one of the biggest lignite mines in Europe, were examined and analysed in relation to stand age as well as stand and tree biometric features. Also, biodiversity and sporocarp production of EMF in the stands of different tree species (*Pinus sylvestris, Quercus robur, Q. sessilis, Q. rubra, Betula pendula, Alnus glutinosa*), growing in various habitat conditions (mineral substrate of different structure, pH and $CaCO_3$ content) but of the same age (17-20 years) were compared. The studies of EMF were based on qualitative and quantitative sporocarp observations in permanent plots. In total, sporocarps of more than 80 EMF species were found. The chronosequence studies revealed a clear pattern of species sequential appearance and relationships between fungal dynamics and stand development. The most species rich were plantations of oak trees.

Physiological, biochemical and molecular events associated with root colonization of *Piriformospora indica* in the medicinal plant *Centella asiatica*: implications on host biotic and abiotic stress tolerance

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An induced symbiotic association was established between the newly described axenically cultivable endosymbiont, *Piriformospora indica,* and *Centella asiatica*, a rich source of the medicinally relevant triterpenoid asiaticoside. *P.indica* colonization also resulted in significant enhancement of host plant growth, which reached a maximum after 45 days of co-culture. The growth was correlated with an increased accumulation of host triterpenoid (asiaticoside) synthesis. The HPLC profile indicated a twofold increase in asiaticoside content (% w/w) both in whole plants and in leaves after co-cultivation. Real-time PCR analysis of key genes in the asiaticoside biosynthesis pathway revealed their strong up regulation (5 and 79 times respectively) in the leaves of the colonized plants. Quantification of phytohormones indicated an increase in auxin and cytokinin content in host plants. Our experiments indicate that low phosphate content of the growth medium (10% and 1% (w/v) of that in normal MS medium) favoured maximum *P.indica* colonization and asiaticoside production, which also resulted in increased acid phosphatase activity. Interestingly, cell wall extract prepared from *P.indica* retained the growth promoting activity and enhanced biosynthesis of asiaticoside, as demonstrated by whole fungal biomass. Our preliminary experiments to assess the promontory effect of *P.indica* symbiosis on host abiotic/biotic stress tolerance are promising and together our results suggest the immense potential of *P.indica* in improving plant production systems with least adverse effects on the environment.

Metal induction of a *Pisolithus albus* metallothionein and its involvement in heavy metal tolerance during mycorrhizal symbiosis

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Metallothioneins (MTs) are cysteine-rich peptides involved in heavy metal tolerance of many eukaryotes. Here, we examined their involvement in intracellular sequestration in the ectomycorrhizal fungus *Pisolithus albus*. A full length cDNA encoding a metallothionein designated as PaMT was cloned and characterized from *P. albus*. The PaMT contains 105bp ORF encoding 35 amino acids with a predicted molecular mass of 3422 Da and pI of 7.52. It showed homology with MTs of different organisms including the ectomycorrhizal fungus *Paxillus involutus*. We determined the transcript levels of PaMT by RT-PCR analysis using gene specific primers. The mRNA accumulation pattern of PaMT was examined with different concentrations of copper and cadmium. The full length cDNA was used to perform functional complementation in yeast mutant strains. As revealed by heterologous complementation assays in yeast, PaMT encode a functional polypeptide capable of conferring increased tolerance against Cu and Cd. We have also tested the role of PaMT in providing the increased tolerance to *Eucalyptus tereticornins* plantlets due to mycorrhizal colonization by *P. albus*. These results show that *P. albus* encode a metallothionein gene (PaMT) that they play critical role in improving the survival and growth of ectomycorrhizal trees in ecosystems contaminated by heavy metals.

Biodiversity and bioremediation functions of arbuscular mycorrhizal fungi in soils contaminated from industrial activities

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The global aim of this study is to understand how AMF communities vary within different soil contamination levels to identify AMF strains that would improve bioremediation processes. This study is part of a large integrative project named GenoRem (http://www.genorem.umontreal.ca/) that is currently being undertaken to investigate the molecular basis and biological processes involved in soil decontamination by plant-microbe associations. AMF community structure of plants growing on trace metals (TM) polluted sites was assessed using molecular fingerprinting techniques. TM contamination decreased AMF diversity and modified community structure. Specific taxa were dominant at high TM levels, including *Glomus mosseae*, while others as *G. irregulare* were frequent at all TM levels. The effect of *G. irregulare* and *G. mosseae* on TM uptake by plants at three Cd soil levels were compared. At low Cd concentration, *G. irregulare* significantly increased shoot Cd and Zn uptake compared to *G. mosseae* or non-inoculated plants. However, at high Cd level, *G. mosseae*-inoculated plants had significantly lower shoot Cd and Zn concentrations than *G. irregulare*-inoculated and control plants. This suggests that these AMF mediate different tolerance strategies to alleviate TM toxicity, and may indicate that some AMF are better suited for phytoextraction while others are more adapted to allow revegetation of contaminated sites. A metagenomic study on field samples from Montreal area covering various contamination levels (mostly PAH, PCB, TE) provides useful information on how AMF and associated rhizosphere microbes vary with mixed soil contamination levels and identify key genes involved in bioremediation of organic and inorganic pollutants.

Functional analysis of gm201 gene from arbuscular mycorrhizal fungus *Glomus mosseae* in the early symbiosis signaling

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The 14-3-3 proteins constitute a family of conserved proteins present in all eukaryotic organisms so far investigated. These proteins have attracted interest because they are involved in important cellular processes such as signal transduction, cell-cycle control, apoptosis, stress response and malignant transformation. Previous findings suggest Gi. 14-3-3 gene is involved in the protection that the arbuscular mycorrhizal (AM) symbiosis confers to the host plant against drought stress. However, neither their function nor action mechanism in early AM symbiosis have been fully addressed. To better understand how 14-3-3 regulate metabolism in early AM symbiosis . We got the full length of Gm201 gene(a 14-3-3 like gene) use 3'RACE and 5'RACE from *Glomus mosseae*. Through gene knock out and other molecular technology we found that Gm14-3-3 has two different 3'UTRs and it's 5'UTR has an important relationship with the increase of host plants' stress resistance. The result of our research provide new evidence about 14-3-3 protein's function in AM symbiosis formation and increase the stress resistance of plants in molecular level.

Genetic diversity of ectomycorrhizal genus *Russula* from Central Himalayan region of Uttarakhand.

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The ectomycorrhizal (ECM) symbiosis represents one of the most prominent and ecological crucial mutualistic associations in terrestrial habitats. Studies addressing ECM diversity in a detailed manner can only stem for basic information of the tropic status of fungi in a given ecosystem. Such knowledge is also needed for investigating wider aspect of ecology in habitats where fungi play a major role and for interpreting the evolutionary history of fungi In the present investigation, high level of genetic diversity (up to 89%) was observed between *Russula* species which demonstrates that the level of genetic variation among the species is substantial and suggests that genetic base is quite broad. The finding of present study suggests that the genetic variability present in *Russula* species was quite appreciable due to change in abiotic factors such as environmental conditions viz. rainfall, sunlight, temperature, salinity, UV exposure, height above sea level etc . This also shows that RFLP-PCR method is capable of revealing appreciable levels of polymorphism in case of *Russula* species. The study indicates that if assay conditions are carefully controlled, the RFLP-PCR methodology may provide a cheap, rapid and effective means to evaluate the genetic diversity among a large number of fungal populations.

Effect of manganese on the ecophysiological and biochemical responses in ectomycorrhizal *Eucalyptus grandis* plants

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The ectomycorrhizae are environmental alternatives to promote the growth of plants under biotic and abiotic stress. This work aims to evaluate the effect of glucose on the growth of *Pisolithus microcarpus* exposed to different concentrations of manganese and analyse the biochemical and ecophysiological responses of association of Eucalyptus-*P. microcarpus* under different levels of manganese (Mn^{2+}). For *in vitro* studies, six doses of $MnCl_2$ were tested under three glucose concentrations (0.1, 1, and 10 g L^{-1}). For in vivo experiment, three doses of Mn^{2+} (0, 200, and 1000 µmol L^{-1}) were used in inoculated and non-inoculated Eucalyptus plants. Fungal growth was stimulated in the presence of Mn^{2+}, especially at the highest concentration of glucose. Plants associated with *P. microcarpus* exhibited the highest growth and dry weight than non-mycorrhizal when exposed to Mn^{2+}. Plants inoculated in the presence of high concentrations of Mn^{2+} showed a higher photosynthetic rate and significant increases in water use efficiency and carboxylation efficiency. The oxidative enzyme, catalase, and glutathione S-transferase were activated when the fungus was exposed to metals. The activity of these enzymes in plant roots was significantly increased only in non-mycorrhizal plants. The evaluation of nutrients in shoots showed a lower content of Mn^{2+} in mycorrhizal treatments. There was an increase in the concentrations of nitrogen and phosphorus in plants associated with the ectomycorrhizal fungus. The *P. microcarpus* showed tolerance towards Mn^{2+}, indicating a possible application of this fungus in reforestation programs in areas contaminated with manganese.

Acid-tolerant arbuscular mycorrhizal fungi provide an alternative pathway of plant nutrient uptake in acidic soil

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High levels of Al inhibit plant root growth and function in acidic soil, and thus deficiency in essential nutrients, especially P, is one of the primary causes that limits plant establishment. We have found that adaptability of plants to acidic soil is greatly improved by arbuscular mycorrhizal (AM) symbiosis and thus hypothesize that AM fungi provide an alternative pathway of nutrient uptake for the plants of which the roots are damaged by Al. Seedlings of a pioneer grass *Miscanthus sinensis* were inoculated either with an acid-tolerant fungus *Glomus* sp. RF1 (RF1) or a susceptible fungus *G. etunicatum* (Ge) and grown at pH 3.2 and 5.2 in the presence or absence of P-fertilizer. After seven and thirteen weeks, shoot dry weight, P content, AM fungal colonization, root length, root tip damage (FDA-PI stain), and Al-deposition on root tips were assessed. At pH 5.2, plant growth and P uptake were significantly increased by the inoculations with RF1 and Ge as well as by P-fertilizer. At pH 3.2, however, only the plants inoculated with RF1 could survive, and those inoculated with Ge or fertilized with P could not. The plants grown at pH 3.2 showed shorter root length, more damaged root tips, and more Al-deposition on the root tips than those grown at pH 5.2 irrespective of AM fungal species and P-application. These observations suggest that acid-tolerant AM fungi play a significant role in plant adaptability to acidic soil via improving plant nutrient status, but not via alleviating root damage.

Influence of AM fungi (*G. fasciculatum* and *G. mosseae*) on growth and biochemical changes in *Macrotylloma uniflora* under salinity stress condition

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This study investigates the effect of Arbuscular Mycorrhizal (AM) colonization by *Glomus mosseae* and *Glomus fasciculatum* on growth and physiological performance of Horse gram (*Macrotylloma uniflora*) grown at different salinity stress conditions. Plant growth was assessed for salinity and Mycorrhiza treatments after 45 and 60 days of AM inoculation. The interaction of salinity and Mycorrhiza significantly improved these parameters after 60 days of AM inoculation. Inoculation of *G. fasciculatum* was found to be more promising to induce growth as compared to *G. mosseae* in Horse gram plants under salinity stress conditions. AM protects the plants from salt stress by increasing the biomass content and photosynthetic activity under salinity stress. This study also report that, AM fungi help in increment of antioxidant enzymes and MDA. Also, praline plays an important role in regulation of growth for positive adaptation of AM horse gram plants to salt stress. Inoculation of *G. mosseae* accumulated more Proline at moderate salinity level (200mM NaCl), which lead to enhances osmotic more adjustment in horsegram plants. In conclusion, application of mycorrhizal species, *G. fasciculatum* and *G. mossea,* as a biofertilizer protect the plants against salinity by alleviating the salt induced adverse effect on horse gram crop.

Enhancement in yield and antioxidant enzyme activity of *Lycopersicon esculentum* by mycorrhizal inoculation under salt stress conditions

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Arbuscular mycorrhizal (AM) fungi are obligate symbiotic soil microorganisms that colonize the roots of the majority of plants and this symbiosis enhances the performance of crop plants in stress condition. In the pot culture experiment, mycorrhizal fungal species *Glomus etunicatum* was used to determine its effects on plant growth, antioxidant activity and yield under salinity stress condition. To study the growth and antioxidant enzyme activities in tomato plants under salinity stress conditions, three different concentrations (50 mM, 100 mM and 150 mM) of NaCl were given to AM inoculated and non-AM inoculated tomato seedlings. In the present study, mycorrhizal tomato plants showed significant increase in fresh weight and dry weight. Antioxidant enzyme activities such as superoxide dismutase and peroxidase were found to be higher in AM inoculated tomato plants as compared to non AM inoculated tomato plants after 60, 90 and 120 days of AM inoculation. The fresh fruit yield, fruit weight and fruit number in a mycorrhizal tomato plant was also found to be higher than in a nonmycorrhizal tomato plant. Mycorrhizal association resulted into helping tomato plant to cope up with salt stress.

Influence of arbuscular mycorrhiza on low temperature stress of petunia and poinsettia

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In Central Europe, the economic efficiency of cultivating ornamentals under greenhouse conditions during cold periods has been challenged due to increasing energy costs. However, seasonal plants like petunia and poinsettia have to be produced for the European market during cold periods. A significant temperature reduction during the cultivation of these plants would be an economic advantage. We investigate low temperature tolerance of petunia and poinsettia induced by symbiosis with arbuscular Mycorrhiza fungi (AMF). In AMF screenings, 15 different AMF isolates were tested for alterations in the chilling tolerance of petunia and poinsettia cultivars, each with a sensitive and a tolerant cultivar against cold temperature. After mycorrhizal establishment, plants were cultivated under standard and cold temperatures.

Results show an interaction between AMF isolate and plant cultivar for both plants. Depending on Mycorrhiza-plant combination, plant growth was significantly improved, independent of AMF colonisation, for instance: For petunia different growth, parameters were analysed; single isolates resulted in an increase in shoot length. The investigations with poinsettia focused on the phase of bracts development. An influence of AMF on bracts dry matter and amount of bract leaves could be observed. These effects will be analysed in further experiments: Mycorrhiza induced tolerance will be characterised by analysing transcriptome and chilling relevant sections in carbohydrate and plant hormone metabolism. Furthermore, the influence of low cultivation temperatures on the occurrence of plant diseases will be investigated. This project is financed within the framework of BMBF-AgroClustEr WeGa by the Federal Ministry of Education and Research, Germany.

Responses of arbuscular mycorrhizal fungal communities to striga-susceptible and -resistant maize cultivars that differ in strigolactone secretion profile

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Strigolactones, a group of sesquiterpene lactones, are secreted from plant roots into the soil, where they stimulate hyphal branching of arbuscular mycorrhizal (AM) fungi and seed germination of parasitic weeds such as *Striga* spp. We have found that a Striga-resistant maize cultivar secreted a much lesser amount of 5-deoxystrigol, the most stable one among the naturally occurring analogues, than a Striga-susceptible cultivar. It was hypothesized, therefore, that the difference in the secretion profile of strigolactones might also be involved in compatibility/selectivity of AM fungi. Two maize cultivars, Striga-susceptible Pioneer 3253 and -resistant KST94, were grown in the field as well as in a greenhouse using the soils collected from two different experimental fields. DNA was extracted from the roots, and AM fungal LSU rDNA was amplified and sequenced by Roche FLX system (field experiment) or by the clone library-based dideoxy method (greenhouse experiment). AM fungal phylotypes were defined based on sequence similarities in the gene. No significant difference in the levels of AM fungal colonization was observed between the two cultivars both in the field and greenhouse experiments. Canonical correspondence analysis on the AM fungal communities revealed that soil chemical properties and growth conditions (field or greenhouse) significantly influenced the community compositions, but the cultivars did not. These observations suggest that 5-deoxystrigol may not be involved in AM fungal compatibility/selectivity.

Enhanced dissipation of polycyclic aromatic hydrocarbons (PAHS) in weathered soil by arbuscular mycorrhizal *Cucurbita moschata* amended with composted cow manure

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A pot experiment was conducted to evaluate the effects of arbuscular mycorrhizal fungi and composted cow manure on the dissipation of PAH in soils cultivated with *Cucurbita moschata* M. var. Jingou. Four treatments included inoculation with *Glomus caledonium* 90036 (AM), application of cow manure (CM), inoculation with 90036 and application of cow manure (AM+CM), and the control (without inoculation or application i.e. CK). All *C. moschata* plants were harvested after 6 weeks of transplanting. Both mycorrhizal fungi and cow manure resulted in a significant increase of the PAH accumulation in roots rather than in shoots, while plant shoots accumulated 2-4 ring PAH only. The combined application of AM+CM significantly enhanced both root and shoot bioaccumulation factors (BAFs). *C. moschata* transplantation could enhance the PAH dissipation in weathered soils, the residual PAH concentration in the soil decreased markedly by 13% in the control and declined by 38% in AM+CM treatment. *C. moschata* associated with mycorrhizal fungi and/or cow manure greatly increased the dissipation of 3-4 ringed PAH, which may be due to increased microbial populations and activities in soils, but did not influence the degradation of high molecular weight PAH. It was concluded that mycorrhizal inoculation and cow manure application might exert significant synergistic effects on the reclamation of weathered PAH-contaminated soils.

Resource transfer between different litter types, ectomycorrhizal fungi, and scots pine seedlings

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Ectomycorrhizal (ECM) fungi are abundant in forest ecosystems, and are important in facilitating nutrient uptake by the host plant. Some ECM fungi have the ability to produce extracellular enzymes, which may help release nutrients from organic material, but the extent of these capabilities and the substrates they are able to act on is unclear. High densities of ECM mycelium are often found in the moss layer of pine forests, suggesting that it may be a key source of nutrients for ECM fungi, and

previous studies have shown transfer of nitrogen from colonised moss tissue to the host plant. In this microcosm study, an isotopic labelling approach was taken to compare the ability of the ECM fungi *Paxillus involutus*, in symbiosis with a Scots pine seedling, to grow into patches of moss and pine litter and subsequently take up nutrients applied to these patches. Both imaging of the microcosms, and quantitative analysis, clearly demonstrate a greater transfer of labelled nutrients from litter patches to the host plant shoot in those microcosms that received moss litter than those that received pine. These results highlight the importance of moss for the nutrition of ECM fungi and trees, while suggesting that the ability of ECM fungi to colonise and obtain nutrients from other litter types may be limited.

Effect of *Glomus fasciculatum* inoculation on concentrations of stevioside and rebaudioside-A in *Stevia rebaudiana*

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The steviol glycosides (stevioside and rebaudioside-A) of *Stevia rebaudiana* are widely used as zero-caloric sweeteners in food and pharmaceutical industries. In this regard, the potential of arbuscular mycorrhizal fungus to enhance their production is being established, however, little is known about the mechanisms that AMF employ to improve their production. With this in mind, a pot experiment was conducted to decipher the contributions and mechanisms of AMF on production of steviol glycosides. Mycorrhizal inoculation imparted a positive impact on the concentrations on stevioside and rebaudioside-A. The higher concentrations of these glycosides in M than NM plants may be attributed to improved growth and biomass, higher trichome density and better nutrition status (P, N, Mg, Cu, Fe, Mn, and Zn). Then again, AMF colonization increased the concentration of chlorophyll, offering the plants an opportunity to increase the rate of photosynthesis and subsequently increase sugar accumulation. Steviol glycosides are formed via glycosylation of sugar therefore; increase in sugar concentration in M plants is another mechanism by which AMF increases these glycosides. The study also revealed a positive correlation between trichome density and jasmonic acid concentrations in mycorrhizal plants suggesting involvement of hormone in the synthesis of these glycosides in *Stevia rebaudiana*.

Nutrient transportation patterns in two types of ectomycorrhizas species

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Ectomycorrhizal (ECM) fungi form mutualistic symbioses with trees in boreal and north temperate forests and have key roles in regulating biogeochemical cycles. The extensive mycelium produced by many ECM fungi enables resources to be transported over large distances. Some ECM fungi are considered to be specialists and have a restricted range of host plant species, whilst others are considered generalists and can form mycorrhizas with a number of species. Little is currently known about the reasons why specialist and generalist mutualists have evolved and so this project aims to investigate the basis for these different strategies. We predict that resource stoichiometry between plant and fungal partners may be a key factor: specialist fungi may be more efficient in obtaining nutrients from litter and transporting these rapidly to host plants and in return may receive larger amounts of plant photosynthate. Here, we focus on using stable and radioisotopes to trace and quantify transfer of carbon and phosphorus, and fine-scale spatial-temporal analysis of amino acid transportation between host plants and fungi. Using Photon Counting Scintillation Imaging (PCSI), we show that amino acids are transported long-distances by ECM fungi intact and that the transportation is highly directional but the speed of transfer varies between species of ECM. We observed that transportation of amino acid and phosphorus from generalist fungus to the host plant is rapid compared to the specialist fungus.

Identification of possible plant traits associated with arbuscular-mycorrhiza (AM) responsiveness in rice (*Oryza sativa* L.)

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Arbuscular mycorrhizal fungi (AMF), abundant in aerobic soils of rain fed uplands, naturally infect upland rice. Varying degrees of AM-responsiveness for phosphorus (P) acquisition in upland rice varieties, constrained by poor P nutrition under drought prone uplands, have been reported. In the present investigation, an attempt was made to identify plant traits possibly associated with AM-responsiveness

in upland rice. The information would provide clues for possible identification of molecular markers linked to these traits associated with AM-responsiveness and subsequently marker-aided-selection of high AM-responsive varieties for P-limiting environments of rain fed uplands covering about 22% of total rice growing areas of India. Varieties with higher growth rate (GR) and high P-demand having low soil exploring capability (low root length density; RLD) were found AM-responsive. Twenty-eight germplasms representing a wide genetic base and varied ecological adaptation were initially selected. Seven varieties were further short listed based on confirmation of GR analysis. Based on three traits (GR, RLD and P-demand) associated with AM-response, the selected varieties were classified into four groups viz., (A) fast GR, strongly AM-responsive with moderate RLD and higher P-demand (Sathi 34-36), (B) fast-medium GR, moderately AM-responsive, with moderate RLD and lower P-demand (Vandana), (C) medium (Jonga) to slow (Thara) GR, AM non-responsive with high RLD and lower P-demand and (D) fast GR, moderately AM-responsive with low RLD and high P-demand (Mashuri). GR, P-demand and RLD were worked out following standard procedures. AM-response was computed based on mycorrhizal inoculation effect (MIE) using *Glomus intraradices* (source TERI, India).

Parasitism as an intrinsic rate variable in mycorrhizal functioning

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Mycorrhizal functioning and the various outcomes of arbuscular mycorrhizal (AM) symbiosis give rise to a fundamental debate and controversy regarding our conceptual views on mycorrhizal symbiosis. New approaches based upon application of techniques that are new and/or new for this field of research, such as molecular biology and dynamic modelling, may make the picture clearer. Based on what has been achieved so far, and considering what we may find in the near future, one can conclude that it might be helpful for our understanding of mycorrhizal functioning to consider parasitism as an intrinsic (ever present, essential) rate variable in the symbiosis. The various outcomes of the plant-AM fungal interactions (see Smith & Smith, Plant and Soil, 2012) and the concept of the mutualism-parasitism continuum (Johnson & Graham, Plant and Soil, 2012) naturally finds its place in this intrinsic rate variable concept. Minor adaptations in the semantic sense may be necessary, for example the preference of using the expression mutualistic-pathogenic continuum instead of the mutualism-parasitism continuum. There is a need for a broad acceptance of the classical meaning of parasitism that includes the understanding of parasitism being a nutritional relationship. This requires a clear discrimination between parasitism and pathogenicity and how these phenomena are communicated. The more dynamic modelling of mycorrhizal interactions receives attention it is likely to find support for the concept of intrinsic AMF parasitism. A similar support may come from increased understanding of host-parasite-pathogen interactions at molecular and physiological level.

Metabolite profiling in wheat during phosphorus uptake from organic and inorganic sources

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Molecular and biochemical processes as well as microbial activity facilitating root phosphorus uptake of crop plants are currently of particular interest since fertilizer resources are limited and alternative methods necessary in order to secure food sustainability. So far, gene expression signalling, the activation of phosphate transporters as well as the release of phytohormones have been investigated under phosphorus starvation conditions and in the presence of orthophosphate. However, very little is known about short and long distance signal events of metabolites like sugars and organic acids in response to phosphorus availability and source type. Moreover, it is not well understood whether and in which way microorganisms such as mycorrhizal fungi affect these signals. Here, we present the first results from a series of experiments that investigate the biochemistry of phosphorus uptake in wheat from different sources and subsequently address the impact of mycorrhizal fungi on this process. In an axenic environment, we grew *Triticum aestivum* L. cv. Cadenza on different organic and inorganic phosphorus compounds and looked into any variations in the metabolite profiles with source type. Furthermore, we examined root exudates that potentially enables phosphorus uptake from less accessible compounds.

Effects of AM fungi and plant growth promoting bacteria on the fruit yield and quality of strawberry under conditions of low fertilization

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Arbuscular mycorrhizal fungi (AMF) and Plant Growth Promoting Bacteria (PGPB) can positively affect plant nutrition and growth. Recent studies have also shown that AMF can result in improved fruit features. Strawberries are highly appreciated for their taste and for their nutritional properties, being a source of fibres, potassium, iron, vitamin C, floats, and flavonoids. In this work, we have tested if the use of AMF and/or PGPB allows for a reduction of the chemical fertilization without a decrease in yield. Furthermore, we have also assessed the effects of the inocula on the quality of the fruits, considering a set of parameters (pH, acidity, biomass, water content, concentration of sucrose/D-glucose/D-fructose, folic acid, ascorbic acid, organic acids and anthocyanins). One mycorrhizal inoculum (Mybasol Srl) and two bacterial strains of *Pseudomonas fluorescens* (called Pf4 and 5Vm1K, and selected because of their ability to solubilize phosphate and to produce IAA and siderofores), have been used, alone or in combination, under conditions of low fertilization (70% of the standard conditions). Uninoculated plants grown either at full or reduced fertilization were used as controls. Double inoculation with U2 and each of the two PGPB resulted in increased production of flowers and fruits, whose size was larger. Any inoculum increased folate, vitamin C, and anthocyanin concentrations in the fruit, with increased antioxidant activity. Our results suggest the use of the selected microorganisms as biofertilizers, allowing to reduce chemical inputs and resulting in improved nutritional and sensorial characteristics of the fruits. BIRS-OASIS Project 0147000368 (Regione Piemonte, Italy).

Temperature induced fungal regulation of nutrient exchange in the AM symbiosis

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In the arbuscular mycorrhizal (AM) symbiosis both partners cooperate and usually benefit from the association. Nutrients acquired by fungi are exchanged for plant carbon. The "fair trade" between symbionts could be affected by external conditions such as increasing temperature in the currently changing climate. The role of arbuscular mycorrhizal (AM) fungi as an important carbon storage has been previously recognised, but the extend of fungal regulation of the trade has been hard to investigate, partly because the fungus is an obligate symbiont. We manipulated the growth temperature of the extra radical mycelium (ERM) of an AM fungus (*Glomus intraradices*) associated with *Medicago truncatula* to increase sink strength and hence photosynthetic carbon flow to the fungus without changing the plant growth conditions, to test the hypothesis that the increased influx of carbon would boost phosphorus transfer to the plant and be reflected in up-regulation of the Mycorrhiza-specific plant nutrient transporters. This hypothesis implies fungal control of the nutrient exchange, with P supply to the host matching the fungal C demand; any excess of P would be stored by the fungus. A growth cabinet experiment was established using a two-compartment microcosm system where only the AM fungus had access to the heated and P enriched compartment. Samples were analysed using physiological (% colonisation, growth rates, P nutrition) and molecular methods (qPCR).

Modulatory effects of silicon on ROS status and lipoxygenase activity in mycorrhizal chickpea plants under salinity stress

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Silicon (Si) supplementation has been professed as a cost effective approach to ameliorate salt stress. In addition, arbuscular mycorrhizal (AM) fungi are considered as important bio-ameliorators of saline soils. Although Si concentrations are low in legumes, recent studies indicate that the effective mycorrhizal symbiosis is able to accumulate Si and enhance its concentration in the host plant. Present study was conducted to determine the effects of Si alone and in combination with AM on plant growth, oxidative status, and lipoxygenase (LOX) activity in chickpea (*Cicer arientinum* L.) genotypes (salt tolerant - HC 3 and salt sensitive -CSG 9505) under salinity stress. Plants were subjected to five salt treatments (0, 40 60, 80, 100 mM NaCl), three Si concentrations (0, 2 and 4 mM potassium silicate), and with /without AM (*Glomus mosseae*). NaCl inhibited the growth of plants as a result of overproduction of ROS (MDA, H_2O_2) which triggered an increase in membrane LOX activity and electrolyte leakage. However, individual applications of Si and AM markedly inhibited Na^+ uptake by roots and improved plant growth under salt stress. The positive effects of treatments were more significant in HC 3 compared to CSG 9505. In addition, AM inoculations significantly mobilized Si uptake by the roots and mitigated NaCl-induced stress through reduced ROS generation, decreased lipid peroxidation and strengthened membrane integrity. The combined treatments of Si and AM were highly effective in reducing the negative effects of salt even at higher concentrations and imparting salt stress tolerance in chickpea genotypes.

Variation in 15N as indicator of resource use in low Arctic plants

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Differences in the natural abundance of the stable heavy isotope of N (15N) in plant material may arise due to (i) direct plant uptake of N pools with different isotopic composition, and (ii) mycorrhiza mediated uptake of N. Direct uptake by roots is unlikely to fractionate the source isotopic composition. Transfer from mycorrhizal symbiont to plant discriminates against the heavier isotope resulting in 15N-depleted signatures in host plants. The use of variations in N isotope natural abundances as indicators of resource use among low arctic plant species was explored. The results show that some low Arctic species (e.g. *Gnaphalium norvegicum, Asteraceae*)thought to be obligatorily mycorrhizal (AM) are non-mycorrhizal under certain field conditions. The wide isotopic 15N range in these species when non-mycorrhizal indicates that the plants may utilize inorganic and organic N pools unassisted by mycorrhiza although frequently AM under field conditions. In the grass *Deschampsia flexuosa*, generally considered facultatively mycorrhizal, AM colonization intensity correlated with increasingly negative 15N values indicating increasing N transfer from the AM symbiont with increasing AM frequency in plant roots. This study shows that in the low Arctic, some AM host plants may be fully functional without AM symbiosis under field conditions.

Quantification of carbon fluxes in boreal forest ecosystem

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Boreal forest trees allocate a considerable part of their assimilated carbon to ectomycorrhizal (ECM) fungi, suggesting that ECM fungi may have a large potential to influence soil carbon fluxes and mediate belowground carbon sequestration. This is highly relevant in the context of predicting and mitigating environmental change and modelling carbon budgets. To assess carbon fluxes in boreal forest ecosystems more accurately, it is critical to investigate how specific components of the plant-ECM-soil interaction regulate belowground carbon flow. Within my PhD project I aim to fill in knowledge gaps of how microorganisms regulate carbon sequestration in forest soils. This will be achieved by coupling measurements of soil carbon dynamics (i.e. ECM biomass production, turnover and respiration) with fungal communities and forest stand properties. The project is part of the strong research environment IMPRESS (Integrating microorganisms in predictive models of carbon sequestration in forest soils), which aims to improve the capacity of ecosystem models to predict future national carbon budgets.

Antioxidant potential of fungi and seedlings during mycorrhizal induction

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Mycorrhizal associations implicate a chemical recognizing process being the establishment of the symbiosis triggered by signals produced by both partners. We evaluated the establishment of mycorrhizal symbiosis between two ectomycorrhizal (ECM) fungi species, *Paxillus involutus* and *Pisolithus arhizus*, and two different symbionts, *Pinus pinaster* and *Castanea sativa*. In a first step, we monitored the growth of the two ECM species in the presence and absence of the symbiont *P. pinaster*. Then we evaluated the antioxidant properties of fungi and seedlings, in response to the symbiotic process, under different contact periods. After, we tested the mycorrhization process between the two fungi species and *C. sativa* seedlings, assessing the production of tocopherols and sugars produced by both partners during the earlier steps of symbiosis. The antioxidant properties were assessed through four *in vitro* assays: Folin-Ciocalteu assay (total phenolics), Ferricyanide/Prussian blue assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and β-carotene/linoleate system assay. Tocopherols and sugars were analysed by HPLC-fluorescence and HPLC-RI, respectively. ECM fungi grew less in the presence of *P. pinaster*, with *P. arhizus* being less affected (in growth) and thus being more adapted to this association. Regarding the mechanisms of oxidative stress in ectomycorrhizal associations, the response of the fungi and plants to the contact with the host is somehow specific, revealing different forms of mutual recognition. Considering bioactive compounds production, *P. involutus* revealed a maximal value of phenolics after 6h in co-culture with *P. pinaster* and *P. arhizus* revealed increased contents of sugars and tocopherols when in contact with *C. sativa.*

Effect of AM fungi on antioxidant enzymes activity in tomato (*Solanum lycopersicum* L.)

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Arbuscular mycorrhizal fungi (AMF) have been used to enhance the plant growth and yield to maintain good health and fertility that contributes great extents to a sustainable product. The study was undertaken to identify the responses of antioxidant enzymes activities in tomato (*Solanum lycopersicum* L.) inoculated with AM fungi. Oxygen free radicals induce damage due to peroxidation of biomembranes, which lead to tissue damage, thus cause occurrence of a number of diseases. Antioxidants neutralize the effect of free radicals through different ways and prevent the body from various diseases. The Arbuscular mycorrhizal fungi (AMF) increased the antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase (CAT) activity in roots of inoculated plants, indicating lower oxidative damage in the colonized plants. It can be concluded that the mycorrhiza helps tomato plants by enhancing the antioxidant activity compared to non-mycorrhizal plants. Therefore, inoculation of indigenous AM fungi is recommended at seedling stage.

Extraradical mycelium: a functional trait to measure symbiotic performance of arbuscular mycorrhizal fungi in tropical ecosystems

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The extraradical mycelium of the arbuscular mycorrhizal fungi is one of the most important structures for nutrient uptake. The mycelium length, spatial distribution and viability are related with the effectiveness of the symbiosis in terms of mineral nutrition and plant growth. However, until now its variation and the possible factors that can explain it, has been poorly assessed in tropical ecosystems. The objective was to analyse the extraradical mycelium variation in patchy vegetation (tropical dry forest, secondary vegetation, shrub land, and grassland) during the rainy and dry seasons. This study was carried out in La Mancha Biological Research Station at Veracruz State, east Mexico. Twenty plots where randomly selected in each vegetation type. The mycelium extraction was performed with Miller and Jastrow's (1998) modified technique and the quantification according to Jakobsen's (1998).

Significant differences were found between vegetation and season ($p < 0.001$). The shrub land in dry season had the highest value (9.59 m/g^{-1}) and the secondary vegetation in rainy season had lowest one (3.87 m/g^{-1}). The results indicate that higher humidity and accumulation of organic matter in the shrub land enhance the production of mycelium and in general, plant diversity is not directly related with the production of this structure.

Impact of photoperiod and temperature on the endogenous rhythmic growth of *Quercus robur* L. during ectomycorrhiza formation

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In temperate regions, temperature and photoperiod vary during the vegetation period and greatly determine the phenology of deciduous tree development. The common oak *Quercus robur* L. is characterized by an endogenous rhythmic growth with alternating root and shoot flushes, which are regularly expressed under optimal growth conditions (16-hour photoperiod and 23°C) and were shown to influence EM formation. Until now, little is known about how temperature and photoperiod affect rhythmic growth and mycorrhization, or what the consequences are for resource allocation in the plant. Two climatic scenarios were simulated, a cold summer (15°C and a 16-hour photoperiod) and a warm autumn (23°C and 12 hour photoperiod). We then studied their impact on the development of *Q. robur* microcuttings DF159 in the presence or absence of *Piloderma croceum* in microcosms. In addition, we used 15N- and 13C-stable-isotope labelling to bridge the observed phenological patterns with nutrient allocation patterns. Our data show that modified temperature and photoperiod have significant effects on mycorrhization and rhythmic development. Mycorrhization was highly increased in plants growing under warm autumn conditions and accompanied by a longer period in the rhythmicity. In contrast, under cold summer conditions plants stopped growing and an entrance toward apical bud dormancy was noted. Sugar and starch allocations were significantly modified with phenology and enhanced mycorrhization. In summary, both oak development and mycorrhization are sensitive to changes in climate.

Detection of acid phosphatase activity in the rhizosphere of arbuscular mycorrhizal plant

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Arbuscular mycorrhizal (AM) fungi increase phosphate (P) uptake of plants. Organic P comprises 30% - 80% of total P in most agricultural soils. Some plant can utilize organic P by secretion of acid phosphatase (ACP) from root. However, utilization of organic P with AM fungi is little known. Objective of this study is to detect ACP activity in the rhizosphere of mycorrhizal plant. Sterilized Andisol was packed in pots that were separated into root and hyphal compartments with nylon net of 30 ¼m pore size. Seedlings of *Allium fistulosum* were inoculated or inoculated. Soil solution was collected with mullite ceramic tubes from 40 days to 55 days. ACP activity of solution was measured. Inoculated or uninoculated Ri T-DNA transformed Chicory (*Cichorium intybus*) and Flax (*Linum usitatissimum*) roots were cultured on modified Strullu-Romand medium including phenolphthaleindiphosphate. ACP activity in the MSR medium was detected 14 days after incubation. Mycorrhizal colonization, shoot dry weight, and shoot P concentration was higher in inoculated *A. fistulosum* than uninoculated plant. ACP activity of soil solution in hyphal compartment was higher in inoculated *A. fistulosum* than uninoculated plant at 40 to 50 days. ACP activity from *C. intybus* was higher in inoculated plant than uninoculated plant. No difference in ACP activity was observed between inoculated and uninoculated *L. usitatissimum*. Our results suggest that AM colonization increase ACP activity in the rhizosphere.

Hyphal-mediated nitrogen transfer between *Medicago sativa* and *Allium firstulosum* or *Dactylis glomerata* by arbuscular mycorrhizal hyphal networks

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Arbuscular mycorrhizal (AM) fungi provide soil phosphorus and nitrogen for host plant. They form hyphal networks between different plant species. This may affect nitrogen transfer between legumes to others. Objective of this study is to examine nitrogen transfer from legume to other plants by mycorrhizal hyphal networks. Two separate pot with plant were separated by a set of two stainless screens (34 µm) separated by 1 cm air gap. Sterilized sub soil of Andosol was added into pot. Alfalfa (*Medicago sativa*) and Welsh onion (*Allium fistulosum*) ; M×A, Alfalfa and Orchard grass (*Dactylis glomerata*) ; M×D were grown in the pots. Plants were inoculated with or without *Gigaspora margarita* and were grown for 90 days in greenhouse. One percent (w/v) $K_{15}NO_3$ (atom%) solution were applied to soil of alfalfa 3~5 days before harvest. Shoot dry weight, AM colonization , hyphal length, 15N content and N concentration were measured. Shoot dry weight, mycorrhizal colonization, 15N contents were higher in inoculated Welsh onion than uninoculated plants. Mycorrhizal colonization of alfalfa (M×A) was higher than uninoculated plants. Mycorrhizal colonization, hyphal length and 15N contents of inoculated orchard grass were higher than uninoculated plants. Mycorrhizal colonization and hyphal length of alfalfa (M×D) were higher than uninoculated plants. These results indicate that fertilized nitrogen was transferred between legume and other plants by mycorrhizal hyphal networks.

Arbuscular mycorrhizal fungi prevent N_2O-emissions from soil

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Global warming is one of the major threats for the ecological equilibrium on our planet, as it exists today. Factors determining greenhouse gas emissions need to be identified to develop strategies for their mitigation. The role of soil microorganisms and their ecological interactions in atmospheric gas exchange processes is still poorly understood. Here we show that one of the most widespread and

abundant group of soil microorganisms, the arbuscular mycorrhizal fungi (AMF), affect greenhouse gas emissions from model microcosms. We focus on N_2O, an important greenhouse gas with a very high global warming potential. Two experiments using two different methods to manipulate the presence of AMF (sterilized and re-inoculated soil & non-mycorrhizal tomato mutants) were conducted. In both experiments, N_2O emissions following a fertilization pulse were increased significantly in the non-mycorrhizal treatments, compared to the mycorrhizal treatments. The results indicate that AMF are important regulators of N_2O emissions. A reduction of their abundance e.g. by intensification of agricultural practices could increase N_2O production and hence further intensify the greenhouse effect.

Stimulation of growth, mycorrhization, nitrogen, and trehalose metabolism by naringenin and AM in chickpea under salt stress

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Flavonoids, the secondary metabolites, play imperative role in tripartite symbiosis of legumes with rhizobia and arbuscular mycorrhizae. Among flavonoids, naringenin has been found to be an effective exudate signalling nodulation and mycorrhization. Salinity limits legume growth and nodulation by modulating physiologically important secondary metabolites. Thus, green house study was intended to investigate the potential of naringenin (0, 2 and 4 μM) and mycorrhiza (*Glomus mosseae*) in enhancing nodulation and nitrogen fixation in *Cicer arietinum L.* (chickpea) genotypes (PBG-5 and DCP92-3) under salt stress (0, 40, 60, 80 and 100 mM NaCl). Uptake of Na^+ ions by the roots had deleterious effects on growth attributes, nodule occupancy and nutrient status of both genotypes, with higher negative effects in DCP92-3 than PBG-5, which could be directly correlated with higher mycorrhizal dependency and lower mycorrhizal colonization. Nodule biomass, leghemoglobin, nitrogenase (ARA) and nitrogen content along with naringenin content declined with increasing salinity. Enhanced uptake of exogenous naringenin by chickpea roots restored effective nodulation and mycorrhization pointing towards its involvement as signal molecule in tripartite symbioses. Mycorrhization and naringenin further enhanced salt induced trehalose 6-P-synthatase and phosphatase but diminished trahalase activity, ensuing higher trehalose biosynthesis in the nodules. Relative assessment of AM and naringenin treatments indicated a more prominent contribution of AM in recuperating root and shoot biomass by reducing sodium uptake and improving phosphorus status albeit nodulation exhibited higher degree of dependency on naringenin. However their complementation suggested promising correlation between fungal endophyte and naringenin in escalating rhizobial and mycorrhizal symbiosis.

Contribution of different AM species to nutrient acquisition and mitigation of ionic imbalance and antioxidant defense in salt-stressed *Cajanus cajan* (l). millsp. plants

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Arbuscular mycorrhizal (AM) symbiosis is a key component in helping plants survive under adverse environmental conditions. The role of different AM fungi on growth, nutrient acquisition and level of antioxidants was investigated in pigeonpea (*Cajanus cajan* L. Millsp) grown under saline stress. Two genotypes of pigeonpea- Paras (salt sensitive) and Pusa-2002 (salt tolerant) were subjected to four levels of salt stress: 0, 40, 60 and 80 mM NaCl, with and without AM. Four AM inoculations were used- indigenous mycorrhiza (Mi), *Glomus mosseae* (Gm), *Glomus intraradices* (Gi), and a mix of *G. mosseae* and *G. intraradices* (Gm+i). While salinity reduced plant biomass in both genotypes and caused a reduction in root to shoot (R/S) ratio, mycorrhization improved plant biomass at all salinity levels. AM inoculations enhanced salt tolerance by alleviating salt-induced reduction of N, P, K^+ and Ca^{2+} uptake while preventing excess uptake of Na^+ ions. Salt stress activated the antioxidant system by increasing the activities of antioxidant enzymes (SOD, POX, APX, MDHAR, DHAR, and GR) to a certain extent, which was further boosted with mycorrhizal inoculations. A significant variability was observed among the fungal species - *G. intraradices* (Gi) alone or in combination with *G. mosseae* (Gm) showed higher tolerance to salinity than Gm or indigenous mycorrhiza (Mi), reflected in terms of higher biomass, stronger antioxidant defence and improved nutrient acquisition. The study indicated a promising role of mycorrhiza in salt stress amelioration, with symbiosis formed by Gi and Gi+Gm exhibiting a more stable viability and efficiency under salt stress.

Anthracene and pyrene dissipation from soil using arbuscular mycorrhizae: impact on physiology and biochemistry of *Glycine max* (soybean)

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Polycyclic aromatic hydrocarbons (PAHs) in soil are of major concern due to their recalcitrance and mutagenic properties. As PAHs also disturb physiological processes in plants, remediation of PAH contamination is an on-going endeavour. Pot study was conducted to investigate a) effect

of mycorrhiza and soil microflora on anthracene and pyrene dissipation; b) responses of soybean to anthracene and pyrene stress (150 mg/kg), in presence of soil microflora [isolated from PAH contaminated soil (SM1) and non-contaminated soil (SM2)] and arbuscular mycorrhizae [(PAH contaminated soil (A1) and *Glomus* mix (A2)] at 4-8 weeks of growth. Dissipation of 5 and 20% in anthracene, 1 and 16% in pyrene at 4-8 weeks respectively was observed in uninoculated non planted soils, which increased remarkably in planted SM1 soils (anthracene: 30-59%, pyrene: 54-85% at 4-8 weeks respectively). Inoculation with *Glomus* mix and SM1 resulted in maximum PAH dissipation (anthracene: 52-70%; pyrene: 74-97% at 4-8 weeks respectively). PAH resulted in decreased root and shoot dry weights, chlorosis and damage of leaf chlorophyll in plants grown in uninoculated soils. Plants experienced oxidative stress, indicated by increased ion leakage, elevated H_2O_2 and peroxidase activity. Inoculation with SM1 and A2 had significant positive effects on alleviation of PAH toxicity, which manifested as increased root biomass and leaf chlorophyll, decreased peroxidase activity, lesser ion leakage and lower H_2O_2. The study indicated synergistic effects of *Glomus* mix and soil microflora isolated from PAH contaminated soil in stimulating PAH dissipation and subsequent alleviation of PAH toxicity in soybean.

Interactions between fungal phosphate metabolism and plant growth benefit in the arbuscular mycorrhizal symbiosis

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The utilization of the arbuscular mycorrhizal (AM) symbiosis in sustainable agriculture has been hindered by our limited understanding of what regulates fungal nutrient uptake and transport. The goal of our work was to examine the effect of the fungal phosphate (P) metabolism on plant growth benefit. We inoculated *Medicago truncatula* plants with 30 different AM fungi and analysed the P contents in root and shoots, the P pool distribution, and correlated our findings to plant growth parameters. There were high inter- and intra-specific differences in the plant growth benefit of different AM associations and plant growth benefit was strongly correlated to an increase in the P uptake by the AM fungus. Low quality and high-quality AM fungi differed in their P handling and particularly in their polyphosphate metabolism. In mycorrhizal roots that were colonized with low quality AM fungi, a higher percentage of P was found in form of polyphosphates, a fungal specific P pool that is largely unavailable for the host. This could indicate a potential P hoarding strategy of these fungi. In contrast, in high-quality AM fungal strains, the percentage of short-chained polyphosphates were relatively low. This could indicate that these fungi remobilize this storage pool faster and made P available for their host. The results will be discussed with regard to different fungal strategies in P handling that determine plant growth benefit and P nutrition of the host.

Carbon partitioning and host specificity in maize mycorrhizal system

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An Open Top Chamber (OTC) experiment was conducted at the department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore to determine the response of maize plants inoculated with various arbuscular mycorrhizal fungal (AMF) species (*Glomus intraradices, G. mossae, G. fasciculatum, G. margarita* and *G. pellucida*) under ambient (390 ppm) and elevated (550 and 750 ppm) CO_2 concentration with replicated four times in a factorial RBD.. During experimentation, root colonization, growth (roots and shoots biomass) and biochemical parameters (chlorophyll, soluble protein and PEPcase activity) were assessed besides active and passive pools of carbon (organic carbon, biomass carbon, humic and fulvic acid carbon). The results revealed that AMF especially Glomus intraradices inoculation increased the root colonization irrespective of different CO_2 levels. Dry weights of shoot and root biomass were higher (2.5 to 3.0 times) at elevated than at ambient CO_2 and in *Glomus intraradices* inoculated than other mycorrhizal maize plants. *Glomus intraradices* increased the plant biochemical changes than the other inoculated *Glomus* species. AMF inoculation improved soil carbon fractions viz., organic carbon (44.4%), biomass carbon (46.3%), humic (47.9) and fulvic acid carbon (62.8) over un-inoculated plants. Among the AMF species, *Glomus intraradices* inoculated soils had higher amounts of glomalin contents (0.449 mg g^{-1}) compare to control (0.218 mg g^{-1}). The overall data suggest that C is considerably sequestered in soils by AMF especially Glomus intraradices inoculation at elevated CO_2 concentration.

Seasonal changes in GRSP and VA mycorrhizal status in the alluvial soils from rhizosphere of selected crop plants from Nanded district, Maharashtra state, India

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Glomalin-related soil protein (GRSP) is a substance produced by vesicular arbuscular (VA) mycorrhizal fungi. In this study, we hypothesised that GRSP pool and VA mycorrhizal status of crop species varies with seasons. The rhizospheric soils samples and plant roots of three crop plants viz. *Saccharum officinarum, Glycine max,* and *Gossypium sativum* were collected from Nanded District, Maharashtra state during winter, monsoon and summer seasons. Soil samples were used to extract two GRSP fractions: Bradford-reactive soil protein (BRSP) and easily extractable Bradford-reactive soil protein (EE-BRSP). The root samples were examined for the percent root colonization. The pH of soil samples was found to be alkaline throughout the year. The alkalinity and EC was high in summer season. The high amount of organic matter, organic C, N, P and Ca was recorded in all the three crop plants during winter and monsoon seasons. The spore density (1359 per 50 gm. of soil) and EE-BRSP (0.353 mg/gm.) were observed to be relatively more in *Gossypium sativum* and *Glycine max* during winter season. However, in *Saccharum officinarum*, high amount of BRSP fraction (50.2 mg/gm.) and percent root colonization (70.12%) was recorded during monsoon season. Pearson correlation data showed that the VA mycorrhizal status and GRSP were significantly influenced by soil moisture, pH, EC, organic matter, C, K, Ca, Fe and Cu. Thus it can be inferred that GRSP content of soil and VA mycorrhizal status of crop plants may be influenced by soil edaphic factors, season and the type of host plant.

Carbon supply to the fungus triggers nitrogen uptake and transport in the arbuscular mycorrhizal symbiosis

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Arbuscular mycorrhizal (AM) fungi can substantially contribute to host plant nitrogen (N) nutrition in exchange for carbon. We studied the effect of carbon supply on N uptake and transport in the AM symbiosis via 15N labelling, enzymatic assays and qPCR analysis of fungal genes putatively involved in N metabolism. We found that an increase in carbon supply stimulated 15N transport and increased the enzymatic activity of arginase and urease in the intraradical mycelium (IRM). The fungus responded to an increase in the carbon supply with an upregulation of genes involved in N assimilation and arginine biosynthesis, but with a downregulation of a fungal urease in the extraradical mycelium (ERM). The effect on fungal gene expression in the IRM was relatively small, but genes involved in arginine biosynthesis were downregulated by an increase in carbon availability. The results indicate that carbon from the host triggers N uptake by the AM fungus, the conversion of N into arginine in the ERM, the transport of arginine to the IRM and subsequent breakdown of arginine via the catabolic arm of the urea cycle. When the fungus had access to a carbon supply independent from the host, we observed reduced 15N transport and a different gene expression pattern, which could suggest that the fungus changes its nutrient allocation strategy when less dependent on the host for its carbon supply. The results are discussed in relation to fungal regulatory mechanisms of nutrient transport in the symbiosis.

Seed germination in orchids: exogenous C and peptone in asymbiotic germination media possibly substituting the mycorrhizal association in nature

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Orchid seeds are unique for their lack of cotyledon or endosperm, minimal nutrient reserves, a reduced, mostly undifferentiated embryo and their dependence on mycorrhizal association for germination. Frank (1885) designated "mycorrhiza" to denote root-fungus and indicated the possibility of a symbiotic association in orchid seeds. Bernard (1899) apprehended the precise requirements for germination and pioneered the first-ever *in vitro* technique called 'symbiotic germination' in which orchid seeds and fungus were co-cultured. Knudson (1922) advocated that mere supply of appropriate sugar in the germination media would suffice and improvised their asymbiotic seed germination method. The present study on asymbiotic germination of five orchid species demonstrated satisfactory germination on modified Knudson-C media containing 2% (w/v) sucrose. Employment of peptone in the media further improved germination in *Dendrobium transparens* but was ineffective for *Geodorum citrinum*, *G. densiflorum*, *Vanda tessellate*, and *V. testacea*. However, peptone effectively overcame protocorm-mortality, which would otherwise cause severe loss in seedling-yield in their asymbiotic germination. Also, peptone stimulated rhizome induction in *Geodorum* spp. and speeded-up their aerial-shoot formation. While peptone triggered an over-all growth stimulation, NH_4NO_3 was ineffective or sometimes, inhibitory. It implies that the required NH_4^+ / NO_3^- ratio was violated with additional NH_4NO_3. The enhancement due to peptone can be attributed to either the additional organic-N or other growth substances from peptone. Different PGRs and modulators of endogenous auxins hardly affected germination. The germinating orchid seeds in nature may depend upon their fungal-partner principally for acquisition of C and organic N.

Arbuscular mycorrhizal fungi and phosphatase activity: stimulating N and P cycling in a mixed plantation of *Eucalyptus grandis* and *Acacia mangium*

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Mixed stands of eucalyptus with leguminous trees have been planted with promising results regarding N and P cycling. The objective of our study was to evaluate root arbuscular mycorrhizal colonization right after planting up to 20 months afterwards, in pure and mixed stands of *E. grandis* and *A. mangium*, and to verify the dynamics of P cycling. Soil and root samples (0-10cm) were obtained at seven, 14, and 20 months after planting pure and mixed stands in a second rotation, with the following treatments: only *E. grandis*, only *A. mangium*, *E. grandis* with nitrogen fertilization, and mixed *E. grandis* and *A. mangium*. High mycorrhizal colonization rates were found at seven and 14 months for all treatments. However, Eucalyptus in mixed cultures presented root colonization rates similar to those of pure and mixed Acacia (80%), significantly higher than in pure Eucalyptus stands (60%). Root colonization rates showed high correlation with the litter P content and with acid phosphatase activity in soil and negative correlation with litter C/N and C/P rates. These data suggest that the presence of Acacia roots stimulates root colonization rate of Eucalyptus roots by AMF and enhances N and P mineralization in a litter with higher contents of these nutrients, while a greater amount of AMF hyphae produces a better linkage between available nutrients and plant roots. Thus, mixed stands of *E. grandis* and *A. mangium* (without N fertilization) show good development and greater sustainability, when compared to pure stands, due to optimized AMF functions.

Diversity and functional aspects of mycorrhizal associations in photosynthetic terrestrial orchids growing in Estonian grasslands

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The establishment of a relationship with mycorrhizal fungi is a requirement for germination and early development in all orchids as they grow from extremely small seeds, nearly lacking in nutritional reserves. Over 200 species remain achlorophyllous and nutritionally dependent upon fungi at the adult stage. Partially mycoheterotrophic orchids use a dual strategy that combines mycoheterotrophy and photosynthesis, obtaining part of their carbon via mycorrhizal fungi under low light availability. The majority of orchids is fully photosynthetic at maturity but to varying degrees remains dependent on fungal infection. Identification of orchid symbionts has been limited until the advent of fungal molecular systematics, whereas the recent application of carbon and nitrogen stable isotope analyses has represented a powerful tool for understanding the orchid-fungus resource exchange. We characterized fungi associated with the photosynthetic terrestrial orchids *Dactylorhiza fuchsii*, *D. incarnata*, *Gymnadenia conopsea* and *Ophrys insectifera* by employing molecular techniques and test whether nutrients are gained from the fungal symbiont by means of stable nitrogen and carbon isotope abundance analyses. For each orchid species, roots were collected from fifteen adult individuals from grassland sites in Estonia. The fungal symbionts, forming pelotons in cortical cells of all orchid roots, were identified by sequencing the fungal internal transcribe spacer (ITS) of ribosomal DNA. Sequences revealed that several fungi belonging to *Basidiomycota* and *Ascomycota* associated with investigated orchids. All orchid species were significantly enriched in fungal-derived nitrogen, as compared with neighboring non-orchid plants, thus indicating that these orchids depend on their fungal symbionts at least for nitrogen nutrition.

Management of arbuscular mycorrhizal fungi with *Petunia hybrida* (L.) mill. as ornamental plant in Saudi Arabia - A case study

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Arbuscular mycorrhizal fungi (AMF) can improve plant growth by up taking P and help to absorb N, K, Ca, S, Cu, and Zn, improving water absorption, drought tolerance, decrease disease incidence, also enhance the salt tolerance of host plants and heavy metal sequestration. *Petunia hybrida* (L.) Mill. is one of the most favorite ornamental plants growing all over the Riyadh city of Saudi Arabia. In the present study, we would like to highlight the Petunia as a mycotrophic plant for the management of mycorrhizal fungi. Roots along with rhizosphere soils of *P. hybrida* were collected from various sites in Riyadh, Saudi Arabia for colonization study and soil samples were collected for assessment of biodiversity of AMF. The data obtained in this study indicated that *P. hybrida* is a very highly mycotrophic plants, as almost all the samples showed a very high infection with mycelium, vesicles, coiled hyphae and arbuscules. The significant variation was found with the occurrence of mycelium and vesicles among the locations but in case of arbuscules more or less same range of occurrence was found. The AMF spore was recorded from the soils of all the locations. The *Glomus* spp. was found in all the locations. Under harsh condition of Saudi Arabia, *P. hybrida* may be grown in Saudi Arabia to manage the plant growth under water stress, saline soils and heavy metal toxicity conditions.

Arbuscular mycorrhizal fungi mediated phytoremediation of Cd-contaminated soil: hyper-accumulating plant or fast-growing plant?

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Cadmium contamination in agricultural soils has become a global environmental issue for years. Phytoremediation using green plants and their associated microbes for the in situ treatment of heavy metal-contaminated soils has recently attracted considerable attention. The objectives of this study were to compare the phytoextraction efficiency of Cd by Alfred stonecrop (*Sedum alfredii* Hance, a hyper-accumulating plant) and Perennial ryegrass (*Lolium perenne* L., a fast-growing plant) from a unsterilized Cd-contaminated (1.6 mg/kg) soil and their responses to two arbuscular mycorrhizal (AM) fungal strains, *Glomus caledonium* 90036 (Gc) and *G. mosseae* M47V (Gm). There were three treatments for each plant species, including control, inoculation with Gc, and inoculation with Gm. Ryegrass and stonecrop were harvested at week 9 after seeding and week 27 after cutting, respectively. Without AM fungal inoculation, the weekly extraction efficiency of stonecrop was about 3.5 times higher than that of ryegrass. Both Gc and Gm significantly increased root mycorrhizal colonization rates and shoot biomasses of these two plant, and tended to increase soil acid phosphatase activities and plant P acquisitions. They had no significant effects on plant Cd concentrations, but all significantly increased ryegrass Cd acquisitions, while only Gc significantly elevated stonecrop Cd acquisition. The weekly extraction efficiency of Gc-inoculated stonecrop was about 5 times higher than that of Gc-inoculated ryegrass. In addition, both Gc and Gm significantly decreased soil Cd phytoavailability by elevating soil pH, causing lower transfer risks after remediation. The results showed potential application of stonecrop associated with Gc in phytoremediation of Cd-contaminated soils.

Effects of land-use and plant biodiversity on arbuscular mycorrhizal communities in roots; problems and preliminary results

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The connection between coexistence mechanisms and phylogenies in communities is a widely discussed topic in ecology. In our project, we attempt to address related questions studying arbuscular mycorrhizal communities in grassland plant roots. As the study is embedded in the German Biodiversity Exploratories design, it is possible to examine the influences of several potentially relevant factors, like land use and plant biodiversity.As arbuscular mycorrhizal fungi are directly influenced by their host plants, our hypothesis is that the identity and specific traits of host plants will cause phylogenetic clustering in AMF communities. Increasing disturbance (e.g. land use) will alter the community structure of AMF, leading to more uniform communities. The aim of the project is to obtain molecular genetic data using next-generation sequencing methods on AMF biodiversity inside plant roots, enabling to test these two hypotheses.

The influence of species and genetic level variation on the outcome of arbuscular mycorrhizal fungal-plant-herbivore interactions

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Awareness of the influence of mycorrhizal fungi on above-belowground interactions is growing, however we are still far from making generalizations due to the great variation observed in most systems. In this talk I aim to address the role of species and genotypic level variation in driving variation in arbuscular mycorrhizal fungal (AMF)-plant-herbivore interactions. Our recent research has focused on species and genetic variation influences on plant responses to AMF and both above- and below-ground herbivores. Unlike most studies, we have manipulated AMF communities, herbivore species and genotype, and plant species and genotype, in particular aphids hosting different bacterial endosymbionts feeding on various potato species and genotypes (*Solanum* spp.) above-ground and different levels of AMF diversity and nematodes belowground. Here we show both variation in aphid genotype and endosymbiont presence influence plant allocation patterns in some, but not all, AMF communities. Nematodes also influenced plant-AMF interactions and resource allocation, but this depended upon potato genotype and species. Aphid and nematode herbivory Interactions also

altered plant resource allocation and AMF associations with host plants, but often these interactions varied with species and genotype. Together this novel research approach shows the incorporation of species and genotypic variation in the study of mycorrhizal fungal-plant-herbivore research in part explains the vast variation in the observed effects in these interactions.

Arbuscular mycorrhizal fungi influence plant tolerance to root herbivory in raspberry

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The presence of arbuscular mycorrhizal fungi (AM fungi) in the roots of plants can have varied effects on insect herbivores. This study focuses on the root feeding vine weevil (*Otioryhnchus sulcatus* Fabricius) which is a pest of the perennial soft fruit crop Raspberry (*Rubus idaeus* L). A glasshouse experiment was conducted with two different cultivars of *R. idaeus, Glen Rosa,* and *Glen Ample,* which were grown with or without AM fungi and then subjected to three weevil treatments: control; high (40 eggs/plant) and low (20 eggs/plant). Plant measurements were taken periodically. Root volatiles were sampled using automated thermal desorption tubes. Ten weeks after the addition of the weevils the plants were harvested, freeze-dried, weighed, and larval mortality of vine weevils on each plant was calculated. Root colonisation and root volatile data will also be presented. Vine weevils feeding raspberry plants were found to have a positive effect on aboveground plant biomass, suggesting a degree of compensatory growth. This plant tolerance increased in the presence of AM fungi depending on the plant genotype and the density of herbivores.

Arbuscular mycorrhiza (AM) fungal spores distribution on a transect line in a Goa (India) primary coastal dune system

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Arbuscular mycorrhiza (AM) fungal spores diversity (1-D), evenness (E), relative abundance and correlation coefficient (r) were assessed on a transect line in a primary dune system in Goa on the west coast of India, post-monsoon 2011 and pre-monsoon 2012. Seven sampling stations were aligned with a distinct plants zonation pattern. Plants rooted frequency was assessed by quadrat survey, nutrients availability analyzed and edaphic factors (pH, EC, sand-grain particle size and climate data) recorded. Nutrients were typically deficient, sand grains mobile but Na relatively low. Graphic

comparison of spores is presented at the generic level (*Acaulospora*, *Gigasporaceae*, and *Glomus*), and pre-monsoon data at species level. Species richness was limited, diversity poor and evenness low, but relative abundance of dominant species high. *Acaulospora* species spores were the most abundant in all stations but that furthest from the shoreline. Relative abundances varied at both generic and species levels. Correlation analysis of the fungal species was significant on only one positive occasion. There were no significant correlations between plants and spores. It is concluded that two dominant spore-number species may be OM and NH_4-N nutrient-function efficient.

Tillage regime and depth shape the community structure of AM fungi colonizing barley

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Increasing demand for food in both quantity and quality challenges growers to improve productivity in a sustainable manner. To achieve this we must use resources efficiently in order to maintain yields whilst reducing inputs and environmental impact. Through the AM symbiosis, plants benefit from several potential advantages including improved phosphorus acquisition, water relations, and pathogen resistance. Although most crop plants are mycorrhizal, effective utilization of the symbiosis requires a much better understanding of the spatial and functional ecology of AM fungi. It is known that conventional agricultural systems harbour less diverse AM fungal communities, although reduced tillage and low input systems may support richer communities. Reduced diversity is likely due to a combination of factors including tillage practices, fertilization, and monoculture. To explore the effects of physical disturbance on the dynamics of AM fungi a survey was undertaken across an established tillage trial with treatments ranging from zero tillage to deep ploughing. Using a combination of T-RFLP and selected sequencing, we have shown that tillage regime significantly affects the structure of the AM fungal communities as well as soil nutrient levels and pH. There are significant differences with depth in both nutrient distribution, soil pH and community structure with apparent shallow and deep communities forming. These communities are shaped by tillage with subsequent upward re-colonisation of the deeper community. Subsequent work will focus on the functional differences between these communities combining molecular and tracer tools to investigate the performance of different AM fungal communities in plant phosphorus uptake.

Arbuscular mycorrhizal community changes along a successional gradient in subarctic

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Deschampsia flexuosa is a widespread grass that is obligately mycorrhizal. It is commonly found in meadows, but it can occur in mature dunes as well as in climax forests where soil and light conditions are favourable. In our study site, located in north Fennoscandia, *D. flexuosa* is found at the initial point (sand dune) and climax point (birch forest) of a successional series. The aim of our study is to investigate I) the structure of AM community in *D. flexuosa* in an subarctic environment II) The difference of AM community in *D. flexuosa* at different successional stages. We sampled 20 individuals of *D. flexuosa* and estimated AM colonization in the grass roots. Colonization rate was significantly lower in the birch forest compared to the sand dune. DNA was extracted from dried roots and the small subunit (SSU) ribosomal RNA gene was amplified using AM fungal-specific primers. 288 cloned fragments were sequenced. Sequences were assigned by BLAST searches against MaarjAM database. 4 different phylotypes were found, with no phylotype in common between the two successional stages. Our results suggest that I) *D. flexuosa* hosts a very simple AM community in the subarctic II) In *D. flexuosa* AM community diversity does not increase along the succession III) AM symbiosis is not only dependent upon the identity of the host, but also on environmental conditions to which the host and the symbiont are exposed to.

Screening of ERM fungal isolates for biocontrol potential against root pathogens

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Our working hypothesis "ERMF can help to protect ericaceous plants against root pathogen infections during the nursery phase" is based on three pre-conditions (PC): 1. ERMF can produce antifungal substances. 2. ERMF can sufficiently colonize the host plant. 3. Antifungal substances are effective against root pathogens in plants under greenhouse production conditions. More than 100 isolates collected from ericaceous plants of different native stands and from commercial substrate were screened. PC 1 was verified in dual agar plate culture tests for *in vitro* antibiosis against *Pythium* and *Phytophthora* spp., PC 2 was tested in mycorrhiza-free rooted cuttings of *Calluna vulgaris* and micropropagated Rhododendron plantlets and rated microscopically after 4-5 weeks culture in greenhouse environment. Selection criteria of 10 ERMF candidates for further greenhouse biocontrol trials were high colonization efficiency in planta plus lasting fungistatic or fungicidal effects *in vitro* plus sufficient growth in axenic subculture for inoculum supply. Biocontrol tests under greenhouse conditions gave inconsistent results due to failure in the development of either pathogens or endophytes. Hence, the hypothesis of a biocontrol effect of ERMF in plant production is not yet verified convincingly. Methodology improvements in biocontrol assays should focus on a consistent interaction period of symbionts and pathogens as well as on a replacement of labour intensive microscopic ratings by a faster, but reliable ERMF quantification method. The abundant biodiversity within the root endophytes encourages for further screenings of promising ERMF isolates.

Biodiversity of ectomycorrhizal (ECM) fungi associated with *Pseudomonotes tropenbosii* (dipterocarpaceae), Amazonia, Colombia

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The ecological importance of tropical ectomycorrhizal (ECM) fungi is poorly understood and relatively little is known about their biodiversity, community structure, and functional roles in tropical ecosystems. The EcM symbiosis of members of the plant family Dipterocarpaceae is well studied in Asian species of the family. *Pseudomonotes tropenbosii*, which is one of two species recently described for the Neotropics, is suspected to have a relation with fungal EcM symbionts. This endemic tree is only known from small populations in the Colombian Amazon, occurring in the southern part of the Guiana shield on very nutrient-poor sandy soil. Since 2010, we document the diversity of ectomycorrhizal fungi associated with *P. tropenbosii* and study their community structure. We have three study areas in primary Amazonian rainforest dominated by *P. tropenbosii*, where we have collected EcM roots tips, basidiocarps belongs to ECM taxa as well as soil samples. Seventy-nine of putative EcM morphospecies have been collected that belong to commonly known families of EcM fungi, Boletaceae (20 species), Russula (19 species), Clavulinaceae (6 species), Cantharellaceae (6 species), Agaricales (*Amanita* 6 species, *Cortinarius* 4 species). Many of these species may represent new species. Fungal species occurring in EcM root tips were further identified using the Internal Transcribed Spacer of the ribosomal DNA and identified a Sebacina type A. The fungal community structure is studied by 454 pyrosequencing of soil samples.

A hot water treatment, a new physical method for controlling white root rot, alters bacterial communities in mycosphere of the pathogen and arbuscular mycorrhizal fungus

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Hot water treatment (HWT) is a method to control white root rot, caused by *Rosellinia necatrix* (Rn), on Japanese pear trees. The disease has brought severe losses in fruit production since the infected trees result in death. HWT is a method with the aim of curing the diseased trees by dripping 50°C of water on rhizosphere to raise soil temperature up to 35°C at 30cm in depth. Effectiveness of HWT to control Rn has been reported, as a relapse rate after two years was about 50%. To understand the control mechanism and impacts on the other soil microbes of HWT, we investigated changes of bacterial community in mycosphere of Rn and arbuscular mycorrhizal fungus (*Gigaspora margarita*, Gm) and in surrounding soil. Water bath treatment mimicking HWT (WBT) showed Rn died in a live soil but survived in a sterilized one. In WBT, embedded Gm spores increased germination rate with the presence of soil microbes. HWT and WBT increased numbers of culturable bacteria in mycosphere and soil. Isolation rate of antagonistic bacteria to Rn was higher in Rn mycosphere than in soil, and the trend retained after HWT. PCR-DGGE based on the 16S ribosomal RNA gene analysis showed bacterial communities of both mycosphere revealed several distinct bands and the patterns were changed by HWT and WBT. These results suggest that HWT alters bacteria communities in Rn and Gm mycosphere and that the changes are involved in the Rn death and the enhancement of Gm spore germination.

Mycorrhizal status of *Enkianthus campanulatus* (Ericaceae)

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Enkianthus is the most basal extant genus in the plant phylogeny of the family Ericaceae. Its members house arbuscular mycorrhiza (AM)-like hyphal structures in their roots. As yet, no study has explicitly surveyed the component of fungal species involved. We first examined the mycorrhizal status of *Enkianthus campanulatus* by microscopy, and then identified associating mycorrhizal fungal flora by both culture-independent and -dependent methods. For the culture-independent method, AM

fungi were identified directly from roots by using AM-specific primers. For the culture-dependent method, root segments were applied for fungal isolations. Roots of *E. campanulatus* were collected at four distantly located study sites in Japan. Intracellular hyphal coils were commonly observed in root cortical cells of all examined roots. Fungal DNA sequences of the partial small subunit ribosomal RNA gene were obtained from the most of the root segments examined. A phylogenetic analysis showed that they all belonged to *Glomus* group A lineage. For the culture-dependent method, 51 fungal isolates were obtained from approximately 600 surface-sterilized root segments from 14 individual trees. DNA sequences of 14 isolates were phylogenetically nested with *Phialocephala* spp. and *Acephala* spp.. Six, 4 and 3 isolates were nested with *Cryptosporiopsis* spp., *Meliniomyces* spp. and the members of Helotiales, respectively, and each closely related with the fungal taxa that have ability to form ericoid mycorrhizae (ERMs) with ericaceous plants. These results implied that *E. campanulatus* associates with both AM and putative ERM fungi.

Bacteria associated with AMF vesicles in native plants growing on extreme petroleum hydrocarbon polluted soil

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Many literature reports clearly demonstrated that arbuscular mycorrhizal fungi harbor bacteria in their mycelia. However, the role of these bacteria in AMF remains unclear. In this study, we investigated molecular biodiversity of bacteria associated with vesicles of AMF extracted from *Solidago rugosa,* a native plant growing on an extreme petroleum hydrocarbon polluted soil of an old refinery basin that was used to dump petroleum wastes over many decades. Roots of *S. rugosa* were surface disinfected and digested using hemicellulases. Vesicles were then extracted by micro-dissecting roots and were subjected to whole genome amplification. PCR, cloning and sequencing were performed using 18S rDNA in order to identify AMF, while Bacteria were assigned to operational taxonomic units (OTUs) using 16S rDNA. We found a remarkable AMF diversity inside *S. rugosa* roots. Out of five vesicles that were genotyped so far, AMF ribotypes were identified as *Glomus eburneum, Archaeospora schenckii* and three uncultured *Glomus* spp. The highest proportion of bacteria OTUs (33 %) associated to *G. eburneum* were assigned to an uncultured bacterium, while 30% of OTUs were identified as *Pseudomonas* sp. However, 23% of OTUs were chloroplast DNA contaminants. Further studies will be undertaken to investigate the role of these bacteria in bioremediation.

Structural and functional dynamics of arbuscular mycorrhizal fungal communities during revegetation by endemic species in a Mediterranean ecosystem

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Arbuscular mycorrhizal fungi (AM fungi) play a key role in the establishment of vegetation in disturbed areas. However, structural and functional dynamics of AM fungal communities during development of vegetation have not yet been well understood. Revegetation trials by endemic plants are being conducted in limestone quarries in Southwestern Australia, which belong to the world biodiversity hotspots. Two hypotheses were addressed in the present study: only AM fungi that are highly tolerant to environmental stress associate with the plants in a primary stage of revegetation, and development of forest improves soil quality and thus diversifies AM fungal communities. Two quarries in a cement factory, Quarry 7 (Q7) and Quarry 5 (Q5) in which planting was conducted in 1996 and 2009, respectively, differing in the developmental stage of vegetation, were chosen, and roots and rhizosphere soils of *Acacia saligna* and *Eucalyptus gomphocephala* were collected in 2010. DNA was extracted from the roots, and the community compositions were determined based on AM fungal LSU rDNA sequences. *A. saligna* was grown in soils inoculated with AM fungi with or without 5% organic matter (peat moss) in a greenhouse. No significant difference was discovered in AM fungal community composition between the quarries. However, the two communities were functionally differentiated: the fungi from Q5 improved plant growth irrespective of the presence of organic matter whereas those from Q7 improved the growth only in the presence of organic matter. The community compositions in the greenhouse experiment are now being investigated.

Arbuscular mycorrhizal and dark septate endophyte fungal associations in some woody tree species from tropical and subtropical forests of Manipur, Northeast India

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Sixteen trees species from six sites (tropical and subtropical natural mixed forests) of eastern Himalayan region were examined for arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal associations. Intensity of AM and DSE fungal colonization and distribution of fungal structures

varied with the host species over a period during the summer season. Of the 74 root samples belonging to 16 trees species examined, 69 samples from 15 species had AM fungal structures. In contrast, DSE fungal structures were found in all the root samples examined. All the tree species had Paris-type AM morphology. Total root length with AM colonization ranged from 9.1% (*Schima wallichii*) to 66.7% (*Dillenia indica*). Root length with hyphae varied between 9.4% (*Dipterocarpus tuberculatus*) and 22.1% (*D. indica*); hyphal coils ranged from 6.5% (*S. wallichii*) to 37.9% (*Alstonia scholaris*); arbusculate coils varied between 1.7% (*Quercus griffithii*) and 20.1% (*Wendlandia tinctoria*) and vesicles ranged from <1% (*Alnus nepalensis, A. scholaris, Castanopsis hystrix, Cedrela serrata, Emblica officinalis, Melanorrhoea usitata, Wendlendia pendula, W. tinctoria*) to 5.1% (*D. indica*). Extent of total root length with DSE colonization varied from 6.6% (*Q. griffithii*) to 33.1% (*D. tuberculatus*); DSE hyphae ranged from 4.9% (*Q. griffithii*) to 25.3% (*D. tuberculatus*); while microsclerotia varied between <1% (*A. scholaris, Hiptage acuminata*) and 9.9% (*M. usitatia*). All the AM and DSE fungal variables varied significantly ($P<0.01$) among different tree species. However, no significant differences among sites existed for fungal variables in *D. indica, M. usitata* and *Tectona grandis* occurring at two sites.

Ectomycorrhizal fungal communities in pot culture seedlings of *Dipterocarpus alatus* introduced by soil inocula from a natural forest and a plantation

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Dipterocarp forests have been much decreased caused by excessive harvests in Southeast Asian countries. It is well recognized that ectomycorrhizal (ECM) fungal symbioses enhance soil nutrients uptake of the host plants, therefore the utilization of ECM fungi are expected to be the key factor for reforestation. Although many of the inoculation techniques have been applied to induce ECM formation of dipterocarp seedlings, using a soil inoculum could be a simple method to introduce diverse ECM fungi. Therefore, we examined a relationship between the ECM fungal community and growth of *D. alatus* seedlings inoculated with soil inocula collected from both a dry natural dipterocarp forest and a plantation of *D. alatus*. Autoclaved soils were also applied to make control seedlings. At harvest, various growth increments of the seedlings were measured, and ECM fungal communities were investigated based on the sequences of ITS rDNA. Furthermore, Principal component analysis (PCA) was applied to detect some effective ECM fungi on growth of the seedlings.

The shoot and root dry weights of seedlings inoculated with forest soil were significantly greater than those of the others. Mycorrhizal root tips were classified into 3 morphotypes, which were then divided into 18 phylotypes base on molecular analysis. Most of the phylotypes were recognized as ECM fungal taxa, i.e. *Laccaria, Lactarius, Tomentella, Pyronemataceae,* and *Trichoromataceae.* Among them, PCA suggested that *Lactarius* sp.1, *Tomentella* sp.2, and *Trichoromataceae* 1, obtained from the forest soil inoculum, might be effective for growth promotion of the *D. alatus* seedlings in the pot culture condition.

Diversity and community structure of ectomycorrhizal fungi along an altitudinal gradient on Mount Fuji

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Limited knowledge exists on microbial diversity patterns and their causal mechanisms. It is critical to advance our understanding of biodiversity-environment relationships to establish conservation strategies of both macro- and microorganisms to rapidly changing environments. Diversity patterns along altitudinal gradients are often studied by ecologists to explore determining factors of biodiversity. We investigated species diversity and community structures of ectomycorrhizal fungi along an altitudinal gradient and examined potential factors influencing the diversity pattern. We also compared richness-altitude patterns between trees and ectomycorrhizal fungi. Ectomycorrhizal roots were sampled at four sites along an altitudinal gradient on the northwest slope of Mt. Fuji, Japan. Ectomycorrhizal root tips were identified by morphological and molecular analyses based on sequences of the internal transcribed space region of nuclear rDNA. We identified 308 fungal species, from which more than 500 species were estimated to exist in the study sites. Ectomycorrhizal fungal diversity increased with mean annual temperature of the site. The diversity of ectomycorrhizal fungi was not clearly correlated to the diversity of trees, suggesting that richness-altitude patterns may differ between above- and belowground organisms. We also revealed ectomyhcorrhizal fungal compositions were distinctively different between the high elevation and low elevation sites. We discuss the relative importance of regional- and local- environmental factors influencing the diversity and community structures of ectomycorrhizal fungi.

Impact of heavy metal pollution on fungal biodiversity and community structure

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In the northern part of Belgium, pyrometallurgical industry has polluted vast areas with heavy metals such as zinc, cadmium, and lead. Many soil born microorganisms and plant species are hence exposed to elevated concentrations of metal ions. Only those organisms that have sufficient metabolic plasticity or in which tolerance mechanisms have evolved, can survive the exposure to elevated concentrations of heavy metals. The most sensitive organisms are quickly outcompeted by more resistant or tolerant species. Through this selection pressure, biodiversity generally decreases with increasing concentrations of metal ions. In the most extreme cases, even almost all organisms disappear, creating so-called metal deserts. To investigate the effect of metal ions on the fungal biodiversity and community structure, we analysed the fungal community using 454 pyrosequencing and a number of soil parameters on a contaminated site and a control site. We hypothesized that the contaminated site would have a decreased biodiversity and that the abundance of tolerant species will increase compared to the control site. We show that both the control field and the contaminated field are dominated by ectomycorrhizal fungi. Furthermore, *Suillus luteus*, a fungus of which we have proven in the past to have zinc and cadmium tolerant subpopulations in this region, became more abundant in the contaminated site. Some other, more sensitive species disappeared almost completely from the contaminated site.

The influence of host genotype and soil conditions on fungal and microbial community of poplars

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The genus *Populus* (Salicaceae) play important roles in various branches of industry as well as it can be used for afforestation of post agricultural lands, recultivation of areas degraded by industry and for production of bioenergy. Poplars and their hybrids are known to be very diverse in terms of their physiological characteristics but little is known about how host genotype influences fungal (ectomycorrhizal and arbuscular) of microbial communities associated with poplar roots and present in the rhizosphere. The objective of our study was to analyse the impact of host genotype and soil conditions on dual (ECM and AM) mycorrhizal colonization of roots and composition of microorganisms in the rhizospere of four selected poplar clones in three 16-19-years-old common-garden experiments localized in polluted and unpolluted area. Our analyses revealed the importance of poplar genotype in the ability of poplar roots to establish symbiotic relationships with mycorrhizal fungi, and tolerance to heavy metal pollution. However, genetic effects were strongly modified by environmental conditions, which influenced fine-root development, the ratio of ECM and AM colonization and community structure of ECM fungi. The results excluded host genotype as a significant factor that affects ECM communities and revealed the dominant role of site characteristics and soil depth in determining the structure and composition of fungal symbionts associated with the roots of poplar clones. On the other hand we found significant genotype effect on biomass of bacteria Gram (+) and Gram (-), Actinomycetes, Protozoa and AM in rhizosphere of studied poplar clones.

Tomentella italica - not only mycorrhizal fungus. tripartite association: fungus-aphid-elm tree roots

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Fifty years ago Polish entomologist Danuta Krzywiec (1962, 1964) described relationship between three organisms: unidentified soil fungus, root-feeding aphid *Mimeuria ulmiphila,* and roots of elm tree (*Ulmus minor*). Recently based on sequencing of the PCR-amplified fungal ITS rDNA, we identified hitherto unknown fungal partner as *Tomentella italica*. Mycelium of *T. italica* produce, yellowish-brown, hollow sclerotia-like cysts (galls), 2-3 mm in diameter, with safe place for the aphids inside. These roundish cysts with apterous exules of *M. ulmiphila*, has been observed all year round (with highest abundance in autumn) on fine roots of *Ulmus leavis* trees grown in the Kornik Arboretum (Poland). The fungal cyst walls are composed with three layers, differing in texture. Close to elm roots, beside cysts with aphids, typical globular, blackish, sclerotia attached to monomitic rhizomorphs has been found. Both, sclerotia and rhizomorphs, were also identified as belonging to *T. italica*. Best to our knowledge it is the first observation of sclerotia formed by *Tomentella.* Fungi from *Tomentella* genus are generally considered as ectomycorrhizal partner of wide range of host plants. However, during our studies, mycorrhizas of T. italica on investigated *U. leavis* trees were never observed. Identification of *T. italica* as a fungal partner in tripartite association with aphid and elm tree roots clearly shown that this fungus may also be involved in multitrophic interactions other than ectomycorrhizal symbiosis. In all probability *T. italica* utilized honeydew produced by the aphids as a carbon source.

Effect of summer rain pulse on ectomycorrhizal community of *Quercus kelloggii* in California mixed-conifer forest

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In Southern California, there is a few but large precipitation event in summer. The event should affect not only pine and oak trees in this area but also the mycorrhizal fungi and function. But detail analysis of the dynamics of ectomycorrhizal fungi has not yet been done. Here, we examined the change in ectomycorrhizal community of *Quercus kelloggii* in California mixed-conifer forest after natural rain and artificial water addition. To monitor soil condition, sensor for soil moisture, CO_2, and temperature were set. Automated minirhizotron was also set to examine the change in fine roots and fungal hyphae. In total, more than 100 ectomycorrhizal fungal species were detected. After 7 days from natural rain or water addition, ectomycorrhizal diversity clearly increased. Number of ectomycorrhizal fungal species was more in water addition treatment than in natural rain treatment. *Cenococcum geophilum* was the most dominant ectomycorrhizal species (24-64%). The number of root tips formed by *C. geophilum* did not change after water addition, but the percentage tended to decrease. It was because that the number of ectomycorrhizae formed by the other species increased. Our results showed that ectomycorrhizal diversity and community structure change after summer rain. However, the abundance of most dominant ectomycorrhizal fungal species did not change. It might mean that summer rain did not change ectomycorrhizal function in this study.

Community structure of ectomycorrhizal fungi in an *Abies religiosa* forest, through an altitudinal gradient

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Worldwide there are 52 species of the genus *Abies* (Pinaceae), ectomycorrhizal fungi have been reported associated with 9 of them. *Abies religiosa* (H. B. K.) Schl. et Cham. (fir), is the most widely distributed species in Mexico where it forms monospecific forests with fragmented distribution in altitudes of 2000 to 3600 m. These forests provide environmental services in carbon sequestration and water recollection. Young trees are used as Christmas tree and mature trees are a timber resource. In other Abies species has been observed high species diversity of ectomycorrhizal fungi associated. This paper describes and analyzes the composition and structure of the ectomycorrhizal community, based on morphological and molecular (ITS rDNA) study of the morphotypes found in an altitudinal gradient. 24 morphotypes were found associated with *A. religiosa*. One corresponds to *Cenoccocum geophilum*, the others morphotypes will be identified by molecular study. The richness, diversity and community structure of morphotypes change along the altitudinal gradient. The richness and diversity decrease with the gradient. This pattern may be related to levels of nitrogen and carbon compounds available, which change along this gradient.

The interaction between ectomycorrhizal fungi and mycorrhiza helper bacteria in the mycorrhizosphere of *Pinus thunbergii*

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Mycorrhiza helper bacteria (MHB) were isolated from *P. thunbergii* mycorrhizosphere soil in Tottori sand dune, Japan. The bacteria isolated were classified into 12 types by using PCR-RFLP and DNA sequencing analyses. The majority consisted of Gram-negative bacteria, such as *Ralstonia* sp., *Pseudomonas* sp., *Burkholderia* sp., *Serratia* spp., and *Rhizobium* sp., and the others were Gram-positive bacteria, such as *Bacillus* sp.1, *Bacillus subtilis*, *Streptomyces* sp., and *Mycobacterium* sp. The effect of the bacteria on the hyphal extension of *S. granulatus* was investigated by two methods: confrontation assay and soaking assay. No bacteria enhanced the hyphal extension in the soaking assay, while the confrontation assay showed that *Ralstonia* sp. promoted the hyphal extension. This result suggested that *Ralstonia* sp. produced some substances to stimulate the hyphal extension of *S. granulatus*. The mycorrhiza formation in *S. granulatus-P. thunbergii* symbiosis was significant stimulated by *Ralstonia* sp. and *B. subtilis* compared with control (without bacteria). Moreover, the importance of *Ralstonia* sp. inoculum dose in the stimulation mycorrhizal formation of *Pinus thunbergii-Suillus granulatus* symbiosis was examined using *in vitro* microcosm. The MHB significantly stimulated mycorrhizal formation at the inoculum dose of more than 108 cells ml^{-1}. Thus, the interaction between mycorrhiza and soil bacteria, especially mycorrhizosphere bacteria, is constructed in Pinus thunvergii-mycorrhizal fungi symbiotic system.

Mycorrhizal fungi associated with *Vanilla planifolia* under different management systems

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Soil samples were collected at sites of *Vanilla planifolia* cultivation under different level of technification (low and high) to study species richness of mycorrhizal fungi associated with this plant species. Spore extraction from soil and observations of mycorrhizal structures in vanilla roots were performed. The samples will be studied morphologically and molecularly (ITS) to identify them. All soil samples showed mycorrhizal fungi spores regardless of the level of technology of cultivation. Spores found in soil could not be identified to species level by morphological description but appear to be specific for vanilla; however we hope will be identified by molecular techniques. The root structures study showed binucleate and multinucleate hyphae, also studying molecular structures indicates that it may be at least one species of Tulasnella and one of Ceratobasidium, but have not been possible to identify the species. The spores in soil indicate that there are more species of mycorrhizal fungi potentially associated with vanilla, but have not been detected by the study of roots. It is necessary to establish trap cultures with sorghum and maize plants as a strategy to better study mycorrhizal fungi species found.

Arbuscular mycorrhizal fungal diversity in saline-alkaline soils of Turkey

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Turkey is a Eurasian country stretches across the Anatolian peninsula in Western Asia and Thrace in the Balkan Region of southeastern Europe. Central Turkey has a huge potential for cereal agriculture. However, salinity-alkalinity is one of the cardinal problems in Turkish Agriculture. The most common approaches in the reclamation of saline-sodic soils are to leach salts from top layers by irrigation or to provide a source of calcium to replace excess sodium. These methods are costly and have environment load. Arbuscular Mycorrhizal (AM) fungi are one of the major members of mycorrhiza and play a huge role in ecosystem. AM fungi have symbiotic associations with most of terrestrial plants and enhance their tolerance for adverse conditions to grow. AM fungi can contribute to improving agricultural performance in saline-alkaline soil conditions; however, there is still lack of information regarding AM fungi in connection with soil chemistry, to evaluate AM fungal effects on plants and to select the suitable AM fungal species for soil reclamation-remediation purposes in such harsh environments. The aim of this study is to investigate AM fungal community compositions under barley agriculture and natural plant cover and to explore the main factors structuring AM fungal community in saline-alkaline soils of Turkey. We selected the study sites that generate saline and alkaline strength graduate in barley cultural area near a saline lake in central Anatolia. AM fungal community structures were investigated with T-RFLP and cloning method, and analysed relationships with soil conditions.

Biogeography of arbuscular mycorrhizal fungal communities in hypoxic soil - evidence from the Slovenian, Italian, and Czech mofette fields (natural CO_2 springs)

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Mofette fields are ambient temperature CO_2-exhaling gas vents occurring in regions of tectonic or volcanic activity worldwide. These specific and extreme ecosystems are characterized by high soil CO_2 and reduced soil O_2 concentrations, leading to a locally hypoxic environment, a long-term abiotic selection pressure affecting present biota. Arbuscular mycorrhiza probably represent the most widespread terrestrial symbiosis and play central roles in terrestrial ecosystem functions, yet information on arbuscular mycorrhizal (AM) fungal communities from mofette areas or in general, hypoxic environments, is limited. The quantification of AM fungi from plant roots sampled in the Stavešinci mofette area (Slovenia), confirmed the presence of apparently unique AM fungal assemblages across a range of soil CO_2 concentrations. In this study, the impact of biogeography on AM fungal communities, unique to locations with high soil air CO_2 concentrations, compared to control areas within and between mofette fields, will be presented. The study has been conducted within mofette areas in there different countries: Slovenian meadow and forest mofettes in Stavešinci, Bossoleto mofette in Italy and Cheb Basin mofettes in Czech Republic, using terminal restriction fragment length polymorphism and metagenetic approaches (454 pyrosequencing).

Impact of climate and nitrogen deposition on ectomycorrhizal fungi of the Scottish native pinewoods

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Ectomycorrhizal (ECM) fungi are intrinsic components of many forest and alpine ecosystems and understanding their responses to the environment is essential in a time of global environmental change. Although we have identified many environmental variables that have the potential to influence ectomycorrhizal communities, there are research gaps in understanding how different environmental drivers, such as climate change and nitrogen deposition, interact to influence the ECM community, and how the importance of these drivers varies at different spatial scales. Assessing the roles of environmental variables in structuring communities' at large spatial scales is important in predicting how environmental change will impact on regional species assemblages. Here we present work conducted to understand the impacts of climate and nitrogen deposition on ECM fungal communities of native Scots pine (*Pinus sylvestris*) at multiple spatial scales. Utilising regional scale gradients in climate and nitrogen deposition in Scotland we show that climate can be influential in structuring communities at a regional scale, and that the impact of climate on ECM communities is greater than that of nitrogen deposition in this study system. We also investigate whether local scale variations in climate, such as along altitudinal gradients, can cause local patterns in the ECM fungal community by intensively surveying a single site with altitudinal variation using high throughput sequencing methods. This work enhances our understanding of the potential impact of environmental change on ectomycorrhizal communities and suggests that climate change may lead to changes in the composition of ECM communities across entire regions.

Fungal communities as determinants of carbon sequestration in forest soils

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Boreal forests play an important role in the global carbon cycling and have been identified as the primary terrestrial sink for atmospheric CO_2 globally. The majority of this C is stored in soil pools. Fungi (saprotrophic - by decomposing soil organic matter, and mycorrhizal - by mobilizing plant nutrients) play fundamental roles in the ecosystem and regulate soil carbon sequestration. Our knowledge of fungal interactions on a molecular level is rapidly increasing, but scaling up from molecular-level to the scale of ecosystems and landscapes represents a major challenge. My PhD project is aiming to understand how stand properties, such as nutrient availability and climate, shape fungal communities and their function, as well as effects of forest management. Cutting-edge molecular tools (454-sequensing, enzyme analyses, and stable isotopes) will be used to derive information about fungal diversity and community composition. Novel isotopic techniques (14C bomb carbon modelling) will be used to estimate long-term carbon dynamics in soils. Multivariate statistics will then be used to yield correlative relationships between fungal communities and soil carbon dynamics. My study aims towards building a predictive statistical model of fungal community composition and its influence on carbon dynamic across the landscape. The project is part of the IMPRESS strong research environment initiated to increase our possibility to better predict the future carbon balance of boreal forest ecosystems, enable prognoses, and provide factual basis for policy decisions about forestry, landscape planning and emission quota.

Arbuscular mycorrhizal fungal dynamics in a range of crops under conventional agriculture

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Agriculture typically relies on high inputs to maintain or improve yields. This has been made possible by ready access to cheap fertiliser and agrochemicals in concert with breeding of varieties that grow well under high input agriculture. This time of plenty is ending with, for example, an increasing limitation of phosphate bearing rock. The arbuscular mycorrhizal symbiosis may aid reduced input agricultural systems but our ability to utilise this symbiosis to aid crop growth is hampered by a lack of knowledge relating to the biology and ecology of the interaction. It is known that the diversity of this group is reduced under conventional agriculture but is higher under reduced tillage or fertilisation although the mechanisms that drive the symbiosis remain obscure. Any low diversity of AM fungi in conventional agriculture may further be compounded by host preference and/or temporal dynamics both of which have been observed in the symbiosis. We have followed the temporal dynamics of indigenous fungi in a range of crop plants grown under conventional agricultural techniques. Using a combination of molecular techniques, we have observed clearly defined differences in colonisation both in different crop species and over the course of the crop cycle. Although patterns are variable over two different non-consecutive years the patterns remain robust and are driven by a limited range of fungi. Types detected include fungi that cluster within *Glomus* groups A and B, *Diverispora, Paraglomus, Archeospora,* and *Ambispora.*

Succession of forest flora and underground mycorrhiza

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Mycorrhizal symbiosis between host tree and mycorrhizal fungi is one form of the mutualistic relationships that plays important roles in regeneration and growth of the tree. Among mycorrhizal trees the family Fagaceae, which includes several dominant species of cool-temperate forests, is of social, and forest ecological importance. Recently a shift from Pinus-Quercus mixed-forest to rather pure, Castanopsis forest has occurred through natural succession in Kyoto, Japan; which may accompany simplification of microbial flora underground. To clarify the symbiotic relationship between Fagaceae trees and their mycorrhizal partners and possible correspondence of their succession, we monitored 1) sporocarp occurrence, 2) mycorrhiza that colonized on Fagaceae seedlings, and 3) mycorrhizal flora in the rhizosphere at both a pure and a mixed forests. As a result, 1) in total 7087 sporocarps comprising 96 species were found in the two forests, each of which consisted of 3 plots. A Bray-Curtis similarity analysis showed that the community of sporocarps in a forest was different from the other, and the species diversity based on Shannon's index was significantly higher in the mixed forest. 2) The mycorrhiza formed on Castanopsis seedlings in the pure forest was more various than those on Quercus seedlings in the mixed forest. 3) A molecular technique-based analysis demonstrated a hidden mycorrhizal community, which was not found in the above tests. Thus, the diversity of sporocarps and mycorrhizal flora in the rhizosphere was inconsistent with that of mycorrhiza, which colonized. In the presentation, a possible mechanism that caused this inconsistency will be discussed.

Diversity of potato associated arbuscular mycorrhizal fungi in root samples and rhizosphere soil from the Peruvian Andes

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Arbuscular mycorrhizal fungi (AMF) are found as root symbionts in the majority of agricultural crops, given the potential benefits of AMF, their characterization as well as the identification of the major driving forces behind AMF community composition could provide valuable information for improving sustainable management practices in agriculture. We characterized the diversity of AMF species associated with potato (*Solanum tuberosum*) in the Peruvian Andes. Root and rhizosphere soil samples were collected from potato fields at 4 different altitudes (ranging from 2,658 to 4,075 m.a.s.l.) at 3 plant developmental stages (emergence, flowering, and senescence). DNA was extracted and a 1500 bp SSUpart-ITS-LSUpart (partial small subunit, internal transcribed spacer, partial large subunit) rRNA gene region was PCR amplified using AMF specific primers. Clone libraries were constructed and at least 40 cloned inserts per sample were screened by RFLPs with three different enzymes. Representatives of diverse patterns were Sanger-sequenced. Phylogenetic trees were calculated in order to identify the AMF at species level. A rough semi-quantitative estimation of diversity was done based on the abundance of RFLP patterns in the clone libraries. A total of 147 distinct sequences were obtained, representing at least 23 different species from 10 different AMF genera, several of them unknown or previously not characterized by DNA sequences. Our preliminary results show that the overall AMF diversity found in the soil and the roots samples is different and the occurrence of certain genera and species varies depending on altitude and plant developmental stage.

Lab-scale quantification of biotic influence on weathering in forest ecosystems

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Increasing demand for bioenergy in Sweden has led to increased harvesting of forest residues, causing concern about the sustainability of supply of base cations necessary to maintain forest growth. More reliable estimates for weathering rates are required at spatial and temporal scales relevant for sustainable forest management. For the last two decades lab and field experiments conducted in Sweden have demonstrated interactions between minerals and mycorrhizal fungi, but there is still uncertainty about their quantitative significance and how they are regulated. Our hypothesis is that biological weathering by symbiotic fungi and associated bacteria make a significant contribution to the mineral requirements of forest trees. This biological weathering is regulated by plants in response to changes in environmental conditions. Different mycorrhizal fungi will be given access to different minerals in partitioned systems in which the mycelium is disrupted or left undisturbed, analyses of mobilized mineral nutrients will be made from micro-lysimeters and from plant nutrient analysis. The distribution and activity of mycorrhizal fungi interacting with mineral substrates in both microcosm and field experiments will be studied with metagenomic and metatranscriptomic approaches utilizing massively parallel 454® and Illumina ® sequencing technologies. These methods will allow patterns of gene expression associated with weathering to be examined on spatial scales ranging from millimeters to hundreds of meters. Selective allocation of carbon to different mycorrhizal taxa will be followed using Stable Isotope Probing. The results will provide small-scale quantification of rates of mineral release for different minerals and fungi.

Arbuscular mycorrhizal diversity in P deficient soils of Tamil Nadu

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Arbuscular mycorrhizal fungi (AMF) form a mutualistic symbiosis with vascular plant roots and exert several benefits including enhanced P and Fe uptake, biotic and abiotic stress tolerance, carbon sequestration, soil nutrients feed-back etc. Understanding the community structure of these fungi in agricultural soils would help to ensure significant contribution to the success of sustainable agriculture. In present investigation, an attempt has been made to identify AMF diversity in P deficient soils of Northwestern Tamil Nadu. The soil samples were analysed for edaphic features, AMF – spore density, root colonization, species richness, and diversity. AMF spore count of the soil was negatively correlated with available P and positively correlated with phosphatase activity. The morpho-typing of AM spores from these soils implied that the diversity of AMF increased with decrease in P availability. Principal Component Analysis of soil variables clearly discriminated the soil samples with a total variability of 62%. The soil organic carbon, pH and alkaline phosphatase showed distinct negative correlation with AM spore count. AMF belong to genera *Acaulospora, Glomus, Gigaspora* and *Scutellospora* were found to be dominant in the native soil. All the dominant AMF were isolated and trap culture was done to identify the species. The P absorption efficiency of these isolates will be assessed to select efficient AMF, suitable as inoculants for P deficient soils. The present study confirms the wide occurrence of AMF in nutritionally poor soils where crops experience short growing period and low nutrient status.

Infectivity and community structure of arbuscular mycorrhizal fungi at different soil depths in high-input agroecosystems of China

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Modern intensive farming practices seem to be a threat to arbuscular mycorrhiza (AM) formation. But some studies showed vertical niche partitioning to be one way by which the high species diversity of AM fungi can be maintained in intensively managed agroecosystems. In the present study, we investigate the mycorrhizal infection potential and molecular diversity of AM fungi at different soil depths of greenhouse vegetable lands, open vegetable lands, and farmlands at three sites in north

China. The results showed that AM fungal propagual abundance was lowest in highest intensive land, and showed a negative relationship with soil depths. But AM fungal diversity in the trapped roots was not always low, especially in the deep layer of greenhouse vegetable lands. Furthermore, land use intensity and soil layer strongly affected community composition as well as the presence and prevalence of many AM fungi. Of the 13 total fungal types investigated, Glo 6 and Div 1 accounted for 72.7% of AM fungal clones analyzed and could be considered as 'generalists' as they were present in each land use and soil layer. An estimated 40% of the 13 observed fungal types could be classified as 'specialists' as exclusively found in specific soil layer and under specific land use intensities. Comparing to AM fungal composition of different soil layers, deeper soil layers still contained some unique fungal types, especially in greenhouse vegetable lands. It implied these deep soil layers may represent a hidden source of additional AMF diversity.

Diversity of ectomycorrhiza of beech seedling growing under different temperature conditions

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Recent warming because of climate change is strongly affecting terrestrial biological systems such as forests, among which impacts of different air temperature on root growth and mycorrhizal diversity are believed to strongly affect belowground processes and carbon dynamics. We have monitored the occurrence of types of ectomycorrhiza (EM) on beech seedlings grown in rhizotrons at four different temperature conditions (15-25° C - NHL, 15-25° C and cooling of roots - DHL, elevated temperature in a greenhouse - RA, outside air temperature - LO). Fine roots growth was studied using Rootfly, types of ECM were identified by anatomical-morphological method after Agerer (1991), sequencing of the ITS1-5.8S rDNA-ITS2 ribosomal region and construction of phylogenetic trees. We found significant differences in root growth parameters between seedlings growing at different temperature regimes. In all four treatments, the dominant ectomycorrhizal fungus belonged to the genus *Scleroderma*. Phylogenetic analysis regrouped all Scleroderma ITS1-5.8S rDNA-ITS2 sequences discovered in this study into the same clade that also contained five *S. areolatum* and two *S. verrucosum* sequences. Low level of intra-clade genetic differences suggests that this clade is homogenous and probably corresponds to a single species. According to available information it was impossible to unambiguously determine which, if any, of the two *Scleroderma* species (*S. areolatum* or *S. verrucosum*) the identified clade represents.

Environmental biomonitoring of radiocesium accumulation by mycorrhizal and saprobic fungi collected in an area with lower fallout after the accident in Fukushima Dai-ichi, Japan

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During the Fukushima Dai-ichi Nuclear Power Plant (NPP) accident after tsunami caused by the earthquake on 11 March 2011 a total released amount of approximately 1.5 x 1017 Bq 137Cs was estimated in Fukushima Prefecture. Although high accumulation of radiocesium (137Cs and 134Cs) in mushrooms and lichens were reported after Chernobyl NPP accident, the mechanism of absorption and accumulation by fungi is still not fully understood. Trends of higher accumulation rates among mycorrhizal than saprobic fungal species were reported. The main purpose of this study is to understand the behaviour of radiocesium in natural ecosystem. The study site is 167 km southeast far from Fukushima Dai-ichi NPP. Around this area the highest radioactivity in the air was 2.5 μSv/h measured few days after the accident. After April 2011, mushrooms were collected almost periodically and measured fresh weight. The concentration of radionuclides was determined by gamma-ray spectrometry using a high purity germanium detector. During April and June 2011, wood rotting fungi as *Schizophyllum commune* (137Cs: 5719 Bq/kg, 134Cs: 5506 Bq/kg) and *Daedaleopsis tricolor* (137Cs: 5377 Bq/kg, 134Cs: 4858 Bq/kg) and saprobe, *Calvatia craniiformis* (137Cs: 2847 Bq/kg, 134Cs: 1782 Bq/kg) were unexpectedly higher than mycorrhizal fungi as *Astraeus hygrometricus* (137Cs: 2847 Bq/kg, 134Cs: 1782 Bq/kg). After autumn, 2011, the highest concentration was measured in a mycorrhizal fungus, *Cortinarius* sp. (137Cs: 840 Bq/kg, 134Cs: 659 Bq/kg). At present, the radiocesium concentration in mushrooms tends to be decrease, but the potential of increase of radiocesium concentration in mycorrhizal fungi should be still considered.

Common mycorrhizal networks amplify size inequality in *Andropogon gerardii* monocultures

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Within communities, arbuscular mycorrhizal fungi can interconnect plant root systems through hyphal common mycorrhizal networks (CMNs). CMNs can influence the distribution of limiting nutrients among interconnected individuals, potentially affecting competition and consequent size hierarchy, i.e., the frequency distribution of plant sizes. Using a microcosm model system, we investigated whether the members of *Andropogon gerardii* monocultures compete via CMNs. We grew *A. gerardii* seedlings with isolated root systems in individual adjacent containers while preventing, disrupting, or allowing CMNs among them. Fertile soil was placed within the containers, which were embedded within infertile sand in microcosms. We assessed mycorrhizas, leaf tissue mineral nutrient concentrations, size hierarchies and the growth of nearest neighbours. We found that plants interconnected by CMNs had greater colonized root length and higher concentrations of phosphorus and manganese than plants never interconnected or severed from CMNs. Interconnected plants were larger on average and had greater size inequality than those never interconnected or with severed connections. Only with intact CMNs were whole-plant dry weights negatively associated with those of their neighbours. Our results suggest that in the absence of root system overlap, CMNs likely promote asymmetric competition belowground, thereby exaggerating size inequality within *A. gerardii* populations. Nevertheless, the increased mean size of plants interconnected by CMNs likely explains why *A. gerardii* does not reject mycorrhizas.

Effects of natural/anthropogenic stressors and chemical contaminants on mycorrhizal symbiosis in wetland plants

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Mycorrhizal fungi, colonizing over 80% of all plants, were long thought absent in wetlands; however, recent studies have shown many wetland plants harbour arbuscular mycorrhizae (AM) and dark septate endophytes (DSE). Recognition of the vital wetland services is accompanied by growing concern for their vulnerability and continued loss, which has resulted in an increased need to understand mycorrhizal symbiosis in wetland plants. Factors regulating this symbiosis need to be better understood to predict plant community response and ultimately wetland functioning when confronting natural and human induced stressors. Our studies focused on the effects of water quality, hydrology, hurricane, and sedimentation on AM and DSE colonization in coastal marsh and effects of an antimicrobial, triclosan (TCS), on AM spore germination, hyphal growth, and colonization in wetland plants. The former, mesocosm studies simulating coastal marsh ran for five years. In the latter studies, AM spores and wetland plants were exposed to 0, 0.4, and 4.0 μg/L TCS concentrations in static renewal and flow through exposures for 21 and 30 days, respectively. AM and DSE colonization were significantly affected by individual and interactions of four variables in mesocosm experiments. Similarly, spore germination, hyphal growth, and AM colonization in wetland plants were significantly inhibited by exposure to the TCS at environmental concentrations. Our results showed that natural and human induced alterations in environmental variables and chemical contaminants significantly impact levels of AM spore density,

spore germination, and colonization in wetland plants. The resulting impacts on plant community structure and ecosystem function require further study.

Effect of heavy metal pollution in the association of arbuscular mycorrhizal (AM) fungi growing in soils contaminated with paper mill effluents

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Arbuscular Mycorrhizal (AM) fungi are known to enhance plant tolerance to a variety of stresses including nutrients, drought, metal toxicity, salinity and pathogens all of which may affect a plants success in a contaminated or polluted soil. The pulp and paper mill industry has been categorized as one of the twenty most polluting industries in India and responsible for soil & water pollution. Our aim was to develop some restoration protocol in such disturbed habitats and to study the beneficial rhizospere fungi like AM fungi that are tolerant to various stresses. This study was undertaken to assess the influence of paper mill effluents on mycorrhizal colonization (%) and endogonaceous spore population (ESP) in polluted and the non-polluted site. *Glomus* was the dominant AM genus isolated during the present investigation. It was isolated from both polluted and non-polluted site. The chemical analysis of soils contaminated with solid and paper mill effluents showed high concentrations of heavy metals. The present experimental findings statistically revealed the significant correlation and regression relationship of mycorrhizal colonization and endogonaceous spore population with various physico-chemical properties of polluted and non-polluted soil. So, the isolated strains of AM fungi can be of great interest since they can be used for inoculation of the plant species that might be used for rehabilitation of contaminated site. This can be also indicated that these AM isolates may partially protect plants against the toxicity of heavy metals due to the higher metal tolerance capacity.

Effect of soil conditions on the spore population and diversity of arbuscular mycorrhizal fungi (AMF) in Kaziranga national park, Assam

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The present work involves the study of influence of certain soil conditions on spore population and diversity of arbuscular mycorrhizal (AM) fungi in grassland and woodland ecosystems of Kaziranga National Park, Assam, India. The soil conditions considered were pH, temperature, moisture content, available nitrogen, available phosphorous, available potassium, and organic carbon. The AMF spore population was positively correlated with the soil temperature and moisture content but negatively related with the soil pH. The spore population, however, was negatively correlated with soil phosphorus, nitrogen and the organic carbon content but it was positively correlated with the available soil potassium content. A remarkable variation in spore population and species composition of AMF in different seasons across the two ecosystems was also obtained. The work so far revealed clear variation in the occurrence of different AMF species in all the four sites and revealed higher spore count and species diversity in grasslands than in woodlands found in Kaziranga National Park. The high diversity of AMF so far observed in the soils of Kaziranga National Park indicated that further investigation might lead to the discovery of new AMF species from this mega diversity zone of the country.

Arbuscular mycorrhizal (AM) profiles of coastal soils in South India: a case study from Karaikal district, Puducherry

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AM fungi are ubiquitous and are found in diverse habitats ranging from arctic to the tropics, arid to aquatic environments and stable plant communities to highly disturbed ecosystems. However, the AM fungal community and AM colonization of plant roots may vary greatly in different soil types. On the other side, soil salinity is a problem of grave concern because it adversely affects growth and development of plants especially in arid and semi-arid regions. In India alone, salinity affects 7 million hectares of land, which is mainly attributed to irrigation with ground water of high salt content, sodic, and alkaline parent material. Additionally, arid lands exhibits high evaporation rate and insufficient leaching of ions due to low precipitation result in a supra-optimal level of accumulation of salts, which render arid and semi-arid soils unproductive. In this case-study, coastal belt of Karaikal located at Puducherry state, India was selected as a study area. 15 species of vascular plants screened for mycorrhizal colonization and 9 species of AM mycorrhizal fungal species were recorded in the rhizosphere soils based on seasonal field surveys. Physio-chemical analyses of rhizosphere soils showed that all the study sites were alkaline, high Electrical Conductivity and ESP levels. Among the AM mycorrhizal genus, *Glomus* and *Scutellospora* populations were dominant in this ecosystem. The potential adaptation of this dominant indigenous AM fungi and their ability to colonize saline tolerant plant communities in the coastal regions will help to remediate the coastal soils with suitable host plants.

Studies on mycorrhizal status of tsunami affected coastal vegetation in South India: post tsunami scenario

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The earthquake of magnitude 9 was occurred off the coast of Sumatra on 26 December 2004 caused a tsunami that traveled from Indonesia to the Indian coast. In South India, the Nagapattinam-Karaikal-Cuddalore shelf on the Coromandel Coast, facing the Bay of Bengal, was the worst affected by the tsunami surge and inundation of seawater. It has caused severe damage to coastal vegetation and soils of the coastal belt of South India turned saline. The present work has studied the floristic changes in the tsunami-affected coastal vegetation of Karaikal district of Puducherry, India and its mycorrhizal profiles during post-tsunami period from 2005 to 2012. Results of present work revealed that adverse impact of tsunami on vegetation within inundated areas of the district was intense and removal of flora was high. Recovery of vegetation was rapid within seven-year period and mainly a process of regeneration rather than primary succession. This is mainly because of higher amount of salts were leached down after the rainy seasons due to high porosity of the sandy soils, creating a favorable environment for plant growth. The comparative account changes in the flora and its mycorrhizal profiles due to tsunami and possible reasons will be discussed in detail.

Arbuscular mycorrhizal fungi of open cast coal mine spoil soil and possible exploitation of its properties in phytoremediation

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Arbuscular Mycorrhizal Fungi (AMF) has been considered as keystone mutualists in ecosystems due to their unique position at the root-soil interface. Open cast coal mining is widely practiced in north-Eastern region of India and consequently leading to soil degradation due to heavy metal contamination and acidification. These degraded soils can be reclaimed using metal tolerant indigenous AM Fungi. Altogether forty-seven rhizospheric soil samples were collected from open cast coal mining areas and fourteen from un-degraded forest of Tinsukia, Assam, India. Average soil pH ranged between 4 to 5 in degraded soil. Concentration of available phosphorus and potassium in degraded soil was lower than that in un-degraded soil. Both the degraded and un-degraded soils had almost similar concentrations of organic carbon, nitrogen, calcium, and magnesium. The order of heavy metal concentration in young spoil soil was Zn>Ni>Pb>Cu>As>Cd>Cr, whereas it was Zn>Ni>Pb>Cu>Cd>As>Cr in old spoil soil. The old rhizospheric soil harboured more spores than the new soil irrespective of seasons. The spoil soil harboured more AM spore in winter season than in rainy. The older soil was richer in AM species (21 species) than the younger soil (7 species). Glomus was the dominant AM Fungi. AMF occurrence frequency was higher for *Glomus* and lower for *Gigaspora*. Total GRSP of the spoil soil was quantified to estimate the hyphal growth and heavy metal stabilization.

Mycorrhizae in the Colombian giant bamboo

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Guadua angustifolia is a woody bamboo from the Andean mountains. Its optimum growth occurs in well drained soils developed from clastic rocks in mountainous terrain from 800 to 2100mosl. Its multifunctional hollow culms grow quickly and have many uses in handmade furniture, handcraft, structural and cladding panels in construction and as decorative elements in architecture. Guadua is part of the colombian culture and is used in recovery of watersheds, ecological restoration, has high potential for carbon capture systems. The pachymorph rhizome of these clumping bamboos is associated with arbuscular mycorrhizal arum, and paris type. The Colombian natural bamboo

forest have different species of *Acaulospora: A. lacunosa, A.mellea, A.colombiana, A. laevis A.rehmii, A.scrobiculata, A.delicata* and *A.tuberculata* and several species of *Glomus*. The amount of external mycelium in soil reaches up to 20.4 mg / g, with a concentration of 14 mg glomalin/g soil. Under natural conditions bamboo forest mycorrhization rates reach 100% even for the non-photosynthetic new shoots. The infective potential of bamboo forest soil is 150 MPN/100g, and in recruitment systems in dilutions 1/40, the sorghum reaches the same mycorrhization at the end of the first month. By inoculating arbuscular mycorrhizal fungi on cuttings in the greenhouse, the bamboo form the association, but has a moderate mycorrhizal dependency of 44% with *G. manihotis* and *A. longula*. The dual inoculation with plant growth promoting bacteria such as *Enterobacter* sp. and *Pseudomonas* sp. promotes mycorrhization and shoot dry mass of the mycorrizhal cuttings

Effect of VAM fungi on growth and survival of water stressed plant babool (*Acacia nelotica*) seedlings under nursery condition

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The effect of vesicular-arbuscular mycorrhizal (VAM) fungi on growth and drought resistance in *Acacia nelotica* seeding was studied in unsterilized soil under nursery conditions. Seedlings were kept under five different soil moisture levels (20%, 16%, 12%, 8%, and 4%), which were maintained by regulating the watering timetable. The seedlings were treated with a mixture of VAM fungi (*Glomus mosseae, G. fasciculatum, G. etunicatum and G. aggregatum*) fertilized with nitrogen (@250gm N/pot) and phosphorus (500gm P/pot) singly or in combination with VAM fungi. Untreated seedlings were considered as control. After three months of sowing different level of drought treatment gradually reduced seedling volume, percent survival, and total dry biomass up to significant level. Fewer than 20% moisture there is no significant difference in seedling volume, dry weight and percent survival of treated seedlings with control, except VAM + fertilizer treated seedlings. On the other hand, at 4% moisture level these parameters differ in all of the three treatments provided. Under extreme moisture, stress condition (4%) VAM fungi treated seedlings significantly differ from the control in their dry weights (44% more) and percent survival (31% more). Application of VAM fungi enhanced root colonization with decrease in soil moisture level, while a reverse trend was observed in control seedlings colonized by native VAM fungi.

Potential of different crop species for Ni and Cd phytoremediation in periurban areas of Varanasi district, India with more than twenty years of wastewater irrigation history

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Heavy metals introduced into soil by indiscriminate dumping along with irrigating with sewage effluent often lead to toxic accumulation of heavy metal ions, which not only impair soil productivity but also cause health hazards by entering in to food chain via soil-plant-animal-atmosphere continuum. To evaluate the potential of different crop species for Ni and Cd phytoremediation fifteen crop species comprising of cereals, vegetables, and flowers were collected from differentially contaminated soils (DTPA-Cd 5.7-6.75 mg kg^{-1}, DTPA-Ni 16.50-20.85 mg kg^{-1}). The tissue metal concentration and relative efficiency of transfer of heavy metals from soil to plant (transfer factor) for various groups of crops

were worked out. The uptake of Cd and Ni increased with contents in soils and the major part of taken up Cd and Ni is translocated to the floricultural crops with maximum accumulation occurred in roots. Values of translocation factor of Cd and Ni were ranged between 0.2 to 0.8 and 0.2 to 1.0 respectively for the different crops studied. The mean total root colonization by arbuscular mycorrhiza (AM) in these soils ranged from 15% for cauliflower to 76% for marigold, suggesting a certain adaptation of these indigenous to such environmental stress. Among the different crops studied marigold with highest translocation factor, mycorrhization and Cd and Ni content in root part holds considered as a potential economic crop for phytoremediation.

Combined application of arbuscular and ericoid mycorrhizal fungi together with compost and biochar products originating from bio-waste

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The EU funded project REFERTIL has the mission to contribute to the transformation of urban organic waste, food industrial by-products, and farm organic residues from a costly disposal process into an income generating activity. The improved output products will be safe, economical and standardized compost and biochar products (biochar originates from different types of carboniferous plant and/or animal waste biomass, it is produced under low temperature carbonization conditions at an average 500°C in the absence of air) containing phosphorous and nitrogen that can be economically and beneficially used by farmers. Within this framework, the Leibniz University of Hannover has the objective to combine these biochar and compost products with inocula of arbuscular and ericoid mycorrhizal fungi. The joined application of mycorrhizal fungi together with biochar or compost shall combine the positive effects on plant nutrition and soil properties the latter have with the known effects of mycorrhizal fungi to improve plant tolerance against abiotic and biotic stress. A number of isolates of arbuscular and ericoid mycorrhizal fungi are tested in combination with biochar and compost in tagetes and rhododendron pants regarding mycorrhizal colonization and functioning. Later field applications will be done with horticultural crops. One additional objective is to improve methodology of mycorrhizal quantification regarding ericoid fungi by molecular methods (quantitative PCR).

Effect of *Glomus intraradices* Schenck & Smith (Arbuscular mycorrhizal fungi) and sewage water irrigation on the growth of two varieties of *Pennisetum purpureum* Schumach (Napier grass).

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Pennisetum purpureum Schumach commonly called as Napier grass belongs to the family Poaceae It is used as fodder grass and the two varieties commonly cultivated in Maharashtra are Hybrid Napier and Giant Napier. Arbuscular Mycorrhizal fungi are important to the vegetative and reproductive success of most of the plants. Fodder crops are important, as it is very difficult to extend the cultivated land for fodder production. The only way out is to increase the biomass production per unit area on the existing cultivated lands. Sewage water is now very frequently used for irrigation. Sewage pollution may be a threat to the indigenously present AM fungi, which play a predominant role in availability of nutrients from the soil. The present experiment was carried out to study the effect of *Glomus intraradices* and sewage water on the growth of Hybrid Napier and Giant Napier and to study the effect of sewage water on AM fungi. The study showed that application of *Glomus intraradices* significantly increased the fresh weight, dry weight, chlorophyll content, nitrogen and phosphorus uptake of the Hybrid Napier and Giant Napier compared to the uninoculated plants. The treatment in which *Glomus intraradices* and sewage water was given also showed significant increase in the fresh weight, dry weight, chlorophyll content, nitrogen and phosphorus uptake but the percent root colonization and spore density was less compared to the treatment, which received only *Glomus intraradices*. Giant Napier showed better growth performance compared to the Hybrid Napier.

Effect of mycorrhization on crops raised on land disturbed by industrial effluents

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In India, more than 53% of the land area is degrades due to many reasons. One of the reasons for this is rapid Industrialization and the disturbance of land by the discharge of effluents. During a survey of VAM, fungi many species of different Mycorrhizal spores encountered from the polluted soil with industrial effluents of the industrial area of Satharia, Jaunpur. Two crops maize (cereal) and fresh water irrigated field have been selected for the study. Both effluent and fresh water irrigated plants showed efficient vesicular arbuscular Mycorrhizal infection (88%). Non VAM infection decreased where as VAM infection increased from initial to find stage of maturity. Mycorrhization plays a strong positive role in excellent growth of crops raised in the disturbed land.

Assessment of incidence of arbuscular mycorrhizal fungi and their efficacy on two species of Amaranthaceae- a disputed member

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Arbuscular mycorrhizal fungi (AMF) are among the best-known soil microorganisms present around the globe and that establish mutual symbiosis with the majority of higher plants. However, some angiospermic families that are reported to be non-mycorrhizal or rarely mycorrhizal which includes Amaranthaceae. The absence of mycorrhiza in such disputed family promoted us to evaluate the mycorrhizal status in amaranthaceae plants. The present investigation was undertaken in order to characterize and quantify the AM fungal association on two disputed species of Amaranthaceae growing in Dharwad district of Karnataka. An attempt was made to screen the effect of AM fungi on growth, biomass, and nutrient uptake on two disputed plants such as *Amaranthus paniuculatus* L. and *Achyranthus aspera* L. under greenhouse condition. Both disputed plants in this present work possessed mycorrhizal colonization. The result showed the percentage variability with the inoculation of different AM fungi on these two experimental plants. *Glomus macrocarpum* was found to be most efficient AM fungus to increases biomass production and P uptake. Therefore, it can be concluded that even disputed plants may be made mycorrhizal by inoculation of indigenous AM fungus.

Synergistic effect of AM fungi and other microbial inoculants on growth response of *Vigna unguiculata* (L) verdc.

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The combined inoculation of selected rhizosperic microorganisms was used to minimize fertilizer application and to maximize plant growth and nutrition. Green house pot experiments were conducted to evaluate the effect of AM Fungi (*Glomus fasciculatum*) along with the dual inoculation of AM fungi (*Glomus fasciculatum*) with *Rhizobium, Azotobacter, Azospirillium* and a tetra inoculation of AM Fungi (*Glomus fasciculatum*), *Rhizobium, Azotobacter, Azospirillium* in *Vigna unguiculata* (L) Verdc. Growth parameters such as plant height, dry weight of root and shoot, spore number, per cent root

colonization, numbers of nodules were recorded, and P and N uptake were estimated at the intervals of 30 and 45 days. Results revealed that inoculation of AM Fungi (*Glomus fasciculatum*) + *Rhizobium, Azotobacter+ Azospirillium* showed increase in all the growth parameters when compared with single and dual inoculation. The combined inoculation of bacteria and AM fungi evidence provide that these two organisms are synergistically involved in beneficial effects of *Vigna unguiculata* (L) Verdc.

AMF inoculation in bioremediation systems VG-biobeds: impact on the efficiency and the sustainability of the system

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VG-biobeds are a bioremediation system designed in 2009 in Switzerland to struggle against the pollution of surface water caused by pesticides use. Effluents are collected in a tank and irrigate vertical modules vegetated and filled with specific substrate. The latter fix the active molecules that are degraded by microorganisms while water is evapotranspired by vegetation. The aim of this research is to determine whether AMF inoculation improve the efficiency and the sustainability of this system. For this purpose, two factorial microcosm experiments were conducted in climate chamber. The first was performed without pesticide by using three factors: substrate (growing media and compost), vegetation (grass and alfalfa), and AMF (*Glomus intraradices*, commercial mixture containing several species of *Glomus* and control). The second experiment was also conducted with three factors: substrate, AMF, and pesticides (two fungicides, herbicide, cocktail of pesticides and control). The results show that mycorrhization rate depends more on the substrate and root biomass than on bio-inoculation. In addition, all pesticides tested, although greatly diluted, show toxic effects on AMF and vegetation. The protective effect of the AMF on vegetation is not certified. Regarding the substrate, the compost has a protective effect stronger than the growing media but this one produce a plant biomass significantly higher, which compensates results. According to this study, bio-inoculation cannot be recommended. However, it is not contraindicated and could be useful to regenerate mycorrhization and vegetation after a season of effluents treatment. Long-term experiments should be useful to determine the real benefits of AMF inoculation.

Arbuscular mycorrhizal fungi strain potential on flax

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Flax is a major crop in the Canadian Prairie provinces with close to 900 000 hectares in culture. This major crop is considered beneficial to animal and human health as oil extracted from flax seeds contains a high proportion of polyunsaturated fatty acids. Moreover, flax is resistant to most cereal diseases, to a diversity of stresses and is highly mycotrophic with arbuscular mycorrhizal fungi. Consequently, flax has been selected for evaluation of its mycorrhizal potential with a diversity of AMF strains propagated at the 'Glomeromycetes *in vivo* and *in vitro* collection' in Agriculture and Agri-Food Canada's collection and with *Piriformospora indica* (Sebacinales) as a symbiont. Under greenhouse conditions, 10 of the best performing strains were tested on flax. Yield, shoot, pod and seed biomass were surveyed and flax response to AMF estimated through root colonization levels. Root parameters were analysed using WinRhizo software. Root colonization levels never exceeded 18% and were obtained with both G. irregulare inoculants. The shoot biomasses, the diameter sizes, and number of roots between 0.5 to 0.1 µm (fine roots) were affected differently by the different inoculants. The best colonized plants produced a lower number of capsules per plant but with a higher total biomass. The best AMF strain tested over flax were *G. irregulare* and *G. lamellosum*. The optimal strain for both number of capsules and weight of capsules was *P. indica*.

Effects of plant growth promoting microorganisms on the fruit yield and quality of tomato under field conditions

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Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth Promoting Bacteria (PGPB) can improve plant nutrition and stress tolerance, thus resulting in healthier plants. Here, we report the selection of some PGPB, their use alone or in combination with two AMF inocula, and the resulting effects on tomato under conditions of reduced fertilization (70% of the standard application), in a field trial. Three PGPB strains of *Pseudomonas fluorescens* and *P. marginalis*, and two mycorrhizal inocula (U1 and U2, Mybasol srl) were used alone or in combination on tomato plants. AMF were added at transplant, while bacteria were provided with the watering system, by drip irrigation. Uninoculated plants grown at full or reduced fertilization were used as controls. The experiment resulted in 21 treatments, consisting of 168 plants each, for 3528 plants. Ripe berries were harvested and analysed

for acidity, pH, water content, concentration of ascorbic acid, soluble sugars, nitrites, nitrates, organic acids, lycopene and βcarotene. In spite of the reduced chemical inputs, yield was not affected. Inoculated plants were healthier and more resistant to pathogens. Berries were larger and, with the inoculum U1, they had higher dry mass. *P. fluorescens* C7 decreased the concentration of nitrates and oxalic acid; *P. fluorescens* 19Fv1T increased the concentration of total sugars. Rhizospheric microorganisms can therefore modulate the qualitative feature of the fruits and promote biomass increase. Different combination of inocula can be designed, according to the final use of the fruit (industrial transformation or direct consumption).

Silver/titanium nanoparticles: effects on mycorrhiza and plants growth

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Silver nanoparticles (AgNPs) represent one of the fastest growing consumer product categories of engineered nanomaterials (ENMs), which have already found application in the nanotechnology industry. Antimicrobial properties of AgNPs encourage its use in a large number of biomedical and environmental applications as well as in growing list of coating materials, textile products, cosmetic and numerous consumer products. Even with its increasing commercialization, much remains unknown about nanotechnology, including some of the environmental and safety impacts of nanoparticles. The presence of AgNPs (50,100 and 200 mg L^{-1}) decreased root and shoot biomass and resulted in root structure changes of *Helianthus annuus* and *Plantago lanceolata* species. AgNPs added to soil caused significant loss of mycorrhizal colonization in roots. The vitality of the mycelium and arbuscules inside roots was evaluated by using Endogenous Alkaline Phosphatase Detection. Silver NPs affected photosynthetic activity of *H. annuus* and *P. lanceaolata*. Moreover, the effect of Ag/Ti nanoparticles on the development of mycorrhiza in *Helianthus annuus* that was cultivated in the presence of radioactive 134Cs was analysed. In addition to mycorrhizal colonization also the effect of radioactivity and nanoparticles on plants were assessed and possible way of attenuation of both by application of active carbon was tested. It is difficult to assess the risk of nanomaterials, but it is essential for nanotechnology leadership to design and institute ways to protect environment from the unintended consequences of ENMs.

Higher intensity land use allows adaptation of few species of AMF improving phosphorus acquisition by soybean

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Argentina is the third largest producer of soybean. This fact has led to the advance of the agricultural boundaries into wilderness areas and livestock farms. We studied the effects of soybean production on soil AMF biodiversity and their relationship with mycorrhizal colonization and phosphorus (P) nutrition in three sites differing in their intensity land use: A (intensive crop); B (crop including rotation with forages for meat production) and C (crop after clearing of native shrubs). During soybean growing season, four samples per site were taken. Density and biodiversity of AMF spore (Shannon-Wiener), AMF root colonization (%); potential infectivity (MSI50), soybean shoot dry matter, and P content and extractable soil P were measured. The treatment A had the highest spore density (Gl. fuegianum)"but the lowest value of biodiversity. In treatments B and C (*Cl. etunicatum; Cl. claroideum; Ac. scrobiculata; Ac. excavata, D. spurca, G. margarita, G. decipiens, S. pellucida, S. heterogama, R. persicae, A. gerdemannii*) were observed. Therefore, B and C had higher Shannon-Wiener index than A (1.7; 1.8 and 0.1, respectively). However, MSI50 was three times lower in A than B and C. The propagule infectivity was correlated with AMF colonization in soybean: A>B>C (65%; 45% and 39%; respectively). Additionally, plants in A reached the highest P content despite the lower values of soil P. We conclude that intensive land use produced the adaptation of few specie of AMF, and probably they improved P acquisition by soybean.

Arbuscular mycorrhizal fungi boosts crop production with reduced chemical fertilizer input

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Food security has become the major concern for the developing world. To meet the growing demand and to maintain the mineral status of soil, farmers are using higher doses of chemical fertilizers. Excessive usage of fertilizers in agriculture has not only increased the crop productivity but impacted environment significantly. World is facing greatest problems of environment pollution like soil and water acidification, contamination of water resources and increased emission of greenhouse gases.

All these environmental issues and low input of crop yield, have forced farmers to think and introduce some new agricultural practices that have lower impact on environment and could be able to increase the productivity. The aim of this study was to test the efficacy of G. *intraradices* (both as seed coating and soil application) to reduce the doses of chemical fertilizers. Field trials were conducted in wheat, gram, barley and citrus at SFCI research farms Suratgarh, Sardargarh in Rajasthan and Hissar in Haryana, India during *Rabi* 2011-12. The experiments were designed in a randomized block with two treatments *i.e.* T1: 100% RDF and T2: 75% RDF + Mycorrhiza to assess the ability of treatments with reduced dosage of N, P, K, fertilizers. Results obtained that there was a significant increase in plant height, number of leaves, number of tillers, grain quality, and yield in mycorrhizal treated plots in comparison with control (100% RDF) plots. It was concluded that mycorrhiza application could reduce the chemical fertilizers up to 25% and increase yield up to 10-15% in different cropping systems.

Mycorrhized root cultures as continuous biological and biochemical factories

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Arbuscular Mycorrhiza (AM) are rhizospheric fungi which colonize plant roots and extend themselves around the plant root system. This symbiotic association formed between roots and AMF plays an essential role in plant growth, bio protection, and production of secondary metabolites having medicinal importance. Rosmarinic acid, known for its antioxidant, anti-inflammatory, antiviral and anticancer properties is a multifunctional caffeic acid ester derivative found in roots and aerial parts of sweet basil (*Ocimum basilicum*). The study describes development of an *in vitro* dual culture between pRi derived transformed roots of sweet basil and CMCCROC3. Developed co culture system provides insights into interaction mechanism between transformed root and AM fungi, its developmental stages and potential of mycorrhiza as a biotic elicitor in comparison to non-mycorrhized root cultures. Thus, effect of mycorrhization on rosmarinic acid content along with mycorrhiza production and utility of these root cultures as continuous biological and biochemical factories would be discussed through this study.

Effect of AMF inoculation on growth, nutrition and production of withaferin-A in *Withania somnifera*

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Arbuscular mycorrhizal (AM) fungi play a crucial role as keystone mutualists in the most widespread symbiotic association found between plant root and fungus. The mutualistic association is known to influence different aspects of plant physiology and phytochemical constituents. *Withania somnifera* (Solanaceae) commonly known as Ashwagandha, is one of the most important medicinal plant in Indian Ayurveda with anti-inflammatory, antioxidant, anti-stress, and anti-tumour properties attributed due to the presence of alkaloids and steroid lactones. Withaferin-A, a steroid lactone is an important phytochemical constituent in the plant known for its anti-inflammatory properties. In the present study comparative assessment of mycorrhiza inoculation and uninoculated plants are studied with germplasm collected from different geographical regions. Thus, this study would discuss the variability in growth, nutrition, and Withaferin- A content as influenced by arbuscular mycorrhizal fungi and its potential aspect as biofertilizer for *Withania somnifera.*

Racing and interaction of AMFs from the same genera under *in vitro*; a tool to define desired inocula for application

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Four species of AMF *Gigaspora decipiens, Gigaspora. margarita, Gigaspora rosea,* and *Gigaspora albida* grown in various combinations. Growth and colonization was found to be varied, aggressive colonizer either inhibit the growth of other AMF under the given culture condition or known to be slow grower AMF itself get suppressed for its growth in presence of a fast growing AMF. Our data from co-culturing experiments amply demonstrate that there is a need of such experiments in order to find compatible and non-suppressive isolates for designing inocula and its desired virulence.

Biofortification of Fe and Zn in maize grain using mycorrhizal symbiosis

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Four sets of experiments (two each under open field and greenhouse conditions) were conducted in order to study the biofortification of Fe and Zn in maize (*Zea mays* L.) grains using arbuscular mycorrhizal fugal (AMF) inoculation. Treatments consisted of two levels of $FeSO_4$ (12.5 and 25 kg ha^{-1}), two levels of $ZnSO_4$ (12.5 and 25 kg ha^{-1}) and two mycorrhizal treatments (with or without inoculum carrying *Glomus intraradices* Schenck & Smith) replicated four times in a factorial RBD. The results revealed that AMF+ soils had significantly higher Fe and Zn in comparison to AMF- soils. Increased availability of Fe and Zn in soil in combination with enhanced concentrations in plants assisted the mycorrhizal plants to maintain higher micronutrient concentrations in grains (Fe M- 31.2, M+ 35.3; Zn M- 45.1, M+ 52.4 mg kg^{-1}). AMF inoculated maize plants produced grains with 10-15% higher Fe and Zn concentrations while an anti-nutritional factor "phytic acid" decreased (M- 1.13; M+ 1.07 mg g^{-1}) regardless of soil types. Overall, the data suggest that AMF inoculation is one of the potential factors assist in biofortification of grains with micronutrients besides circumventing the impact of anti-nutritional factors.

Screening of plant growth promoting rhizobacteria (PGPR) and their relation to the ectomycorrhizal development in Japanese black pine rhizosphere

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Pinus thunbergii has long been closely related to our life in Japan as windbreak and sand shield, especially in the coastal area. It is known that *P. thunbergii* is associated with soil microbial community, and not only ectomycorrhizal (ECM) fungi but also rhizobacteria may potentially contribute to *P. thunbergii* growth. In this study, a screening of plant growth promoting rhizobacteria (PGPR) obtained from the rhizosphere of *P. thunbergii* was carried out, and the effect of co-inoculation of the PGPR and an ECM fungus *Suillus granulatus* was evaluated. We isolated 160 bacterial strains from the rhizosphere soil of 15 *P. thunbergii* seedlings in the coastal area in Tottori, Japan. They served the first screening process using *Arabidopsis thaliana* to characterize their effect on both plant growth and postembryonic root development. As a result 15 strains increased the lateral root number, which were found *Bacillus, Burkholderia, Leifsonia, Pantoea, Ralstonia, Raoultella, Rhizobium,* and *Streptomyces* based on 16S rRNA sequence. These PGPR candidates were then used for the second screening to assess their ability to improve *P. thunbergii* growth and ectomycorrhization. In addition, the biochemical properties such as aminocyclopropanecarboxylic acid (ACC) degradation, auxin production, nitrogen fixation, potassium solubilisation, and phosphate solubilisation were tested to validate that each of them was consistent with the PGPR ability. Based on our results, the potential use of PGPR as mixed inoculants for stimulating growth of *P. thunbergii* in coastal plantation, especially the Great East Japan Earthquake and Tsunami-devastated area, will be discussed.

Selection of efficient AM fungi for *Lawsonia inermis* L., a medicinal plant raised through stem cuttings

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Sustainability of medicinal plants requires, a balanced and functional microbial inoculation to understand this, green house experiments were undertaken in order to evaluate the efficiency of six arbuscular mycorrhizal fungi (AMF) namely, *Glomus macrocarpum, Glomus bagyarajii, Glomus geosporum, Glomus fasciculatum* and *Acaulospora laevis* under mist chamber using sterilized soil. Of six AM fungi, *Acaulospora laevis* was found to be best for improved plant growth, number of leaves, fresh weight of shoot and root, shoot: root ratio, stem diameter, shoot biomass, biochemical changes such as total phenols, tannins, acid and alkaline phosphatase and peroxidase activities and shoot phosphorous content. Analysis performed 40 days after the inoculation, showed that maximum beneficial effect over the non-inoculated plants. It can be concluded that early inoculation of *Acaulospora laevis* enhances early plant development and crop productivity of *Lawsonia inermis* L., seedlings.

Arbuscular mycorrhizal colonization enhanced early growth of *Samanea saman* and *Mallotus paniculatus* under nursery condition in East Kalimantan, Indonesia

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High timber demand, forest fire, and open cast mining increased deforestation in Indonesia. Therefore, reforestation is urgently needed. Natural reforestation requires long time and consists of initial, middle, and climax stages. *Samanea saman* and *Mallotus paniculatus* are native tropical tree species and potential to promote initial stage of reforestation. This study aimed to determine the effect of arbuscular mycorrhizal (AM) fungi on the growth of *S. saman* and *M. paniculatus* under nursery condition. Seedlings were inoculated with *Gigaspora decipiens* and *Glomus clarum*, or uninoculated and grown in nursery at Berau, East Kalimantan, Indonesia. Compost was sterilized by firewood for 3 hours to grow seedling. Seedling height and leaf number were measured in 2, 3, and 4 months after sowing. AM colonization, shoot biomass, shoot phosphorus (P) and nitrogen (N) concentration were measured 6 months after sowing. *G. decipiens* and *G. clarum* colonized 53% and 53% of *S. saman* seedling, and 90% and 54% of *M. paniculatus* seedling, respectively. G. *decipiens* and *G. clarum* increased seedling height, leaf number, shoot P and N uptake, and shoot dry weight of *S. saman*. While *G. decipiens* increased seedling height, leaf number, shoot P and N uptake, and shoot dry weight of *M. paniculatus*. Positive correlation was observed between shoot N and P uptake and shoot dry weight. These results suggest that AM fungi are effective in accelerating initial stage of reforestation by improving nutrient uptake and plant growth.

Ericoid mycorrhizal fungi and potential for inoculation of commercial berry species (*Vaccinium corymbosium* L.)

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Ericaceous plants species dominant the fynbos vegetation of South Africa. The fynbos biome is characterized by highly leached acidic soils and low mineral nutrients. Ericoid mycorrhizal fungi associate with Erica species enabling them to acquire nutrients from organic sources essential for their survival. The aim of this study was to select local Ericaceae plant species, isolate, identify the endophytes and assess their potential as inocula for commercial berry species. Two ericaceous species, *Erica cerinthoides* L. and *Erica demmissa* Klotzsch ex Benth., were collected from the Grahamstown, South Africa. Root staining and microscopy was used to confirm the mycorrhizal status of both plants. Endophytes were isolated in pure culture. Microscopy was used for initial identification; two potential ericoid isolates were selected for molecular identification. DNA was extracted and PCR amplified using ITS1 and ITS4 fungal primers. The DNA template was then sequenced and analyzed by comparison to sequences in the GenBANK and UNITES databases. *Lachnum* species and *Cadophora* species were identified as endophytes of *E. cerinthoides* and *E. demmissa*, respectively. The isolates were inoculated onto micropropagated *Vaccinnium corymbosum* L. plantlets of 5 different varieties. *Lachnum* species infected roots showed potential ericoid structures while the *Cadophora* inoculated roots had ericoid mycorrhizas. The growth responses were variable within varieties and between varieties. Inoculation

significantly enhanced the shoot growth of Brightwell and Elliott varieties while Bluecrop and Elliott varieties inoculated with *Cadophora* and *Lachnum* accumulated more root biomass. Treatments with *Cadophora* and *Lachnum* species inocula showed potential in the promotion of Blueberry growth.

Arbuscular mycorrhizal fungi enhanced hardening and post hardening water stress tolerance of under mist system rooted semi-herbaceous olive cuttings

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Inappropriate hardening leads to high mortality of cuttings rooted under mist-system thereby limiting extensive plants propagation. This work attempts to evaluate the effect of arbuscular mycorrhizal fungi (AMF) on the survival to the acclimatization and transplanting shock and post-hardening water stress tolerance in semi-herbaceous olive cuttings rooted under mist-system. Rooted cuttings of olive (Moroccan Picholine) were inoculated or not with *Glomus intraradices*, *G. manihotis* or *G. fasciculatum* when transplanted under hardening greenhouse. Ten weeks after ex-mist-system transfer the surviving plantlets were subjected to two watering regimes 75% of field capacity and 25% of field capacity for another ten weeks. Results showed that AMF inoculation significantly increased the number (98 %) of plantlets surviving to ex-mist-system transfer. AMF improve plantlets growth (shoot height, root length, number of leaves and branching) and biomass production (shoots and roots dry weights). Moreover under severe post-hardening water deficit (25% FC), AMF-plantlets accumulate more mineral nutrients (P, Na^+, K^+) and maintained their water use efficiency, their relative water content and their stomatal conductance at a higher level. Data obtained by performing pressure-volume curves showed that symplastic water fraction, water potential, osmotic potential at full turgor and osmotic potential at turgor loss point were higher in AMF-plantlets. The positive effects of AMF were more spectacular in plantlets inoculated with G. manihotis. Biochemical analysis emphasized the role of AMF in olive water stress tolerance by increasing antioxidant enzymes (SOD, CAT, PO, and APX) activities, sugars, protein accumulation, and reducing MDA and H_2O_2 accumulation.

Reclamation and revegetation of extremely disturbed lands and assessment of mycorrhizal inoculums potential of re-constructed soils

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Surface mining of oil sand produces massive areas of disturbed lands in Alberta and considered challenging areas for reclamation and revegetation. Plant establishment is difficult by the harsh conditions in areas affected by oil sands by-products. Several amending materials have been used to amend these disturbed lands to support native plant community establishment. One of the key components for successful land reclamation is the functional soil microbial community crucial for plant growth and survival. Mycorrhizal fungi play an essential role in plant nutrient uptake, water relations, buffering environmental stresses and ecosystem establishment. However, anthropogenic disturbance of landscape may result in destruction of mycorrhizal fungal network in soil system and re-installation of these networks is an essential approach of disturbed habitat restoration. The approach for application of mycorrhizal fungi from a biotechnological perspective may be an effective tool that can improve reclamation of eroded lands and establishment of native plants. In this study, seedlings pre-inoculated with either single or consortium of ectomycorrhizal (ECM) fungal species was outplanted on oil sands reclamation sites. Another approach of this study was to assess natural arbuscular mycorrhizal (AM) and ECM inoculum potential and diversity of re-constructed soils for reclamation. The field responses of seedlings to ECM inoculation varied between plant species, inoculation treatments, and measured parameters. The indigenous inoculum levels of the test materials were low.

Infection process of *Glomus mosseae* in *Dendrocalamus strictus*: a critical step in tropical wastelands afforestation

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India has taken up an ambitious mission of greening the country. Planting of area-specific species on wastelands will be one of highlights of this decade long project. Bamboos, almost 13% of Indian forests, will be preferred profusely for their wide distribution and multifarious uses. Moreover, they sequester 35% more carbon than other trees with short rotation of 3-5 years. Bamboos are

mycotrophic due to their shallow roots and fast growth. However, they are monocarpic and multiply vegetatively (macroproliferation). Tropical soils are P fixing and, therefore, association of bamboos with AM becomes all the more useful as these fungi help in P mobilization. Fortification of bamboos with endomycorrhiza will not only improve their field survival and growth but also help in soil amelioration through improved soil aggregation. For integration of mycorrhizal fungus, *G. mosseae* in the macroproliferation of *D. strictus* , a very popular species of bamboos, and an experiment was designed to capture the various events of colonization on temporal scale. All the stages namely, penetration of the host roots, arbuscular initiation/disintegration, formation, and proliferation of extrametrical hyphae, vesicle formation and maturation and finally, chlamydospore formation were microphotographed on time scale. The whole process took almost 60 days. This period also matches with the raising of bamboo seedlings to their transplantable stage/ age- a crucial step in large-scale afforestation effort like National Green Mission.

Effect of previous mycorrhizal crop on AMF and bacterial wilt incidence of tomato

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Bacterial wilt, a soilborne disease caused by *Ralstonia solanacearum*, one of the world's most important phytopathogenic bacteria, is responsible for high economic losses in horticultural crops, especially in tropical and sub-tropical areas. In Martinique (French West Indies), since 1999, a highly virulent population has been identified on solanaceous and cucurbitaceae crops (Wicker, 2007). Agronomic French research team (INRA and CIRAD) study different agro-ecological strategies to maintain health and productivity of tomato in the fields. However, tomato benefits from mycorrhization when challenged by root pathogens (Gianinazzi, 2010). Effect of previous mycorrhizal crop was assessed by sampling soils from neighbouring agricultural plots one cultivated with tomato, the other one with chive (*Allium fistulosum*) and compared to a sterilized control soil. Tomato, cv Heatmaster, was cultivated on these soils under greenhouse conditions. Tomatoes grown on *Allium fistulosum* previous crop showed significant higher rates of mycorrhization. Moreover, no disease incidence was observed on tomatoes grown after *Allium fistulosum*. On the contrary, the percentage of disease incidence reached 80 % on tomatoes grown after tomatoes. This experiment highlights the potential impact of AMF on tomato bacterial wilt bioprotection.

Diversity analysis of endobacteria harboured within *in vitro* grown spores of different arbuscular mycorrhizal fungi (AMF)

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Arbuscular mycorrhizal fungi (AMF) from the phylum Glomeromycota are obligate symbionts of plants forming an association with a majority of them. They move nutrients such as phosphorus, nitrogen and other micronutrients through the roots into the plants thus enhancing plant growth. Spores from the fungi and mycelia from the infected roots can further colonize new host roots. Apart from enhanced nutrient uptake, resistance to pathogens is also exhibited. As the rhizosphere has a rich diversity many microbes are found lodged on the AMF spore walls and the hypha. The AMF supports some of these microbes which cluster together to form a polysaccharide containing biofilm and co-habitation occurs. Endobacteria have also been identified in the cytoplasm of the AMF spores. These cohabiting microbes may be imparting a synergistic effect to the AMF as well as plants. In our work, we selected *Gigaspora margarita, Gi. decipiens, Scutellospora heterogama* from the *in vitro* culture and *Glomus coronatum* from pot cultures. The spores were crushed to be able to access DNA of culturable as well as non-culturable bacteria. The bacteria were identified using DGGE as well as 16S rDNA partial sequencing. These results will allow us to better understand the role of the harboured microbes in the tripartite relationship between microorganisms, AMF and host roots.

Long-term effect of bio-fertilizer application on wheat yield and quality

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The agriculture sector in India employs 69% of the workforce, engages 50 million households, and contributes more than one fourth of the GDP of India. However, the land under cultivation has almost reached its saturation point with respect to productivity. Farmers overusing fertilizers and insecticides pay high costs have lower efficiency and soil and water become polluted. The research trials for wheat-legume and wheat-rice rotations were undertaken between 2005 and 2010. Two fertilizer levels were tested: farmers' practice: FP and zero fertilization: F0 with 7 inoculation treatments. Both

AMF (consortium of 4 different strains) and PGPR (2 fluorescent *Pseudomonas* strains; R62 & R81) were derived from wheat rhizosphere from Bhawanipur region. The following results are derived from 8 harvests of wheat. The grain yield in AMF and PGPR inoculated FP – plots surpassing non-inoculated controls by 26% and 24% upon application of these biofertilizers respectively. The maximum grain yield in FP – plots was obtained with dual inoculation of AMF+PGPR surpassing non-inoculated controls by 29%. Although the average grain yield in F0 – plots was lower than in FP - plots by 38.6%, the effect of AMF+PGPR inoculation was higher in the F0 - plots with up to 55% increment. Besides the yield, AMF and PGPR inoculated plots showed improvement in nutrient use efficiency and soil & grain quality. We conclude that the joint inoculation of AMF and PGPR could be adopted for achieving higher yield of wheat with improved grain quality at reduced fertilizer inputs.

Bioremediation of Cr contaminated soil using mycorrhized plants & ATA-SG: a feasibility study to assess the economic viability of the practice

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Chromium (Cr) contamination in soil due to various industrial activities has been an increasing worry to the farmers and plant producers around the world. Reclamation of soil using mycorrhized plant is well known and is in practice since decades, but nothing substantial has been done till date to manage the waste generated through the practice in the form of heavy metal laden plant biomass, which has made the current situation very alarming. Considering this, the study was conducted to find out the possibility of using mycorrhized plants for bioextraction of Cr from contaminated soil and recovery of pure form of Cr metal from metal laden plant biomass as a value addition. Further, the feasibility study was conducted to assess the economic viability of the practice. Under this study, a multidisciplinary research approach was followed to develop a sustainable biopackage to remove and recover Cr metal from the plant biomass. The biopackage includes a very specific combination of mycorrhizae, plants and metal selective chelating resin (ATA-SG) developed through a green chemistry technique. The developed chelating resin is very specific to the Cr (sorption capacity: 4400 µg of Cr/g of resin) and can be successfully regenerated at least 50 times without loss of chelating sites. In terms of Cr removal from contaminated soil, the selected mycorrhized plants are able to accumulate up to 190 mg Cr /g of dry biomass and in terms of recovery of pure form of Cr metal from plant biomass using chelating resin, up to 96.40% of recovery is possible. The cost economics analysis of the study showed the significant economic viability of the practice.

Interaction of micropropagated corymbia hybrid (*C. torelliana* muell x c. Citriodora hook) with *Laccaria laccata* under ***in-vitro*** condition

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Establishment of ectomycorrhizae on micropropagated plantlets is suggested to reduce environmental stresses and hence mortality upon transplantation to in-vivo conditions. Therefore, the present work was undertaken with the objective of integrating *Laccaria laccata,* an ectomycorrhizal fungus, with micropropagated plantlet of Corymbia hybrid before transplantation. Nodal segments of the hybrid containing axillary buds from newly developed fresh shoots were taken, surface sterilized and incubated in Murashige and Skoog (MS) medium supplemented with 3% sucrose, BAP (2 mg/l) and NAA (1 mg/l) for axillary bud induction and shoot initiation. Shoot multiplication was achieved in the same medium and regular subculturing was carried out on MS with BAP (1 mg/l). After 4-6 subculturing sufficient juvenility was achieved and thereafter half strength MS medium devoid of plant growth regulators was used to get elongated shoots to induce rooting. Best rooting was observed in half strength MS medium supplemented with IBA (0.1 mg/l) and 2% sucrose. *Laccaria laccata* was multiplied using Modified Melin Norkrans (MMN) medium and inoculated into rooted plantlets in vermiculite having MMN/ MS medium or in soil under in-vitro conditions. Plantlets died within 1 and 2 months of inoculation in vermiculite and soil respectively. This may be attributed to the ready availability of nitrogen and phosphorus in MMN and MS media and more competency of mycorrhiza in retrieving these nutrients as compared to the plantlet. The soil, where plantlets lived longer may be a useful medium for integrating Laccaria laccata into Corymbia hybrid before transplantation.

Mycorrhiza network at TERI since 1988: A journey of progress and achievements

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Mycorrhiza Network was set up at TERI on 1st April 1988 with seed support from IDRC and was subsequently supported by the Department of Biotechnology, Government of India (for the period 1993-1998 and 2010-2013). The general objective of the Network is to strengthen research, encourage cooperation, promote exchange of information and germplasm, and facilitate transfer

of technology to the field through the establishment of a mycorrhiza research network. Within the Network, a Mycorrhiza Information Centre (MIC) has been set up with support from the Department of Biotechnology, Government of India, with the following objective. The Centre functions as a specialized centre for collection, compilation, and dissemination of information and resources on mycorrhiza. Over 2500 classified references sourced from national and international journals have been made available in the Literature Abstracts Database. The main objective of this programme is that the scientists working on mycorrhiza can obtain information at macro level on broad subjects under a particular category of mycorrhiza or on a specific topic of research on which they may formulate their projects. The Mycorrhiza Network at TERI publishes, since 1988, a newsletter—*Mycorrhiza News*— on a quarterly basis to provide a forum for dissemination, acquisition, interaction, and communication of scientific information on mycorrhizal research and activities. Wide diversity exists within the AM fungi, which has over 210 species within the order Glomales, the diversity that shows up in morphology, physiology, and functionality. To conserve and exploit this diversity, the Centre for Mycorrhizal Culture Collection (CMCC) was established in 1993 with the seed support from the Department of Biotechnology, Government of India. The Network has developed a database of cultures to help researchers obtain information on specific cultures of interest. The information in the database includes the Germplasm Bank Code against each fungus name (genus and species). A Directory of Mycorrhizologists is being developed based on the information collected through specially designed questionnaire. Scientists/researchers working in the area of mycorrhiza are invited to become members of the Network without any cost or obligations.

Study of mycorrizal fungi inoculums produced in different systems on strawberry development in relation to water use and nitrogen input. Profiling and analysis of contaminations present in production systems that coexist in the final inoculum

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Mycorrhiza are the most common underground symbioses. The organisms involved are multi-functional, assisting the plants in nutrient acquisition, water uptake, mediating carbon transfer and protecting roots from pathogens. The arbuscular mycorrhizal (AM) fungi symbiosis is the most widespread and is thus of particular interest in general plant and crop production. Many different cultivation techniques of AM fungi have been developed in the last decades. The relatively new '*in vitro*' method of cultivation requires less space and the final product is more applicable to seed coating being mainly spore based and free from unwanted organisms. In contrast, vegetative manufacture allows for the production broader variety of species and inoculum is grown in a near natural 'stressed' environment. In the present study, we compare the effects of two inoculums produced in different systems on strawberry development in relation to water use and nitrogen input. Another aspect of our study is the analysis of the sporadic contamination present *in vitro* culture. Experiments have been set up to determine source of contaminant, isolation and identification of microorganisms as well as their location in the system.

Symbiogenetics towards food production

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Soil fungi play a major role in ecological and biogeochemical processes leading to a strong direct influence on crop productivity. As such, understanding how the structure of soil fungal communities are related to crop yield is crucial for optimizing productivity. In this work we test the hypothesis that even under conventional agriculture, in which fertilizers are used, the existing soil fungal diversity provides insights for the development of ecosystem management methodologies to increase food production. We assessed the structure of the fungal community, especially of arbuscular mycorrhizal fungi, naturally colonizing roots of the five most productive maize varieties grown in Portugal. We performed Tag encoded 454 pyrosequencing of the nuclear large ribosomal subunit (LSU) as well as the determination of crop productivity. Fungal community assessment showed a considerable diversity of AMF in agricultural fields. Fungal community structure and its relation with crop yield will be discussed, as a function of maize varieties.

Maternal and paternal effects on offspring performance: importance of plant gender and arbuscular mycorrhizal fungi

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In gynodioecious plants, females are predicted to produce more or better offspring than hermaphrodites in order to be maintained in the same population. In the field, both sexes are usually colonized by arbuscular mycorrhizal (AM) fungi in their roots. These mutualistic fungi are expected

to improve not only host performance but also the quality of the offspring. We examined the maternal and paternal effects of AM fungal colonization and host gender on seed production and posterior seedling performance in *Geranium sylvaticum*, a gynodioecious plant. We hand-pollinated females and hermaphrodites inoculated with either *Glomus claroideum*, *G. hoi* or in non-mycorrhizal condition and measured seed number and seed mass and posterior seedling survival and growth in a greenhouse experiment. We confirmed the prediction that females produce more and larger seeds than hermaphrodites. However, these larger seeds produced by females did not germinate, survive, or grow more than seeds mothered by hermaphrodites. Furthermore, we could partially verify our hypothesis that mycorrhizal plants would be larger and will produce more seeds than non-inoculated plants due to the well-known mycorrhizal benefits. Our experimental design allowed us also to check how AM fungal inoculation of the pollen donor influences reproductive output. Differing from the beneficial effects on mothering, AM fungal inoculation of the plants did not affect fathering abilities related to any of the parameters analysed. This study shows that AM fungi affect offspring performance by affecting maternal but not paternal reproductive capacity.

Increased alkalinity tolerance of *Jatropha curcas* by dual inoculation of arbuscular mycorrhizal fungi and *Azotobacter* bacterium

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The utilization of non-edible oil seeds energy crops as a source of renewable fuels is a concept with great relevance to current ecological and economic issues at both national and global scales. Jatropha curcas a eco-friendly, non-toxic, biodegradable biofuel producing plant has attracted worldwide attention as an alternate sustainable energy source for the future. This study investigated the influence of inoculation with arbuscular mycorrhiza (AM) fungi alone or in combination with a plant growth-promoting rhizobacterium, *Azotobacter chrococcum* on growth, solute accumulation and antioxidant enzyme activity in *Jatropha curcas* affected by different levels of alkalinity stress (0-0.4% Na_2CO_3). Alkalinity stress decreased *Jatropha* growth, regardless of treatments applied and of the salt stress levels. Alkalinity level of 0.3% caused reduction in shoot height (34.1%) and shoot diameter (25.2%) over control plants after 180 day of planting. Increasing alkalinity stress raised significantly the antioxidant enzyme activities, including those of SOD, POD, APX and GR activity, of Jatropha leaves over non-stressed controls. AM colonization in roots of Jatropha and Azotobacter colony count were found to be decreased with increased alkalinity. The combined inoculation of AM fungi and

Azotobacter induced greater root, shoot biomass, improved accumulation of proline, proteins, sugars and chlorophyll contents in Jatropha leaves and an increase in antioxidant enzymes in comparison to non-inoculated plants up to 0.4 % alkalinity. These bioinoculants combination could serve as a useful tool for alleviating alkalinity stress in Jatropha growing on stressed soil.

Transformation of *Solanum tuberosum* var Pukhraj and its study with Arbuscular Mycorrhizal Fungus for inoculum production potential *in vitro*

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The current study involved the transformation of a potato plant tuber (variety pukhraj) with *Agrobacterium rhizogenes* strain Ri 1600. We obtained a transformation efficiency of 40% after 8 days of co-cultivation and the root inducing (Ri) T-DNA transformed hairy roots could be successfully maintained on a hormone-free minimal medium (M). These were confirmed for the transformation using rolA and rolB gene specific primers. The convention Ri T-DNA carrot roots (*Daucus carota* var pusa kesar) were co-cultivated with *Glomus intraradices* (CMCCROC7) and compared with the Ri T-DNA transformed potato roots (*Solanum tuberosum* var pukhraj) co-cultivated with *Glomus intraradices* (CMCCROC7) as arbuscular mycorrhizal root organ cultures (AM-ROC dual cultures) for studying their symbiosis potential for sporulation *in vitro*. Around 60,250 spores / jar could be harvested with around 38,314 extraradical spores / jar and around 21,936 intraradical spores / jar. The new method is promising for mass production of mycorrhizal biofertilizer. The viability of the spores with potato roots was nearly 100% and more than half of the roots had been colonized after inoculation at harvest time.

Impact of chemical and mechanical defoliation strategies on Arbuscular Mycorrhizal fungi associated to potato plants

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Arbuscular mycorrhizal (AM) fungi are obligate symbionts which rely exclusively on their host plants to complete their life cycle through the provision of C resources in exchange of mineral nutrients.

This access of plant C can however be perturbed (e.g. by agricultural practices) and consequently affect the development of their fungal partners. Under *in vitro* conditions, we compared three different defoliation practices, (i) mechanical defoliation, and chemical defoliation with (ii) a contact herbicide (diquat formulation), or (iii) a systemic herbicide (glyphosate formulation) on *Rhizophagus irregularis* MUCL 41833 associated to *Solanum tuberosum* L., var. Bintje. As an indicator of fungal development, spore production dynamics were assessed, and modelled. Results show that the systemic herbicide affected the spore production more rapidly than the other defoliation strategies. We hypothesized that the systemic herbicide had a stronger effect because of its general disruption of plant C metabolism, and potentially of plant capacity to direct toward and transfer C to the AM fungal structures. The contact herbicide and mechanical defoliation had a less marked effect on spore production as plant might have kept their capacity to mobilize C from reserves and to ensure the transfer of these resources towards the AM fungi.

Ectomycorrhizal colonization of black pine seedlings in nursery

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The aim of this study is to produce mycorrhizal black pine [*Pinus nigra* Arnold. subsp. pallasiana (Lamb.) Holmboe] seedling which can be used in semiarid land afforestation. Black pine seedlings were grown in a tree nursery with mycorrhizal fungi. These fungi (*Rhizopogon luteolus, Chroogomhus rutilus* and *Russula rauoltii*) were collected from black pine stands in semiarid zones of backwards of Mediterranean. Then sporocarps were isolated for pure mycelia inoculum under laboratory conditions. Mycelia were obtained from the cultures in MMN medium. Mycelia were added to peat-vermiculite mixture to obtain inoculum. The study was conducted in the laboratory of the Eastern Mediterranean Research Institute. These fungal cultures were used to inoculate the regularly used potting mixture of forest soil, humus and sand. After one growing season, root colonization of the seedlings was measured. In the result of study, were investigated the percentage of seedlings with ectomycorrhizae and degree of ectomycorrhizal development by three fungi. After 1 year were found ectomycorrhizae on approximately half of inoculated seedlings. Mycorrhizal colonization, respectively, were formed by *R. rauoltii, C. rutilus* and *R.luteolus* species.

Arbuscular mycorrhizas and soil microbial interactions in sugarcane agriculture in Kwazulu-Natal, South Africa

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A holistic approach was used to study the mycotrophic nature of commercial sugarcane varieties grown in this region. All five varieties were mycotrophic, but N12 had the highest overall mycorrhization and was selected for a pot study to assess the growth response of sugarcane to inoculation with indigenous AMF and microflora. The pot study suggested that sugarcane will respond positively to inoculation with AMF, but the effects are most clear in the early phase of growth and less obvious in later elongation phases. This observation, taken together with the ability of sugarcane to grow well in sterile soil without microflora additions suggests that the plant may be facultatively mycotrophic. A multivariate analysis determined the nutrient relationships between soil and corresponding leaf nutrient levels on 72 sugarcane field plants, categorised according to either high or low percentage colonisation. Highly colonised plants were found to have more positive nutrient correlations compared to lower percentage colonised plants. AMF were identified from spore morphology and AM bacteria identified by 16s rDNA analysis. Bacteria, *Brevibacillus reuszeri* isolated from *Scutellospora nigra*, *Bacillus megaterium* and *Stenotrophomonas maltophilia* isolated from *Glomus geospora*, *Paenibacillus chitinolyticus* and *Bacillus cereus* isolated from *Acaulospora mellea* and *Gigaspora margarita* spores respectively, were tested for biocontrol capability against pathogenic nematodes of Paratrichodorus, Meloidogyne and Pratylenchus genera. Meloidogyne was the least susceptible to AM bacteria biocontrol and Paratrichodorus the most susceptible. These studies have elucidated the role of AM in sugarcane agriculture.

Dual inoculation of mycorrhiza and *Glucanoacetobacter* to promote zinc availability in vertisols

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In order to improve the availability of Zn in vertisols, a green house experiment was conducted at the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore. It is hypothesized that dual inoculation of Arbuscular Mycorrhizal fungus (AMF) in combination with *Gluconoacetobacter* is known to improve the Zn availability through rhizospheric acidification besides preferential solubilization and transport to the host plant. The experimental design consists of AMF

inoculation and *Gluconoacetobacter* individually or in combination and replicated 5 times in a CRD. During the experimentation, the availability of Zn, fractionation of Zn in soil and Zn related enzymes in the maize plants were examined. Treatment inoculated with dual culture exhibited prominent increase in the leaf area index and chlorophyll content of plants and the response to mycorrhizal inoculation was higher than that of *Glucanoacetobacter* and AMF alone inoculated soil. The results clearly indicated that AMF + *Gluconoacetobacter* enhanced the DTPA extractable Zn to the tune of 44.4% in comparison to individual inoculation (AMF: 18.9%, *Gluconoacetobacter*: 12.4%) and correspondingly enzymes related to Zn uptake (Carbonic anhydrase activity; 351, 313 and 284 EU g^{-1} in AMF + *Gluconoacetobacter*, AMF alone and *Gluconoacetobacter* alone respectively) has shown increase in dual inoculum treatments. Further, Dual inoculation assists in solubilization of tightly bound residual form of Zn which is rarely available to the uninoculated plants. Overall, the data suggests that dual inoculation improves the availability of Zn in the zinc deficient vertisols.

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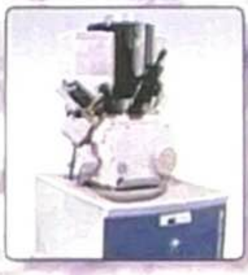

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Mycorrhiza Network

MYCORRHIZA NETWORK TERI , NEW DELHI

The quarterly newsletter of Mycorrhiza Network at TERI publishes state-of-the-art papers/research findings from eminent scientists covering the biology, ecology, and other related aspects of mycorrhiza including biodiversity and conservation of mycorrhizae. It also features notes on important breakthroughs with brief accounts of new approaches and techniques while providing information on forthcoming events on mycorrhiza and related subjects, and also highlights the activities of the Networks' Centre for Mycorrhiza Culture Collection. The newsletter newsletter caters to the needs of young and enthusiastic workers in the field of mycorrhiza research while updating their understanding of mycorrhizae research at national and global level.

The newsletter is useful to general, non-scientist readers including farmers, agriculturists, foresters, policy makers, and others who are interested in utilizing mycorrhiza as biofertiliser.

MYCORRHIZA NETWORK

Set up in April 1988 with seed support from International Development Research Centre, Canada

IDRC CRDI
International Development Research Centre
Centre de recherches pour le développement international

From 1993 to 1998; 2010 to the present Department of Biotechnology, Government of India

Department of Biotechnology, Ministry of Science and Technology, Government of India

Objectives

Help scientists carry out research in Mycorrhiza

Establish

(i) Mycorrhiza Information Centre

(ii) Centre for Mycorrhizal Culture Collection

Publish quarterly newsletter – Mycorrhiza News

Promote communication among mycorrhiza scientists

Contact:

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