



Soil Biological Communities and Aboveground Resilience

COST Action FP1305 BioLink

***Linking belowground biodiversity and ecosystem function
in European forests***

Proceedings of the 3rd Annual Meeting

Rome, 17-19 November 2015





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*Soil Biological Communities and Aboveground Resilience
The 3rd Annual Meeting of FP1305 Biolink Cost Action
Rome, 17-19 November 2015*

Programme

November 17th

Villa Celimontana (Italian Geographical Society),

Via della Navicella 12, Roma

- 16:00-18:30 **Registration**
- 17:00-17:15 **Anna Benedetti - Opening Ceremony and Meeting Introduction**
Head of the Research Centre for the Soil-Plant System, CREA-RPS, Italy
- 17:15-17:30 **Pierfrancesco Sacco - Soil global partnership**
Italian Ambassador to FAO, Italy's Permanent Delegation, Organizations of the United Nations
- 17:30-18:15 **Stefano Grego - Transdisciplinary approaches for building a new sustainable and resilient agroecosystem**
WAHF, World Agricultural Heritage Foundation, Research Council of Italy, CREA-RPS, Rome
- 18.15-18:30 **Discussion**
- 18:30-20:00 **Refreshment**

November 18th

CNR, Piazzale Aldo Moro 7, Roma

Aula Convegni

- 09:00-09:30 **Welcome**
Mauro Gamboni, DISBA CNR, Biolink WG3 Coordinator
Francesco Loreto, Head of DISBA, CNR, IT
Massimo Iannetta, Head of Biotechnologies and Agro-Industry Division, ENEA, IT
Stefano Bisoffi, Head of Council for Agricultural Research and Analysis of Agricultural Economics, CREA, IT

Plenary Session, Part 1

Chairs: Douglas Godbold, Aurelio Ciancio

- 09:30-09:55 **Brian Griffiths - An overview of the resilience of soil microbial processes and the interactions with above-ground processes**
SRUC, Crop and Soil Systems Research Group, Edinburgh, UK



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- 09:55-10:20 **Erica Lumini** - *Unravelling soil fungal communities from different land-use backgrounds*
CNR-IPSP, Institute for Sustainable Plant Protection, Turin, IT
- 10:20-10:40 **Beat Frey** - *Resistance and resilience of the forest soil microbiome to logging-associated compaction*
Swiss Federal Research Institute WSL, Birmensdorf, CH
- 10:40-11:00 **Luis Gonzaga García-Montero** - *Could mycorrhizae perform “ecosystem engineering” on the host plants? Basis on rhizoculture of agroforestry systems to improve their resilience*
Forest Engineering Dept., E.T.S.I. Montes, Technical University of Madrid (UPM), Madrid, ES
- 11:00-11:30 **Coffee break**

Plenary Session, Part 2

Chairs: Johannes Rousk, Anna Barra Caracciolo

- 11:30-11:55 **Jose Julio Ortega-Calvo** - *Microbial and plant influences on bioavailability of hydrophobic organic pollutants in soil*
Instituto de Recursos Naturales y Agrobiología de Sevilla, CSIC, Sevilla, ES
- 11:55-12:20 **Andrey Yurkov** - *Diversity, distribution and functions of yeasts in soils*
Leibniz-Institute DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, DE
- 12:20-12:40 **Petr Baldrian** - *Tracing the activity of individual microbial taxa in forest soils*
Laboratory of Environmental Microbiology, Institute of Microbiology of the ASCR, Prague, CZ
- 12:40-13:00 **Luis Merino-Martín** - *Understanding the factors driving soil aggregate stability: the role of plant-soil-root-microbe interactions*
INRA, UMR AMAP, Montpellier, FR and Centre for Ecology & Hydrology, Crowmarsh Gifford, Wallingford, Oxfordshire, UK
- 13:00-14:00 **Lunch**
- 14:00-16:30 **WG Meetings, Part 1:**
(RESERVED TO BIOLINK COST MEMBERS)
WG1: Aula Convegna
WG2: Aula Golgi
WG3: Aula Silvestri
WG4: Aula Giacomello
- 16:30-17:00 **Coffee Break**
- 17:00-18:00 **MC Meeting**



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November 19th

CNR, Piazzale Aldo Moro 7, Roma

Aula Convegni

08:30-09:20 **Guided tour of Selected e-Posters**

Chair: *Alessandra Lagomarsino*

Plenary Session, Part 3

Chairs: *Gabrielle Deckmyn, Annamaria Bevivino*

09:30-09:55 **Mark Tibbett** - *Microbial functional diversity in restored biodiverse forest soil*

School of Agriculture, Policy and Development, Reading University, UK

09:55-10:20 **Jorge Curiel Yuste** - *Forest fragmentation and drought as engines of change of the plant-soil-microbial ecological interactions in the Mediterranean basin*

Museo Nacional de Ciencias Naturales, CSIC, Madrid, ES

10:20-10:40 **Mona N. Högberg** - *How does soil microbial community composition and nitrogen supply change with ecosystem age in primary boreal forests?*

Forest Ecology and Management Dept., Swedish Agricultural Sciences, University of Umeå, SE

10:40-11:00 **Relena Rose Ribbons** - *Microbial communities, functional genes and nitrogen cycling processes in forest floors under four tree species*

Forest and Conservation Sciences Dept., Faculty of Forestry, British Columbia Univ., Vancouver, Canada; School of Environment, Natural Resources and Geography, Bangor Univ., Wales, UK; Geosciences and Natural Resource Management Dept., Copenhagen Univ, DK

11:00-11:30 **Coffee break** (including poster session)

Plenary Session, Part 4

Chairs: *Olaf Schmidt, Paola Grenni*

11:30-11:55 **Hans Göransson** - *Effects of geometrid moth attacks on belowground processes in sub-arctic birch forests*

Forest ecology, Natural resources and life sciences Univ., Vienna, AT

11:55-12:20 **Franco Nigro** - *Does the soil microbial community affect the endophytes diversity into the xylem and the resilience to vascular wilt diseases? The olive tree as a case study*

Università degli studi Aldo Moro, Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (DiSSPA.) Bari, IT



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- 12:20-12:40 **Jérôme Cortet** - *The use of traits for soil invertebrates: state of the art and perspectives*
CEFE UMR 5175, CNRS, Université Paul-Valéry Montpellier, FR
- 12:40-13:00 **Oskar Franklin** - *Modeling plant - soil interactions in an ectomycorrhizal forest*
IIASA, International Institute for Applied Systems Analysis, Laxenburg, AT
- 13:00-13:20 **Carla Cruz-Paredes** - *Using bacterial and fungal responses to evaluate the mechanisms by which ash application impact forest soils*
Terrestrial Ecology Section, University of Copenhagen, DK
- 13:20-14:20 **Lunch**
- 14:20-15:00 **e-Poster Session**
- 15:00-16:45 **WG Meetings, Part 2**
(RESERVED TO BIOLINK COST MEMBERS)
WG1: Aula Convegna
WG2: Aula Golgi
WG3: Aula Silvestri
WG4: Aula Giacomello
- 16:45-17:15 **Coffee break**
WG participants meeting and briefing by WG leaders (Aula Convegna)
- 17:15-18:00 **Closing Remarks**



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Scientific Committee

- Mauro Gamboni** CNR-DiSBA, National Research Council of Italy,
Department of Biology, Agriculture and Food Sciences
Rome, Italy
mauro.gamboni@cnr.it
- Anna Barra Caracciolo** CNR-IRSA, National Research Council of Italy,
Water Research Institute, Rome, Italy
barracaracciolo@irsa.cnr.it
- Anna Benedetti** CREA-RPS, Council for Agricultural Research and
Analysis of Agricultural Economics,
Research Center for the Study on Relationships between
Plant and Soil, Rome, Italy
anna.benedetti@entecra.it
- Annamaria Bevivino** ENEA, Italian National Agency for New Technologies,
Energy and Sustainable Development, Rome, Italy
annamaria.bevivino@enea.it
- Aurelio Ciancio** CNR-IPSP, National Research Council of Italy,
Institute for Sustainable Plant Protection, Bari, Italy
aurelio.ciancio@ipsp.cnr.it
- Alessandro Frandi** CNR-DiSBA, National Research Council of Italy,
Department of Biology, Agriculture and Food Sciences,
Rome, Italy
alessandro.frandi@cnr.it
- Paola Grenni** CNR-IRSA, National Research Council of Italy,
Water Research Institute, Rome, Italy
grenni@irsa.cnr.it
- Alessandra Lagomarsino** CREA-ABP, Council for Agricultural Research and Analysis
of Agricultural Economics,
Agrobiology and Pedology Research Centre,
Florence, Italy
alessandra.lagomarsino@entecra.it



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INTRODUCTORY REMARKS

The predicted increase in the occurrence of extreme weather events due to climate change calls for an improved understanding of the capacity of ecosystems to absorb disturbance. The ability to maintain structure and function of many terrestrial ecosystems will be tested, chiefly due to the fluctuation of climatic conditions that have not experienced in recent past. There are numerous factors that contribute to ecosystem resilience, effective biological activity in the soil is certainly one of them. Belowground species and functional group diversity underpin soil function, which in turn is the keystone of ecosystem productivity and stability.

The role of belowground biodiversity in promoting ecosystem resilience and stability will be the main focus of the 3rd Annual Meeting of COST Action BioLink. The Meeting entitled ‘Soil biological communities and aboveground resilience’ will take place in Rome, from the 17th to 19th November 2015. The Meeting will build upon that network of research and personal relationships created by the BioLink community and explore the current state of knowledge of the connection between soil function as mediated by soil biota and aboveground ecosystem productivity on which humankind depends for its survival. Specifically, the Meeting will focus on forests and tree ecosystems to explore the role of soil biodiversity in systems dominated by vegetation type with multi-decadal life span. Advancing our understanding of the link between soil biota and trees will certainly be of use in creating a more effective and sustainable forest and tree crop management. Since tree ecosystems are extraordinarily important in the provision of several key ecosystem services, there is a high potential for the Meeting to contribute to what is a lively and interesting debate.

Martin Lukac and Mauro Gamboni



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3rd Annual Meeting
“Soil Biological Communities and Aboveground Resilience”

COST Action FP1305 BioLink
***Linking belowground biodiversity and ecosystem function
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Abstracts for Oral Presentations



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1-OP

Transdisciplinary approaches for building a new sustainable and resilient agroecosystem

Stefano Grego (Invited Speaker)

World Agricultural Heritage Foundation (WAHF), Rome; University of Tuscia Viterbo, Italy

grego@unitus.it

Improving the sustainability of humanity's relationship with Planet Earth is firmly established as a societal goal for the 21st Century. To achieve it we need a better understanding of how to govern "the process of moving towards greater sustainability, with a new style of governing which is more pluralist and decentralized than the conventional state-centered government style.

With climate change, biodiversity loss, global water and energy crises, the growing problem of desertification, the phenomenon of massive urbanization and many other manifestations of global environmental change becoming more and more evident, there is a widespread and increasing feeling in the society at large that the concept of sustainable development is not sufficient to counteract the complex and problematical situations. How to manage the difficult global socio-economical-environmental changes and make the agroecosystem resilient and sustainable? It is readily evident that we are facing many urgent sustainability challenges, including poverty, epidemics, violent conflicts, economic crisis, beside climate change, aggressive agriculture and biodiversity.

These problems range in scale from global to local and are expected to affect future generations. We considered all the changes that are currently occurring in our environment and in our society in a sectorial way. Promoting sustainable development requires research on wide range of social, economic, institutional and environmental issues. This vision requires transdisciplinary research approaches creating new conceptual, theoretical, methodological and translational innovations that integrate and move beyond discipline-specific approaches to address a common problem. The aim to understand the dynamics of coupled social-ecological systems stimulated an innovative, problem driven research that has been called *Sustainability Science*. Like "*agricultural science*" and "*health science*", Sustainability Science is a field defined by the problems rather than by the disciplines it employs. From its core focus on advancing understanding of coupled human-environment systems, Sustainability Science has reached out with focused problem-solving efforts based on urgent human needs.



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2-OP

**An overview of the resilience of soil microbial processes
and the interactions with above-ground processes**

Bryan Griffiths (Invited Speaker)

SRUC, Crop and Soil Systems Research Group, West Mains Road, Edinburgh EH9 3JG, UK

bryan.griffiths@sruc.ac.uk

Soil is increasingly under environmental pressures that alter its capacity to fulfil essential ecosystem services. To maintain these crucial soil functions, it is important to know how soil microorganisms respond to disturbance or environmental change. In this presentation I will summarize the recent progress in understanding the resistance and resilience (stability) of soil microbial communities and discuss the underlying mechanisms of soil biological stability together with the factors affecting it. Biological stability is not solely owing to the structure or diversity of the microbial community but is linked to a range of other vegetation and soil properties including aggregation and substrate quality. This suggests that resistance and resilience are governed by soil physico-chemical structure through its effect on microbial community composition and physiology, but that there is no general response to disturbance because stability is particular to the disturbance and soil history. The interaction between the plant community and soil resilience, as well as the reciprocal interaction between soil resilience and the above-ground community will be explored.

Keywords: biodiversity; decomposition; disturbance; ecosystem services; nitrogen cycling

Acknowledgments: SRUC acknowledges funding by the Rural and Environment Science and Analytical Services Division of the Scottish Government.



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3-OP

Unravelling soil fungal communities from different land-use backgrounds

Erica Lumini¹ (Invited Speaker), Andrea Berruti¹, Valeria Bianciotto¹, Alberto Orgiazzi²

¹*CNR-Istituto per la Protezione Sostenibile delle Piante, UOS Torino (CNR-IPSP), c/o Dipartimento di Scienze della Vita e Biologia dei Sistemi, Viale P.A. Mattioli, 25, I-10125 Torino, Italy;*

²*European Commission, Joint Research Centre (JRC), Institute for Environment and Sustainability, Via E. Fermi 2749, I-21027 Ispra, VA, Italy*

erica.lumini@ipsp.cnr.it

Identifying a soil core microbiome is crucial to appreciate the established microbial consortium, which is not usually subject to change and is likely to be resilient to disturbances and edaphic shifts. Soil fungi are indispensable for carbon cycling and soil aggregate formation, mainly operating as decomposers, pathogens and mycorrhizal mutualists. Due to their large diversity, specialization, and important ecological functions, fungi are also excellent bio indicators (Lemanceau et al. 2015). For these reasons, investigating the fungal diversity and the ecological factors that underlie the dynamics of fungal species distribution becomes crucial for the ecological characterization of any given ecosystem.

In this context, tag-assisted Next Generation Sequencing (454 GS FLX/Illumina) targeting the 18S or ITS region of the fungal rDNA represents a powerful approach for the analysis of soil fungal diversity and, specifically, for the disclosure of fungal taxa that are key to ecosystem functioning.

Since 2010, we have been investigating fungal communities from different land-use backgrounds, including soils subjected to anthropogenic impact in a typical Mediterranean landscape (natural cork-oak forest, pasture, managed meadow, vineyards) (Lumini et al. 2010), European agroecosystems subjected to high input practice (maize, wheat, and rice), and soils from the native and naturalized range of broadleaf evergreen ornamental plants.

These investigations have provided an overview of soil fungal diversity at the examined sites and important backbone data on the impact of environmental conditions (soil types, cover vegetation, and human activities) on fungal microbiome composition and structure. The characterisation of soil biodiversity in different types of soils may help in identifying practices that allow to preserve soil organisms. Such knowledge may be transferred to ensure sustainable economic, social and environmental development and therefore used for the implementation of development policies.

Keywords: fungal diversity; fungal communities; metabarcoding; microbiome; land use

Acknowledgments: Research was financially supported by SOILSINK (FISR), PRO-LACTE (Regione Piemonte), RISINNOVA (AGER Foundation) Projects, EU Projects PURE (FP7-265865) EcoFINDERS (FP7-264465) and ForESTFlowers (FP7-PEOPLE-2010-IRSES-269204).

References:

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- Lumini E., Orgiazzi A., Borriello R., Bonfante P., Bianciotto V. (2010) Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. *Environmental Microbiology* 12, 2165-2179.



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4-OP

Resistance and resilience of the forest soil microbiome to logging-associated compaction

Beat Frey (selected abstract), Martin Hartmann

*Rhizosphere Processes Group, Swiss Federal Research Institute WSL,
CH-8903 Birmensdorf, Switzerland
beat.frey@wsl.ch*

Soil compaction is a major disturbance associated with logging, but we lack a fundamental understanding of how this affects the soil microbiome. We assessed structural resistance and resilience of the microbiota using a high-throughput pyrosequencing approach in differently compacted soils during a time-course of eight years after compaction. Compaction significantly reduced abundance, increased diversity, and persistently altered the structure of the microbiota. Fungi were less resistant and resilient than bacteria; clayey soils were less resistant and resilient than sandy soils. The strongest effects were observed around 6 to 12 months in the severely compacted wheel tracks where air and water conductivities were reduced permanently to 10% or even lower of the original conductivities of undisturbed soils. Eight years post-disturbance showed resilience in lightly but not in severely compacted soils. Bacteria capable of anaerobic respiration, including sulfate, sulfur, and metal reducers of the Proteobacteria and Firmicutes, were significantly associated with compacted soils. Compaction detrimentally affected ectomycorrhizal species, whereas saprobic, parasitic, and mutualistic fungi proportionally increased in compacted soils (Hartmann et al. 2014). Structural shifts in the microbiota were accompanied by significant changes in soil processes, resulting in reduced carbon dioxide, and increased methane and nitrous oxide emissions from compacted soils. Monitoring response and resilience of key microbial groups over time and after restoration measures, helps to predict the extent and persistence of soil damage and facilitate the development of efficient restoration strategies of compacted soils.

Keywords: forest soil compaction; soil physical characteristics; microbial diversity; ribosomal pyrotags; greenhouse gas fluxes; soil function

References:

Hartmann M., Niklaus P.A., Zimmermann S., Schmutz S., Kremer J. et al. (2014) Resistance and resilience of the forest soil microbiome to logging-associated compaction. *The ISME Journal* 8, 226-244.

5-OP

**Could mycorrhizae perform “ecosystem engineering” on the host plants?
Basis on rhizoculture of agroforestry systems to improve their resilience**

Luis G. García-Montero¹ (Selected Abstract), V. Monleon², D. Myrold³, T. Kuyper⁴, J. Trappe², J. Baham³, I. Valverde⁵, X. Parladé⁶, A. Álvarez Lafuente⁷, L.F. Benito-Matías⁷, J.R. Quintana⁵, A. Quintana¹, P. Andrés¹, M. Morcillo⁸, C. Menta⁹, S. Pinto⁹, P. Díaz¹⁰, C. Lefevre³, D. Luoma³, C. Turkmen¹¹, J.A. Domínguez¹⁰, A. Santiago⁵, L. Pinto¹, P. Pita¹⁰, F. García-Robredo¹, S. Ortuño¹, J. Pera⁶, E. Verrecchia¹²

¹Dept. of Forest Engineering, E.T.S.I. Montes, Technical University of Madrid (UPM),
Ciudad Universitaria s/n, Madrid 28040, Spain;

²USDA Forest Service, USA;

³Oregon State University, USA

⁴Wageningen University, The Netherlands;

⁵University Complutense of Madrid, Spain;

⁶IRTA, Spain;

⁷National Forest Genetic Resources Centre El Serranillo, Spain;

⁸Micofora, Spain;

⁹University of Parma, Italy;

¹⁰Technical University of Madrid (UPM), Spain;

¹¹University of Çanakkale, Turkey;

¹²University of Lausanne, Switzerland

luisgonzaga.garcia@upm.es

Agroforestry has traditionally been focused on plant growth following plant-centric and anthropocentric points of view. Both paradigms have caused global problems such as soil loss, declining biodiversity and the stock of C in the soil, and the high cost and pollution associated to fertilizers. Both paradigms may have also limited the understanding of mutualistic symbioses of plants assuming that the role of non-photosynthetic symbionts is to mobilize the nutrients necessary for the plants which appear to be the dominant members of the symbiosis. We propose an alternative approach introducing a new 'rhizo-centric' point of view (where non-photosynthetic partners could be the dominant members). This rhizo-centric approach could be modelled by a comparison with the lichens, because (1) their fungal partner is in most cases the dominant member of the symbiosis and (2) some authors proposes that lichenic fungi 'farm' the photobionts in a controlled parasitism. Thus, the present hypothesis proposes to explore a ground-breaking hypothesis suggesting that many mycorrhizal fungi could farm their host plants in a controlled parasitism similarly lichenic model. Within this conceptual framework, the main objective of this hypothesis is to analyze whether 'Ca paradox' (which means that Ca acts simultaneously as nutrient and as stressor of plants) could explain the hypothesized dominance of mycorrhizal fungi on plants on a global scale. This Ca paradox could be provoked by an accumulation of Ca salts (and increased pH) in the rhizosphere associated to mycorrhizal rock-eating activity (and also encouraged by soil fauna). In the event that this hypothetical approach bears fruit it would provide the necessary basis to start to change our agroforestry technology by introducing the 'rhizoculture' based on soil microbiology management (following lichenic model), soil Ca management (using liming), mycorrhizal rock-eating capacity, and soil fauna and livestock management.

Keywords: *ectomycorrhizae; rhizosphere; calcium; rock-eating activity; agroforestry*



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6-OP

Microbial and plant influences on bioavailability of hydrophobic organic pollutants in soil

Jose Julio Ortega-Calvo (Invited Speaker), C. Jimenez-Sanchez,
R. Posada-Baquero, J.L. García, M. Cantos

Instituto de Recursos Naturales y Agrobiología de Sevilla, CSIC, Spain
jjortega@irnase.csic.es

The occurrence of slowly desorbing fractions of hydrophobic pollutants such as polycyclic aromatic hydrocarbons (PAHs) is well explained today by environmental organic chemistry. The association with semisolid, NAPL-like hydrocarbon materials (e.g. creosote), black carbon, and aged residues often leads to slowly desorbing pollutants with a modified bioavailability and persistence. However, the microbial and plant interactions with such residues are less understood. Microorganisms may exhibit a unique set of activities relevant for mobilization and biodegradation, such as biosurfactant production, adhesion and behavioural responses. Plants can also modify the bioavailability of soil pollutants, either directly through physical and chemical actions occurring in the rhizosphere, or indirectly by interacting with soil microorganisms. Indeed, the high levels of microbial biomass associated with the rhizosphere, the carbon turnover caused by root exudates and their microbial utilization as co-substrates for the co-metabolism of PAHs and chemotaxis, as well as the migration of dissolved contaminants within the soil matrix as a result of interactions with dissolved organic matter, are all factors that promote the bioavailability of PAHs (Tejeda-Agredano et al. 2013; Jimenez-Sanchez et al. 2015). These influences are relevant not only for innovation efforts in bioremediation but they have also connections with the determination of bioavailability of organic chemicals in risk assessment and regulation (Ortega-Calvo et al. 2015).

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7-OP

Diversity, distribution and functions of yeasts in soils

Andrey Yurkov¹ (Invited Speaker), José Paulo Sampaio²

¹*Leibniz-Institute DSMZ - German Collection of Microorganisms and Cell Cultures,
Inhoffenstraße 7B, 38124 Braunschweig, Germany;*

²*UCIBIO, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia,
Universidade Nova de Lisboa, 2829-516 Caparica, Portugal*

andrey.yurkov@dsmz.de

Almost a half of the total terrestrial carbon is concentrated in boreal and temperate forests. These stocks are located in soils and dead plant material in form of complex substances, decomposed largely by fungi. Fungi living in soils can be divided in two functional groups: filamentous fungi that are multicellular and can form large mycelial networks and yeasts that are predominantly unicellular. Yeasts are an artificial group of fungi and belong to both the Asco- and Basidiomycota. While filamentous fungi may transport nutrients and water over long distances, yeasts are more locally dependent on environmental conditions but normally respond faster to environmental changes.

Yeasts inhabit soils worldwide and basidiomycetous yeasts are among the dominating fungi in soil substrates as revealed by culture-independent surveys. Since traditionally yeasts were considered as able to degrade only simple carbon compounds like sugars, their occurrence in soils was originally considered to be haphazard. However, unlike the typical saccharolytic phenotype often attributed to yeasts, basidiomycetous species are able to utilize a wide spectrum of carbon sources, including intermediates of lignin degradation, phenols and heterocyclic compounds. Assimilation of lignin and cellulose derivatives, oligotrophy, and psychrotolerance imply that yeasts could play a role in the decomposition process, especially in cold regions or at low temperatures. Formation of polysaccharide capsules further helps yeasts to sequester and concentrate nutrients and sustain low water activity or freezing. These compounds also play a role in soil aggregation and stability.

Recent studies on soil yeasts have shown that climate, basic soil parameters and rainfall determine the size, diversity and structure of the yeast community. However, in a single biome, forest properties and land management have a superior role over basic environmental factors. In forests, aboveground deadwood deposition support development of typical soil-borne species. With a selection of case studies we will exemplify how biodiversity assessments are enlarging our knowledge of yeast ecosystem services.

Keywords: soil; fungi; ecosystem services; decomposition; nutrient cycles

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8-OP

Tracing the activity of individual microbial taxa in forest soils

*Petr Baldrian¹ (selected abstract), Salvador Lladó-Fernández¹, Rubén López-Mondéjar¹,
Tomáš Větrovský¹, Adina Chuang Howe², Katharina Riedel³, Lucia Žifčáková¹*

¹*Laboratory of Environmental Microbiology, Institute of Microbiology of the ASCR,
Prague, Czech Republic;*

²*Iowa State University, Ames, USA;*

³*Ernst-Moritz-Arndt Universität Greifswald, Germany*

baldrian@biomed.cas.cz

In forest soils, microbes are important drivers of soil processes, because they mediate decomposition as well as nutrient transfer from primary producers into soil. Metatranscriptomics, metaproteomics or enzyme activity assays can give a fair picture about the functioning of whole microbial communities, but they are per se not able to identify individual microbial species, participating in the soil processes. Here we addressed the functional potential of dominant microbial species in forest topsoil and to track their real activity in the ecosystem by the combination of shotgun metatranscriptomics, strain isolation and characterization and proteomics of their cultures. This helped us to define the roles of dominant microbes in forest topsoil and to analyze whether their transcription *in situ* differs among horizons and seasons with tree photosynthetic activity and no activity. Isolation has yielded 20 bacterial strains representing some of the dominant molecular OTU from the ecosystem, some of them likely represent novel taxa at the genus level. Among dominant bacteria, interestingly, strains belonging to Acidobacteria showed the highest production of decomposition-related enzymes and the highest counts of glycosyl hydrolases in genomes, in contrast to Bacteroidetes and Proteobacteria. When metatranscriptomic reads were mapped to the genome sequences obtained, it was recorded that their transcript profiles *in situ* differ among horizons and among seasons. The same was noted for fungi from the same ecosystem. Furthermore, culture-based studies followed by proteomics of bacteria showed that by far not all predicted genes involved in decomposition are expressed in the presence of their substrates and that their decomposition systems are variable and complex, and may contain components that were previously not noticed. The results show that a combination of contemporary methods is able to answer the question about the role of individual taxa in their environment.

9-OP

Understanding the factors driving soil aggregate stability: the role of plant-soil-root-microbe interactions

Luis Merino-Martín^{1,2} (selected abstract), Amandine Erktan¹, Yogan Monnier¹, Catherine Roumet³, Yves Le Bissonnais⁴, Rob Griffiths², Alexia Stokes¹

¹INRA, UMR AMAP, Montpellier, France.

²Centre for Ecology & Hydrology, Crowmarsh Gifford, Wallingford, Oxfordshire, United Kingdom.

³C.N.R.S., UMR CEFE, Montpellier, France.

⁴INRA, UMR1221 LISAH, Montpellier, France

luismerinomartin@gmail.com

The soil aggregate stability, described as the resistance of soil aggregates to breakdown under disruptive forces, is a key soil property affecting several ecosystem processes such as infiltration, carbon storage, nutrient availability and erodibility (i.e. the resistance of soils to erosion). Recent studies have highlighted the importance of microbes (e.g. bacteria and fungi) to soil aggregate formation and stabilization. The effects of microbial communities on soil aggregates have mainly been described for fungi, in particular for Arbuscular Mycorrhizal Fungi (AMF). However, the importance of bacteria on soil aggregate formation has also been highlighted (Caesar-TonThat et al. 2008). Furthermore, the role of roots on aggregate stability have also been widely discussed. The trait based approach, developed in plant ecology, has recently been pointed as a promising way to explore the drivers of soil aggregate stability (Rillig et al. 2015). Identifying which root and microbial aspects have a primary influence on soil aggregate formation and aggregation would help to improve our mechanistic understanding of these processes. In our multidisciplinary research team, we are working on understanding the factors affecting soil aggregate stability and more specifically, the role of soil, root and microorganisms and their interactions affecting aggregate stability. In this talk, the different approaches undertaken by our research team will be presented: 1) a correlative approach with field studies in different agro-forests, in a successional gradient of restored road-slopes and agro-ecosystems and forests under different climates; 2) an empirical approach with experiments manipulating microbial communities and plants in soils and inoculating soil aggregates with chemical compounds.

Keywords: soil aggregate stability; soil microbial communities; soil erosion; root traits

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10-OP

Microbial functional diversity in restored biodiverse forest soil

Mark Tibbett (Invited Speaker)

*School of Agriculture, Policy and Development, University of Reading, Whiteknights, PO Box 237,
Reading, RG6 6AR, UK
m.tibbett@reading.ac.uk*

Substantial effort has been put into the restoration of biodiverse forests following major disturbances such as large scale mining (Tibbett, 2015; Spain et al. 2015; Collins and Brundrett, 2015). Aboveground ecological succession has been studied extensively, particularly with regard to flora and fauna (e.g. Grant, 2006; Nichols and Nichols, 2003) yet little is known of the rejuvenation of the soil microbial function - a vital component of the ecosystem known to directly and indirectly influence aboveground diversity.

In order to determine the trajectory of soil microbial development towards a native forest benchmark, restored sites at a bauxite mine in the Jarrah forest of Western Australia were selected providing a time-series for tracking the trend of microbial development over 16 years. The restored sites were treated as a chronosequence (space-for-time substitution) in which soil microbiological function could be analysed overtime and compared to native, undisturbed, benchmark sites.

Soil microbial function was measured in terms of biomass and activity. Primary functional microbial analysis employed MicroResp multiple substrate induced respiration, which showed that the development of the chronosequence towards the native benchmark was driven by quantity of C substrate utilisation. Levels of microbial biomass equivalent to native forests were reached by the oldest year of the chronosequence as measured by microbial biomass C, as well as total organic nitrogen and total carbon (George et al., 2010; Lin et al. 2011). Substrate induced and basal respiration rates significantly surpassed native levels by the oldest year of the restored chronosequence and non-metric multidimensional scaling plot supported with analysis-of-similarity showed that functional diversity appeared to increase with time

Keywords: soil microbiology; biodiversity; Jarrah forest, MicroResp

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11-OP

Forest fragmentation and drought as engines of change of the plant-soil-microbial ecological interactions in the Mediterranean basin.

Jorge Curiel Yuste¹ (Invited Speaker), J. Barba², D. Flores-Rentería¹,
F. Valladares¹, A. Rincón³, F. Lloret²

¹Museo Nacional de Ciencias Naturales (MNCN, CSIC). Serrano 115 dpdo, Madrid 28006, Spain;

²Centre de Recerca Ecologica i Aplicacions Forestals (CREAF). Bellaterra (Barcelona); 08193
Cerdanyola del Vallès, Spain;

³Instituto de Ciencias Agrarias (ICA); Serrano 115 dpdo, Madrid 28006, Spain

j.curiel@mncn.csic.es

Microbial communities are responsible for half of the CO₂ emissions from terrestrial ecosystems and for many other functions essential for the functioning of terrestrial systems. How these hyperdiverse and multifunctional communities will be affected by ongoing environmental perturbations associated with global change (e.g. habitat fragmentation or climate change) will surely have strong, although largely unknown, impact on the resilience and dynamics of Carbon (C) and essential nutrients such as nitrogen (N) of terrestrial ecosystems. In this regard, the Mediterranean basin, due to the large history of human intervention as well as the already notorious observed changes in climate (increases in summer droughts) induced by climate-change, offers one of the best natural labs to study how, and at which extent, different global change engines (and their interaction) may affect the ecology of microbial communities and its potential impact over ecosystem functioning and biogeochemical cycles. I here want to give you an overview on recent advances in our understanding on how global change engines may influence the ecology of soil microbial communities, its interactions with other ecological compartments of the natural systems and how this might be associated with potential implications over biogeochemical cycles. Based on both experimental and field-based monitoring of both the taxonomic and functioning of these communities, we here argue that to understand future trends in C emissions and N dynamics, it is of utmost importance to deepen both, the capacity of microbial communities to physiologically acclimate to local conditions and the complex mechanisms of plant-soil-microbial ecology as main drivers of C and N flow within the system.

Keywords: *microbial communities; carbon dynamics; resilience; global change; plant-soil-microbial interactions*

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12-OP

How does soil microbial community composition and nitrogen supply change with ecosystem age in primary boreal forests?

Mona N. Högberg¹ (selected abstract), Róbert Blaško¹, Tamara Walsky², Stephanie A. Yarwood²

¹Department of Forest Ecology and Management, Swedish University of Agricultural Sciences,
SE-90183, Umeå, Sweden;

²Environmental Science and Technology, University of Maryland, College Park, MD, 20742, USA
mona.n.hogberg@slu.se

To test the role of microorganisms as key immobilisers of available N in the soil we followed changes in C and N cycling with soil age (up to 560 years) in a replicated chronosequence of boreal forest ecosystems created by iso-static rebound after the last glaciation. Previous studies along the Gulf of Bothnia have shown that under such circumstances microbial community composition changes. We focus more than the previous studies on the early and potentially rapid changes of relations between ecosystem age and microbial community composition. Data on organic matter ¹⁴C suggested that the largest input of recently fixed plant C occurred in the younger coniferous forest ecosystems. With increasing ecosystem age, the ratio of microbial C to total soil C was constant but the ratio microbial N to total N increased at the same time as the gross N mineralization declined. Microbial community and ¹⁵N data suggest an increasing role of ectomycorrhizal mycelium and associated microorganisms as sink for N and the creation of a tight N cycle typical of boreal forests.



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13-OP

Microbial communities, functional genes and nitrogen cycling processes in forest floors under four tree species

Relena R. Ribbons^{1,2,3} (Selected Abstract), David J. Levy-Booth¹, Sue J. Grayston¹, Morag A. McDonald², Lars Vesterdal³, and Cindy E. Prescott¹

¹*Department of Forest and Conservation Sciences, Faculty of Forestry, University of British Columbia, Vancouver, British Columbia, Canada;*

²*School of Environment, Natural Resources, and Geography, Bangor University, Wales, United Kingdom;*

³*Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark
rerr@ign.ku.dk*

How nitrogen (N) is cycled in forests is important for understanding ecosystem productivity, and the role of forests as sinks and sources of greenhouse gases. To determine the effects of tree species on N cycling, we studied two sites within a common garden experiment. We used the ¹⁵N pool-dilution method to estimate gross and net rates of N ammonification and nitrification in forest floor soils from western red cedar, western hemlock, Douglas-fir, and Sitka spruce. We used qPCR to determine the bacterial and fungal abundance under each tree species, and the abundance of functional genes associated with nitrification (AOA *amoA*, AOB *amoA*) and denitrification (*nirS*, *nirK*). Cedar had the highest rates of gross N ammonification and ammonium consumption, followed by spruce, hemlock, and Douglas-fir. All tree species showed net immobilization of nitrate. Site differences in C:N ratios appear to be the primary influence on forest floor microbial biomass C:N dynamics, nitrification and denitrification gene abundance, N ammonification and nitrification rates. Nitrogen ammonification rates were associated with microbial biomass, including microbial C and the abundance of bacterial 16S rRNA genes. Nitrification rates were associated with microbial C and the abundance of AOA *amoA* genes, as was the forest floor genetic potential for denitrification. Microbial communities for AOA *amoA* and *nirS* differed amongst tree species, while AOB *amoA* and *nirK* did not, which suggests that tree species foster different abundances of nitrification and denitrification functional groups, and pools of N. The genetic potential for denitrification was strongly influenced by the abundance of ammonia oxidization potential within forest floors. Functional genetics can be used to explore the mechanistic link between tree species effects on soil microbes, and the nitrogen cycling processes regulated by those microbes

Keywords: ammonia oxidizers; nitrifiers; ¹⁵N pool dilution; tree species effects; forest nitrogen cycling



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14-OP

Effects of geometrid moth attacks on belowground processes in sub-arctic birch forests

*Hans Göransson¹ (Invited Speaker), Taru Lehtinen², Lars Ola Nilsson³, Sari Stark⁴,
Mathias Mayer¹, Boris Rewald¹*

¹Forest ecology, University of Natural resources and life sciences, Vienna, Austria (BOKU);

²Institute for Sustainable Plant Production, Department for Soil Health and Plant Nutrition, Austrian
Agency for Health & Food Safety (AGES), Vienna, Austria;

³Norwegian Institute of Bioeconomy Research (NIBIO), Svanhovd, N-9925 Svanvik, Norway;

⁴Arctic Centre, University of Lapland, Rovaniemi, Finland

hans.goeransson@boku.ac.at

Climate change is predicted to have the largest effects at high latitudes. Increased temperatures have been suggested to increase the frequency of insect outbreaks in sub-arctic mountain birch (*Betula pubescens* ssp. *czerepanovii*) forests. Severe outbreaks of geometrid moths can defoliate entire birch stands including the ground cover of *Vaccinium* sp.; repeated outbreaks in consecutive years frequently cause tree death. Defoliation and especially tree death will have consequences for belowground processes affecting soil carbon quality, pools and fluxes.

Plots affected in varying degrees by moth outbreaks; from undisturbed, partly disturbed to highly disturbed (trees killed) by repeated moth attacks were selected at 5 locations in northeast Norway, north Finland and northwest Sweden. Soil CO₂ efflux was measured and soil cores taken for soil analysis below the point of soil CO₂ efflux measurements.

In the humus layer the C:N-ratio was lower in the disturbed stands than in the undisturbed likely caused by a decrease in litter input. Chitinase enzyme activity indicated a decrease in microbial nitrogen demand in the dead stands and a loss of ectomycorrhiza. Living woody fine root biomass decreased by more than 50% in dead stands; this loss was only partly compensated by an increase in grass and herb root biomass. As a consequence, soil CO₂ efflux was up to 50% lower in the dead stands - independently of the time since stand dieback. Soil CO₂ efflux was explained to 51% by living fine root biomass and potential chitinase activity. Thus, geometrid moth attacks significantly affect soil C quality, pools and fluxes of sub-arctic birch forests. Birch stands in the sub arctic have in general been found to be a sink of CO₂, a future increase in moth outbreaks may turn sub-arctic birch forests into a carbon source, creating a positive feedback to climate change.

Keywords: soil respiration; insect outbreaks; enzyme activity; root biomass

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15-OP

**Does the soil microbial community affect the endophytes diversity into the xylem and the resilience to vascular wilt diseases?
The olive tree as a case study**

Franco Nigro (Invited Speaker), Ilaria Antelmi

*Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente,
Università degli Studi di Bari - Aldo Moro, via Amendola 165/A, 70126 Bari, Italy
franco.nigro@uniba.it*

Asymptomatic plant tissues host a variety of fungal, bacterial, or actinobacteria endophytes that are often called “latent pathogens”. However, the type of interaction between tree and endophyte can vary from neutral association to mutualistic, saprotrophic or pathogenic lifestyle. Under suitable conditions, certain microbes can adopt anyone of these life-styles, adding further complexity to the biodiversity inside plant. Endophytes have a great potential to shape and modulate the biotic and abiotic stress tolerance in host plants, directly by inducing defence responses in the plant or indirectly through competition for nutrients. In this paper, data available on fungal and bacterial community occurring in the xylem of tree plants will be reviewed, and new information about the fungal and bacterial community associated with the quick decline syndrome of olive trees will be provided. Moreover, the relationship between xylematic microbial community and the development of vascular wilt diseases will be discussed.

16-OP

The use of traits for soil invertebrates: state of the art and perspectives

Jérôme Cortet¹ (Selected Abstract), Apolline Auclerc², Mickael Hedde³,
Johanne Nahmani¹, Benjamin Pey⁴

¹CEFE UMR 5175, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier, EPHE,
Bat Fabre, route de Mende, 34199 Montpellier Cedex 5, France;

²Université de Lorraine, INRA, Laboratoire Sols et Environnement, UMR 1120,
TSA 40602, 54618 Vandœuvre-lès-Nancy, France;

³INRA Centre de Versailles-Grignon, UR251 Physicochimie et écotoxicologie des sols
d'agrosystèmes contaminés, RD 10, F-78026 Versailles, France;

⁴Ecolab, ENSAT, av. de l'agrobiopole, BP32607, 31326 Castanet-Tolosan, France
jerome.cortet@univ-montp3.fr

Traits have been widely used for several years for the study of plant or benthic invertebrate communities. For few years, groups of researchers, particularly within Europe, have started to apply this concept to soil invertebrates (mainly earthworms and collembolan). For that purpose theoretical frameworks have been proposed (Pey et al. 2014) and databases are being to be built (Pey et al. 2014). One of the main ideas of the trait concept is to explore the functional aspects of organisms, which seems very promising for the study of the effects of environmental changes on soil biota and ecosystem services assessment. First results have shown applications for the comprehension of the distribution of communities at large scales (Salmon et al. 2014), effects of agricultural practices or environmental management (Vandewalle et al. 2010; Pelosi et al. 2014), spatial ecology at local scales (Widenfalk et al. 2015) or urban ecology (Santorufu et al. 2014; Santorufu et al. 2015). In this presentation we will focus on the main results obtained and perspectives for soil functional ecology.

Keywords: soil; traits; collembolan; earthworms; soil functioning; ecosystems services

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17-OP

Modeling plant - soil interactions in an ectomycorrhizal forest

Oskar Franklin (Selected Abstract)

IIASA, International Institute for Applied Systems Analysis, Laxenburg, Austria

franklin@iiasa.ac.at

Ectomycorrhizal symbiosis is omnipresent in boreal forests, where it is assumed to benefit plant growth. However, in contrast to previous beliefs, experiments have shown that ectomycorrhizal symbiosis does not alleviate plant nitrogen (N) limitation (Näsholm et al. 2013). One possible explanation for symbiotic stability despite inconsistent plant benefits is that competition among trees for scarce N and competition among fungi for carbon (C) provide strong incentives for symbiotic trading at the individual level. Due to N immobilization in the biomass of mycorrhizal fungi the symbiosis further aggravates nitrogen limitation, ultimately reducing plant growth compared to a hypothetical-non mycorrhizal forest. This feedback mechanism has been proposed to maintain the strong nitrogen limitation ubiquitous in boreal forests (Franklin et al. 2014) and it may contribute to long-term soil carbon storage (Averill et al. 2014). However, the long-term stability and potential future consequences of these hypotheses have not been evaluated. Here we explore the hypotheses in a dynamic long-term perspective by incorporating our mycorrhizal model in a more complete ecosystem framework, considering the interactions with other microbes and the dynamics of soil C and N. In particular we elucidate the role of different forms of nitrogen, organic and inorganic, for the mycorrhizal ecosystem and how this may affect soil carbon dynamics and tree productivity under increasing atmospheric carbon dioxide and N deposition.

Keywords: boreal forest; mycorrhiza; ecosystem; carbon cycle; nitrogen cycle

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18-OP

Using bacterial and fungal responses to evaluate the mechanisms by which ash application impact forest soils

Carla Cruz-Paredes¹ (Selected Abstract), Rasmus Kjølner¹, Johannes Rousk²

¹Terrestrial Ecology Section, University of Copenhagen, Universitetsparken 15,
2100 Copenhagen Ø, Denmark;

²Microbial Ecology, Department of Biology, Lund University, Ecology Building, 22362 Lund, Sweden
carla.paredes@bio.ku.dk

Wood ash has been proposed as a liming agent and a fertilizer for forest soils. However, the use of ash has been questioned because of the risk associated with heavy metals. Soil pH is one of the most influential factors that structure microbial communities in soil, and it is also well-known that heavy metals strongly affect microbial communities. Ash application to soil results in parallel effects, including increases in pH and elevated metal concentrations. This makes it a challenge to link effects to causal mechanisms. The aim of this study was to evaluate the effects of wood ash on soil microbial communities by: assessing (1) responses of the microbial community structure (PLFA) and of bacterial and fungal growth in soils where different concentrations of wood ash were added, and (2) how ash application modulates the pH dependence and cadmium (Cd) tolerance of microbial communities. In parallel microcosm studies we compared the effect of wood ash contrasted with the effects of Cd and lime in a factorial design. These experiments were designed to disentangle the pH and toxicological effects of Cd in ash. Ash application strongly affected the microbial community structure. In addition, bacterial growth increased and fungal growth decreased with higher rates of ash application. The bacterial stimulation was probably caused by a direct pH effect, while the fungal responses could be related to competitive interaction with the stimulated bacteria. The ash application also modulated the pH relationships of bacterial communities, consistently shifting them toward more alkaline values. These results suggested that a substantial fraction of the microbial community response was a direct pH-effect. We were able to induce tolerance to Cd in the microcosm systems exposed to a Cd solution, but found no evidence suggesting that the ash affected the microbial tolerance to Cd. We conclude that ash affected microbial communities via the increase of pH but exerted no Cd-toxic effects.

Keywords: *ash; pH; cadmium; PLFA; microbial community*

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1-eP

Impacts of water stress on fine root dynamics and soil C storage in altitudinal forests of Himalayan Bhutan

Iftekhhar U. Ahmed, Yonten Dorji, Norbu Wangdi, Georg Gurtzer, Douglas Godbold

*Institute of Forest Ecology, Universität für Bodenkultur (BOKU),
Peter Jordan Strasse 82, Vienna 1190, Austria
iftekhhar.ahmed@boku.ac.at*

The forests of Himalayan ecosystems are vulnerable to global climate change and the evidence showed that the impacts in the regions are three-fold higher than the global average. In Eastern Himalayan Bhutan, altitudinal spectrum and local climatic conditions create a unique forest vegetation gradient and is influenced mainly by soil water regimes. The response of these moisture induced forests to climate change especially, delay or failure of Asian summer monsoon, is uncertain. Whilst the effects of monsoon failure on the agro-environment and subsequent livelihood of the vast population of the region are well documented, the impact on forest ecosystems, particularly on belowground functioning is seldom available. Therefore, the objectives of the present study are i) to explore fine root biomass and soil C storage in coniferous and mixed broadleaved forest of Himalayan Bhutan and ii) to assess the effects of water stress on fine root dynamics under the manipulated drought conditions. A throughfall exclusion experiment was setup to study the impacts of climate change and delay or failure of Asian summer monsoon on two forests of sub alpine coniferous dominated by hemlock (*Tsonga dumosa*) and temperate mixed broadleaved dominated by oak (*Quercus lanata* & *Quercus griffithii*) at the altitudes of 3300 m and 2300 m respectively. The standing fine root biomass was estimated by sequential coring technique and the turnover of fine root by litter bag experiment. The fine root biomass of oak dominated forest was 620 g m^{-2} which was much higher than the coniferous forest (460 g m^{-2}) and after exclusion of throughfall (4 months) in first year no significant variation was observed due to drought treatment. The organic C stocks in top 50 cm soils of coniferous and broadleaved forest were 20 and 11 kg m^{-2} , respectively. The vertical distribution of fine root biomass and soil C indicates strong influence of dominant tree species and soil properties of the forest ecosystems. Finally, these findings will contribute to evaluate the vulnerability and resilience of Himalayan forest responses to global climate change.

Keywords: altitudinal forest; fine root biomass; drought; soil C; climate change



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2-eP

Fine root biomass and production in characteristic land use types of the Ethiopian highland

Dessie Assefa¹, Boris Rewald¹, Hans Göransson¹, Abriham Abiyu², Douglas Godbold¹

*¹Institute of Forest Ecology, Department of Forest and Soil Sciences,
University of Natural Resources and Life Science, Austria;*

²Amhara Agricultural Research Institute, Bahir Dar, Ethiopia

dessie.assefa@boku.ac.at

Although fine root turnover constitutes a major pathway of carbon from plants to soil in all ecosystem, information on root biomass and its turnover are still scarce-especially for highland ecosystems in Africa. Thus, fine root stock and production were studied in one of the few remaining natural, diverse highland forests in Gelawdios, Amhara region, NW Ethiopia. The natural forest was compared to Eucalypt plantations, grazing land and cropland in close vicinity to see how the extensive land use change in northern Ethiopia effects the carbon dynamics.

Three different methods were used to determine fine root production: sequential coring, ingrowth cores and ingrowth nets. Soil cores for sequential coring analyses were gathered in quarterly intervals, while ingrowth cores and nets were harvested monthly. A clear vertical gradient in fine root stock existed along soil depth; for example, fine root biomass in the forest topsoil (0-10 cm) was about 60% higher than in subsoils (10-20 cm). Based on sequential coring estimation, root mass stock in natural forest and eucalyptus plantation were affected by season which, showed peak values in dry time and lowest values occurred at the end of rainy season. Depending on the method determined, fine root production and turnover rates varied greatly. From ingrowth core and net estimation methods, fine root production was significantly less in grazing and crop lands than eucalyptus and forest stands. The importance of natural, diverse forests for high carbon sequestration rates will be discussed.

Keywords: root biomass; necromass; root production; root turnover

Acknowledgments: This work was funded by Austrian ministry of agriculture, forestry, environment and water management through Carbo-Project.

3-eP

Effects of a single microbe versus a complex microbial inoculum on grapevine roots

Raffaella Balestrini¹, Alessandra Salvio², Mara Novero², Giovanni Gabelli², Samuele Voyron²,
Matteo Chialva², Alessandra Dal Molin³, Fabio Marroni⁴, Paola Bonfante²

¹CNR-IPSP, Istituto per la Protezione Sostenibile delle Piante, UOS Torino, CNR, Torino, Italy;

² UniTO, Dipartimento di Scienze della Vita e Biologia dei Sistemi, Torino, Italy;

³ UniVR, Dipartimento di Biotecnologie, Ca' Vignal 1, Verona, Italy;

⁴ Istituto di Genomica Applicata (IGA), Udine, Italy

raffaella.balestrini@ipsp.cnr.it

Plant functionality is highly dependent on the associated microbiota, and among root-associated microbes the symbiotic fungi that form arbuscular mycorrhizae (AM) are very relevant component. *Vitis vinifera*, cultivated for both fruit and beverage, represents one of the most economically important fruit crop worldwide and is a model organism for fruit trees, thanks to its sequenced genome (Jaillon et al. 2007; Velasco et al. 2007). It is cultivated as a perennial crop, traditionally non-irrigated and with a diffused distribution over a wide area of dry or semi-dry ecosystems. Vineyard soils support indigenous AM fungi (AMF) and it is well established that grapevine roots are colonized by AMF (Balestrini et al. 2010). AMF colonization of vines roots can result in increased growth, enhanced nutrient uptake and improved tolerance to biotic and abiotic stresses (Hao et al. 2012). This work is aimed to study the impact of an AM fungal species (*Funneliformis mosseae*) and a commercial inoculum (potentially containing a mixture of AM fungi, *Trichoderma* sp. and bacteria) on the root transcriptome of Pinot noir plants grafted on 110 Richter rootstock. A HiSeq experiment has been performed on RNA extracted from roots of plants maintained in three conditions: non-inoculated plants, mycorrhizal plants and plants inoculated with the commercial inoculum. After 3 months, roots resulted to be mycorrhized exclusively after the *F. mosseae* treatment, and consequently RNAseq analysis revealed several AM marker genes to be up-regulated. The commercial inoculum did not lead to any colonization by AMF, but elicited a more important transcriptional regulation, which was probably due to the dominant presence of plant-growth-promoting bacteria. Fourteen selected genes were validated by RT-qPCR. GO-enrichment and KEGG analyses are in progress to verify the metabolic pathways elicited by the single AM versus the complex inoculum.

Keywords: AM fungi; grapevine; soil microorganisms; commercial inoculum; transcriptomics; RNAseq

Acknowledgments: The authors thank the project VIT-INNOVA (Val D'Aosta) for the funding and all the partners for the collaboration (CCS with Dr G. Giovannetti and S. Volpato, Vivai Roero, CERVIM, and IAR with Dr O. Zecca).

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4-eP

Spatial and Temporal Dynamics of Methane Oxidation across Two Contrasting Forest Chronosequences

Aisha Coggan¹, Alex Dumbrell¹, Elena Vanguelova², Corinne Whitby¹

¹School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, UK;

²Forest Research, Alice Holt Lodge, Farnham, Surrey, UK

acogga@essex.ac.uk

Methane (CH₄) has caused 42% of the total anthropogenic radiative forcing, and is therefore the second largest driver of climate change, after carbon dioxide. The global terrestrial methane sink is estimated to be ~30 Tg CH₄ha⁻¹ y⁻¹, with forest soils contributing significantly to this sink (Smith et al. 2000). Previous work in forest chronosequences has shown changes in soil parameters known to affect methane flux with forest age (Benham et al. 2012); however, little is currently known about how the activity, abundance and community structure of methane oxidising bacteria responsible for creating the sink changes with forest age and, in turn, how this impacts on methane flux. Here, we sampled an oak and a Sitka spruce forest chronosequence across a seasonal cycle, to examine how methane flux and the methane oxidising bacteria community change with forest age. Soil methane flux was measured using gas chromatography on headspace subsamples from static gas chambers. Soil samples were also collected and a range of physicochemical variables were measured to determine the causes of changes in methane flux using linear mixed effects models. Methane consumption did not change with forest age in the oak site. In contrast to this, in the Sitka spruce there was a significant decrease from high methane production in the youngest stand to negligible production the older stands. Soil moisture content has emerged as the primary driving factor of methane oxidation in both sites. These findings show the effect of forest age is dependent on forest type and suggest management strategies should be tailored to reflect this, in particular management of Sitka spruce must consider the potential for methane production in young stands.

Keywords: methane; methane oxidising bacteria; chronosequence; forest soil

Acknowledgements: The authors thank the NERC for project funding.

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5-eP

The role of fungi in the decay of coarse woody debris of European beech (*Fagus sylvatica* L.) in the secondary Virgin Forest Rajhenavski Rog

Domen Finžgar¹, Marko Bajc¹, Matej Rupel¹, Miha Humar², Hojka Kraigher¹

¹*Slovenian Forestry Institute, Vecna pot 2, 1000 Ljubljana, Slovenia;*

²*Biotechnical Faculty, Department of Wood Science and Technology,
Rozna dolina Cesta VIII/34, 1000 Ljubljana, Slovenia*

domen.finzgar@gozdis.si

In a limited view, forest trees sequester carbon until the culmination of their annual increment. After an equilibrium, this role is changed and trees have functions of carbon source due to the decomposition of tree tissues (Nijnik, 2010). However, since trees are never isolated but coexist with other species in the ecosystem, this ideal model is in reality significantly skewed. It is therefore crucial to assess decomposition processes in order to better understand carbon cycle in forest ecosystems (Stokland and Siitonen, 2012).

Researching the diversity and role of fungi in the decay of coarse woody debris (CWD) of European beech was divided in three parts. Determining the loss of CWD density during the decay was performed in part one, in the second part the distribution and succession of fungi in the decaying CWD was investigated, using the denaturing gradient gel electrophoresis (DGGE). There is also an ongoing third part, in which we are aiming to identify fungal communities involved in the CWD decomposition using the Illumina MiSeq next generation sequencing platform.

To study the suitability of other, less toxic, density measuring techniques than the standard mercury one, samples were measured in a mercury medium as well as in a water medium. For water measurements Sartorius MSA 323S scale with YDK01 Density Determination Kit was used for the first time in our labs. Basic, absolute and saturated densities were determined. Lower densities in higher decay phases were detected, as expected.

Based on the DNA extracted from fungi inside CWD, we conducted a DGGE analysis, resulting in fungi community profiles for each sample. According to the results, different decay stages host different types of fungal species, indicating a shift in composition of fungal community in relation to stage of decay. The number of different fungal ITS1 sequences per sample increases with higher decay phases.

Keywords: wood decomposition; DGGE; NGS; density; fungi

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6-eP

Effects of wood ash and liming on ectomycorrhizal fungal abundance, diversity and community composition - review and meta-analysis

Rasmus Kjøller¹, Carla Cruz Paredes¹, Karina Clemmensen²

¹*Terrestrial Ecology Section, University of Copenhagen, Denmark;*

²*Forest Mycology and Plant Pathology, SLU, Sweden*

rasmusk@bio.ku.dk

Liming or wood ash amendments are common practices to counteract acidification in forests. Additionally, wood ash also returns some of the nutrients removed after harvest or clear cutting. Here we review and synthesize previous work on liming and wood ash effects on ectomycorrhizal fungal abundance, diversity and community composition. Negative effects on fungal biomass could negatively feedback on the nutrient capturing capacity of the mycorrhizal fungi and thus of the trees. Negative effects on species richness or community shifts could negatively influence the functional diversity and resilience of the mycorrhizal fungal community. We show that while fungal abundance and species richness most often is not influenced, community composition in contrast changes. The community changes are to some extent predictable with certain genera decreasing (e.g. *Lactarius* and *Russula*) or increasing (e.g. *Tuber* and *Amphinema*) in abundance with liming or ash treatment. We discuss whether the observed community shift is expected to change the function of the ectomycorrhizal community.

Keywords: ectomycorrhizal fungi; liming; wood ash; forests; pH



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7-eP

Complementary shifts of roots, mycorrhizal networks and rhizobiome drive ecosystem adaptation in changing climate

*Ivika Ostonen, Marika Truu, Katrin Rosenthal, Kaarin Parts, Leho Tedersoo,
Jens-Konrad Preem, Krista Lõhmus, Jaak Truu*

Institute of Ecology and Earth Sciences, University of Tartu, Estonia

ivika.ostonen@ut.ee

The root-mycorrhizosphere interface of forest trees is the nexus of a variety of associations between bacteria, fungi, and host plant, the health of these root dependent consortia may play a key role in adaptation of forest ecosystems in global change. Root rhizobiomes and associated mycorrhizal networks affect not only forests sustainability, but contribute significantly into C sequestration in soil. We aimed to analyse the complementary shifts in the community of ectomycorrhizal fungi and rhizosphere bacteria in relation to the changes in root tip morphology and biomass partitioning in host trees. We compared free-air manipulative studies and climate gradient studies to ascertain the universal trends in root traits.

The EcM root traits, the structure of bacterial and EcM fungal communities in bulk soil and rhizosphere from climate manipulation experiment and the data from *Picea abies*, *Pinus sylvestris* and *Betula* sp stands along the climate gradient from boreal to temperate forests in Europe were analysed simultaneously.

The results of our studies revealed strong relations between the EcM root morphology, dominant EcM fungal colonisers and the bacterial community structure in the bulk soil and rhizosphere. Species-specific differences in root morphology were driven by site conditions and by the successional stage of host tree (Ostonen et al. 2013). A clear shift in EcM colonizers towards the dominance of hydrophilic morphotypes and towards symbionts with the long-distance exploration type in humidified stand and in temperate coniferous forests, respectively (Parts et al. 2013; Ostonen et al. 2011). We found significant relationships between bacterial phylotypes proportions and root traits.

Complementary mechanisms within roots, mycorrhizal networks and rhizobiome lead to adaptation of fine root system under global change, but may cause essential changes in C allocation and cycling of forest ecosystems.

Keywords: forest; climate change; root traits; ectomycorrhiza; rhizosphere; bacteria

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8-eP

Atheliaceae fungi are potential keystone species building up the belowground growth of Norway spruce

Taina Pennanen, Sannakajsa M. Velmala, Tiina Rajala

*Natural Resources Institute Finland (Luke), Bio-based Business and Industry,
Jokiniemenkuja 1, FI-01370 Vantaa, Finland
taina.pennanen@luke.fi*

Our studies focus on the relationships of ectomycorrhizal (ECM) fungi and the genetic and phenotypic traits of Norway spruce (*Picea abies*), which is one of the most dominating tree species in boreal forests of Europe. High ECM species richness, that may provide increased nutrient mobilization potential due to functional complementarity of the fungal community, was associated with well-growing spruce phenotypes. Especially the ECM fungal species belonging to the family *Atheliaceae* were found to abundantly colonize the well growing spruce origins (Korkama et al. 2006). These ECM fungi were effective in production of exoenzymes related to N and P uptake (Velmala et al. 2014). *Atheliaceae* are inconspicuous sporocarp forming basidiomycetes, which have long time been merely associated to decomposer communities inhabiting decaying wood in old-growth forests. Furthermore, we have found *Tylospora*, *Amphinema* sp. and *Piloderma* sp. to be the dominant fungal lineages in roots of very young, naturally regenerated and vital spruce seedlings. Other common lineages were e.g. *Tomentella*, *Meliniomyces*, *Russula* and *Lactarius*. These ECM were isolated and inoculated to the seedlings (Pennanen et al. 2015). Modification of the seedling growing conditions by altering nutrients and growing media enabled successful colonization of the isolated ECM, and promoted also dispersal of *Atheliaceae* fungi from the surrounding environment (Vuorinen et al. 2015). In inoculation studies ECM fungal species belonging to *Tylospora* and *Piloderma* increased seedling performance, especially their root/shoot (R/S) ratio more than other ECM species (Pennanen et al. 2015). *Piloderma* colonization was also found to alter root system structure towards less ramified with longer lateral roots (Velmala et al. 2014). *Atheliaceae* dominated seedlings possessing high R/S ratio grew also fast after outplanting to the field (Vuorinen et al. 2015). Our ECM community studies suggest that *Atheliaceae* are important players in the ecology of Norway spruce in boreal forests because of their good nutrient uptake potential and they may promote the host resource allocation towards roots.

Keywords: ectomycorrhiza; Picea abies; forest soil

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9-eP

***Clathrus archeri*, *Suillus lakei* and *Boletellus projectellus*
as a model organisms in fungal invasion studies**

Marcin Pietras

Faculty of Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

mpietras@man.poznan.pl

Invasive species are one of the main threats for biodiversity loss and ecosystem functioning. For a long time, studies on anthropogenic introductions have mainly focused on plant and animal invasive species. The invasion ecology of fungi has attracted much less attention. Therefore, the information on the introductions and spread of fungal invaders is scarce. The objective of this work is to provide a general framework of a project dealing with three invasive fungi: *Clathrus archeri*, *Suillus lakei* and *Boletellus projectellus*. The proposed research consists of six main steps. First step is to made a collection of vouchers, create a dataset of geographical data and molecular library of sequences obtained for each fungus. Next steps are: to design species-specific primers for each targeted fungal species, phylogeographical analysis of sequences obtained and field surveys connected with measurements of environmental factors. In fifth step the maximum entropy method will be used to create models of the suitable niche distribution and to test the niche conservatism of each species. In sixth and final step created distribution models combined with advanced molecular approach (primers design) will be used to define current and potential distribution of analysed fungi in an invasion range. This research will provide strong basic informations about invasion biology, ecology and biogeography of fungal invaders.



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10-eP

Synthesis of siderophores by *Streptomyces* sp. strains under cadmium stress

*Michał Złoch¹, Dominika Thiem¹, Sonia Szymańska¹,
Renata Gadzała-Kopciuch², Katarzyna Hryniewicz^{1*}*

¹*Department of Microbiology, Faculty of Biology and Earth Sciences,
Nicolaus Copernicus University, Lwowska 1, 87-100 Toruń, Poland;*

²*Chair of Environmental Chemistry and Bioanalytics, Faculty of Chemistry,
Nicolaus Copernicus University, Gagarina 7, 87-100 Toruń, Poland*

**hrynk@umk.pl*

Microbial siderophores are low-molecular mass organic compounds chelating the ferric iron (Fe^{3+}) increasing its bioavailability under iron limitation conditions. Apart from function in iron mobilization, siderophores can also chelate other heavy metals such as Al^{3+} , Zn^{2+} , Cu^{2+} , Pb^{2+} and Cd^{2+} , and thereby affect heavy metal tolerance of microorganisms and plants.

The main goals of our studies were selection of the most efficient in siderophores synthesis bacterial strains originated from metalliferous areas and analysis their ability to siderophores synthesis under different Cd^{2+} concentrations (0, 0.5, 1, 2 and 3 mM). We hypothesized that level of siderophore synthesis in selected bacterial strains can be stimulated or inhibited by increasing Cd^{2+} concentration and type of secreted siderophores depend on the heavy metal concentration. In the analysis we used a spectrophotometric chemical assays, high performance liquid chromatography (HPLC) as well combined techniques - LC-Q-TOF/MS.

Our studies proved that the most effective in synthesis of siderophores strains belonged to the genus *Streptomyces* derived from rhizosphere. Investigated strains were able to secrete three types of siderophores (hydroxamates, catecholates and phenolates) under Cd^{2+} stress which amounts generally increased after Cd^{2+} addition.

Keywords: siderophores; cadmium; Streptomyces

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11-eP

Effect of mycorrhization and pH substrate on nursery growth of *Castanea* hybrids

Amaya Álvarez Lafuente¹, Luis F. Benito-Matías¹, Beatriz Cuenca², Luis Ocaña²

¹CNRGF "El Serranillo" Ministerio de Agricultura, Alimentación y Medio Ambiente
crta Fontanar, s/n, 19004 Guadalajara, Spain;

²TRAGSA. Dpto de Mejora Agroforestal. Crta Maceda-Valdrey, km. 2, 32700 Maceda, Ourense, Spain
lusfbm@gmail.com

Actually, plant-mycorrhizal interactions are being revised. A lot of studies has been demonstrated that mycorrhizae increase seedling survival and growth rates. But another studies suggest that fertilization could have the same effect in plants. Some authors define the mycorrhization like a semiparasitoid relation. Ectomycorrhizal fungi could be use some nutrients (calcium for example) to stress their host. In this work, we compared the nursery performance and physiology of ectomycorrhizal and non-ectomycorrhizal chestnut seedlings grown in fertilized pear moss with different pH (6.5, 7.0, 7.5, 8.0 and 8.5).

Two hybrid chestnuts (*Castanea crenata* x *Castanea sativa*) selected for their resistance to on disease were produced in nursery. The propagation was made by cuttings. Cuttings was rooted in a growth chamber during 6 weeks, and transferred to a greenhouse where it was hardened progressively under a fog system. When cuttings has one year-old, were transplanted to 400 mL forest container. A combination of fungi was inoculated in the substrates of ectomycorrhizal seedlings. For control ones, no inoculation was done.

One year later, for each sample shoot height, root collar diameter, shoot dry weight, root dry weight, Fv/Fm ratio and mycorrhization rate (classes I to V, depending on percentage of mycorrhizal root tips) was analyzed. Concentration of nitrogen, phosphorous and potassium was checked too.

Data indicated that mycorrhization rate and pH substrate was an general negative effect in physiological and morphological characteristics.

Keywords: *Castanea* hybrids; pH substrate; mycorrhization



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12-eP

**Field performance and ECM community structure of Scots pine
(*Pinus sylvestris* L.) seedlings 5 years after pine forest litter addition**

*Algis Aučina¹, Maria Rudawska², Tomasz Leski², Marta Kowalska²,
Audrius Skridaila¹, Darius Ryliškis¹*

¹*Botanical Garden of Vilnius University, 43 Kairėnų Str., LT-10239 Vilnius-40, Lithuania;*

²*Institute of Dendrology, Polish Academy of Sciences, 5 Parkowa Str., 62-035 Kórnik, Poland*

algis.aucina@gf.vu.lt

Scots pine (*Pinus sylvestris* L.) is a tree species widely planted for reforestation and often used for afforestation of sites with unfavorable environmental conditions. Success of seedling establishment after outplanting is largely depended on mycorrhiza development. In nursery management practices, the application of forest litter for nursery-grown seedlings can be an attractive factor enhancing ectomycorrhizal (ECM) colonization and further performance on the field sites. The present investigation was focused on the effect of pine litter pre-treatment during nursery stage on survival and ECM community structure of Scots pine seedlings outplanted on four different sites: into a forest clear-cut (S1), a site contaminated by chemical emissions (S2), abandoned agricultural land (S3), and coastal sand dunes (S4) in Lithuania. Seedlings non-treated by forest litter and grown in nursery mineral soil have been used as a reference. Seedling survival was significantly higher in variant with litter pre-treatment than in variant without litter addition during three years after outplanting at sites S1, S2 and S4. Root systems of all pine seedlings were colonized by nursery-adapted ECM fungi and by indigenous ECM fungal communities characteristic for each study site. Twenty two, 17, 14 and 15 fungal taxa were detected on sites S1, S2, S3 and S4, respectively. *Wilcoxina mikolae* and *Thelephora terrestris* dominated ECM communities on seedlings from sites S1-S3, while *Cenococcum geophilum* was most abundant on seedlings from site S4. The results suggest that use of forest litter in nursery practice enhance seedling establishment in different environmental conditions after outplanting and may be useful approach to obtain high-quality planting stock with improved field performance.

Keywords: *forest litter; Scots pine; outplanting; seedling establishment; ectomycorrhiza*

13-eP

Plant-microorganism interactions in recovering a multi-contaminated area of Southern Italy in presence of poplar

Valeria Ancona¹, Paola Grenni², Anna Barra Caracciolo^{2}, Martina Di Lenola², Claudia Campanale¹, Vito Felice Uricchio¹, Angelo Massacci³*

¹CNR-IRSA, National Research Council of Italy, Water Research Institute, Bari, Italy;

²CNR-IRSA, National Research Council of Italy, Water Research Institute, Rome, Italy;

³CNR-IBAF, National Research Council of Italy,
Institute of Agro-Environmental And Forest Biology, Rome, Italy

* barracaracciolo@irsa.cnr.it

Plant-based clean up technologies (phytotechnologies) are gaining popularity as a sustainable technology for the remediation of contaminated soils (Peuke and Rebbenberg, 2005). Phytotechnologies can provide removal or degradation of pollutants together with additional benefits such as soil quality improvement, soil carbon sequestration and biomass for energy production. Due to the difficulty to remediate sites characterized by multiple pollutants (e.g. organic and inorganic toxic compounds), the study of plant-microbial interactions became a new interesting challenge to discover more sustainability soil recovery strategies. Consequently, in recent years, several microbial and plant species have been tested for the remediation of soil contaminants (Abhilash et al. 2012, 2013).

In this work the results of the first year of a Research Project in which poplar-microbial interactions in a multiple-contaminated area in Southern Italy were studied are reported. The investigated area was used for several decades as an unsupervised waste disposal and it is contaminated by PCBs as well as by heavy metals. A specific poplar clone (Monviso) with capabilities in promoting organic pollutant degradation (Meggo et al. 2013; Bianconi et al. 2010), was used in this area (785 m²). Six hundred poplar cuttings (Monviso clone) were planted in the area previously fertilized with compost. After 1 year, soil at different depth, roots and leaves of trees were sampled in selected plots and analysed in order to identify PCB and heavy metal contamination (HM). Chemical analyses were conducted using GC-MS and ICP-MS. At the same time, the microbial cell abundance, viability and dehydrogenase activity (DHA) of the autochthonous microbial community were evaluated to investigate the role of microorganisms in the remediation processes. The comparison of the initial chemical and microbiological analyses of the contaminated area with those performed after one year from the poplar plantation, highlighted interesting and promising results which support that in this area plant-microbial interactions play a key role in promoting degradation of organic pollutants (PCBs) and phytostabilization of the inorganic ones (HMs).

Keywords: *plant-microorganism interaction; PCB; poplar*

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14-eP

Ecosystem services provided by microbial communities in belowground soil

Anna Barra Caracciolo, Martina Di Lenola, Maria Ludovica Saccà, Paola Grenni

*CNR-IRSA, National Research Council of Italy, Water Research Institute,
Via Salaria Km 29,300, I-00015 Monterotondo (Rome)*

grenni@irsa.cnr.it

Ecosystem services are the contributions that ecosystems make to human well-being. They are seen as arising from the interaction of biotic and abiotic processes, and refer specifically to the 'final' outputs or products from ecological systems. Soil, that has a large proportion of the Earth's biodiversity, provides the physical substrate for most human activities. Although soils have been widely studied and classified in terms of physical and chemical characteristics, knowledge of soil biodiversity and functioning are still incomplete. Soil organisms are extremely diverse and contribute to a wide range of ecosystem services that are essential to the sustainable functioning of natural and managed ecosystems.

Microbial communities (Bacteria, Archaea, viruses, protists and fungi) are vital to soil ecosystem functioning. This is because they exist in enormous numbers and have an immense cumulative mass and activity. Most of the phenomena observed in the visible aboveground world are steered directly or indirectly by species, interactions, or processes in the belowground soil. In particular, being microbial communities, involved in nutrient cycling and organic matter degradation, they can affect productivity and biodiversity of phytocenosis. Moreover, the occurrence of some microorganisms can stimulate or inhibit certain plant species (e.g. through the production of toxic compounds) more than others. Microbial communities are the main responsible of soil homeostatic capabilities removing contaminants and providing key ecosystem regulation and supporting services such as soil fertility and resilience and resistance to different kind of stress. Vice versa, the biomass and activity of microorganisms is enhanced in the rhizosphere as a result of compounds exuded by the roots. The composition of root exudates varies from plant to plant and affects the relative abundance of microorganisms in the vicinity of the roots. Plants not only can provide nutrients for microorganisms, but some plant species also contain unique antimicrobial metabolites in their exudates affecting belowground diversity.

Understanding ecosystem functioning, and predicting response to global changes, calls for much better knowledge than we have today about microbial processes and interactions, including those with plants in the rhizosphere.



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15-eP

A metagenomic study on the effect of plant cover on soil bacterial diversity

Mariantonietta Colagiero, Isabella Pentimone, Laura Cristina Rosso, Aurelio Ciancio

CNR-IPSP, Istituto per la Protezione Sostenibile delle Piante, Bari, Italy

aurelio.ciancio@ipsp.cnr.it

To study the effect of cropping and plant cover on soil microbial diversity three adjacent sites characterized by the same soil, but differing for land use and cover, were sampled at Carovigno (Brindisi). Soil samples were collected from a traditional greenhouse producing horticultural crops, a close olive grove and an adjacent mediterranean (*Quercus ilex*) forest spot. The soil bacterial communities from replicated samples were identified with a metagenomic Next Generation Sequencing (NGS) approach. Total RNAs were extracted from 2 g soil subsamples and the V3-V4 hypervariable regions of the 16S rRNAs were sequenced with the Illumina MiSeq technology. A total of $2.46 \cdot 10^6$ reads was produced from 13 samples, of which 85% passed the quality threshold, yielding an average of $97 - 239 \cdot 10^3$ reads per sample. Almost all (99%) sequences belonged to the Kingdom Bacteria, and 30% were informative up to the species level. Using the Greengenes classification system, the average number of species per sample was around 10^3 . Most represented phyla in all samples were *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Planctomycetes* and *Verrucomicrobia*, with α -, β - and γ -*Proteobacteria* as most represented classes. NGS data showed that samples from cultivated soils (olive and vegetables) had higher frequencies (5-10%) of *Bacillales*, which were under-represented in the mediterranean forest. Data analysis at the species level is under course to identify changes in the bacterial composition at deeper taxonomic levels, as related to agricultural practices.



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16-eP

Eco-physiological diversity of soil bacteria communities in relation to tillage system and nitrogen fertilizer

*Claudia Dalmastrì, Annamaria Bevivino**

*Department for Sustainability of Production and Territorial Systems, Biotechnologies and
Agro-Industry Division, ENEA Casaccia Research Center, Rome, Italy*

**annamaria.bevivino@enea.it*

Soil is a complex and dynamic ecosystem whose functionality is related to the equilibrium existing among chemical, physical and biological parameters and the resident microbial communities. The relative abundance of *r-K* strategists, that have a theoretical relationship with a specific functional trait, can contribute to explain microbial community's resistance and resilience to soil disturbances (de Vries and Shade 2013). Then, in the present work, we aimed at assessing the impact of tillage system and nitrogen fertilizer application on soil bacterial communities in a soil that was managed under a *Triticum durum* and *Zea mais* rotation. Sampling was performed at two different seasons in a 13-years experimental field located in the coastal hills of Agugliano (Ancona, Italy). A split-plot with randomized blocks was designed to compare conventional tillage system (CT, 40 cm deep ploughing) with no tillage (NT, sod seeding with chemical desiccation and chopping) and two fertilizer treatments (70 Kg/ha P₂O₅ with or without 90 Kg/ha NH₄NO₃). All soils had similar texture (clay soil), pH values (8.1-8.4) and low C/N ratio (7.82-8.67). Differences were present in organic matter and nitrogen amounts; in particular, NT plots had a higher amount of organic matter than CT plots, and showed the most richness in N-tot (1.4 g/kg), while nitric N was higher in CT soils than NT soils. Soil bacterial population was investigated by using the *r/K*-strategy concept and Eco-Physiological (EP) index. The total microbial density ranged from 7.89 x 10⁵ to 1.16 x 10⁷ cfu g⁻¹ of soil. A high diversity of colony morphology was observed in no-till system. No significant difference in the EP index among samplings was found between the two seasons (P>0.05). In spring, a significant effect of nitrogen fertilization on the abundance of *r-k* strategists in CT soils was found (P=0.0002). Variation of EP index was significant in conventional tillage system with N-fertilization when compared to unfertilized soil (P<0.05). In conclusion, our results suggest that, among the factors investigated, N-fertilization affected the relative abundance of *r-K* strategists in conventional tillage system as well as the evenness of population distribution and richness of the microbial community.

Keywords: soil; N fertilization; tillage; bacterial communities; r-K strategist

Acknowledgment: This work was financially supported by the Italian Ministry for Education, University and Research through the National Project funded by National Program (FISR) SOILSINK: Climate change and agro-forestry systems, impacts on soil carbon sink and microbial diversity.

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17-eP

Mycorrhizal status of European white elm from Veliko Ratno Ostrvo island, Belgrade (Serbia)

Jovana Devetaković¹, Leszek Karliński², Marcin Pietras², Tomasz Leski², Maria Rudawska²

¹*Department for Seed science, nursery production and afforestation, Faculty of Forstry-University of Belgrade, Kneza Višeslava 1, Belgrade, Serbia;*

²*Laboratory of Symbiotic Associations, Institute of Dendrology of the Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland
jovana.devetakovic@sfb.bg.ac.rs*

European white elm (*Ulmus laevis* Pall.) is a tree species which is most common in riparian deciduous forests across Central and Eastern Europe. Natural habitats of this species include different types of soil, from alluvial to clay soil with very variable levels of groundwater and specific flood regimes. During last century the population of European white elm significantly decreased. Main reason for elms dying is the pandemic of Dutch elm disease, but additional factor is the anthropogenic pressure on natural European white elm habitats. Due to its fast growth, ornamental value and tolerance to soil compaction, de-icing salts and air pollution, white elm has long been used for amenity plantings in towns and on road sides. As most trees, European white elm is dependent on mycorrhizal fungi in order to produce its maximum growth but mycorrhizal status of elms is not very well recognized. Therefore this study was undertaken. We analysed fine roots of seedlings and mature trees of European white elms originating from natural populations in Serbia. The seedlings were produced in the nursery Manić (E 20°25'41"; N 44°30'54") near Belgrade and mature trees were grown on the Veliko Ratno Ostrvo island (E 20°25'40"; N 44°50'18"), on the confluence Sava and Danube river in Belgrade. In total 12 samples were collected (5 samples from mature trees and 7 samples from seedlings) in the second half of January 2015. Analysis of fine roots revealed dual colonization of mature trees by arbuscular and ectomycorrhizal fungi (*Tuber* sp., *T. maculatum* and *Cortinarius atrocoeruleus*) and only presence of arbuscular mycorrhizae on nursery seedlings. Dual mycorrhizal colonization of fine roots of European white elm is a new information. Colonization of roots by both groups of mycorrhizal fungi was rather low. It partly might be the result of winter samples collection or specificity of European white elm. Future studies of European white elm mycorrhizae need to consider the effects of season and environmental condition on mycorrhizal establishment.

Key words: european white elm; mycorrhizal status; ectomycorrhizae; arbuscular mycorrhizae



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18-eP

Complementary approaches of studying soil biodiversity

*Martina Di Lenola¹, Paola Grenni¹, Diogo Neves Proença^{2,3},
Paula V. Morais^{2,4}, Anna Barra Caracciolo^{1*}*

¹*CNR-IRSA, National Research Council of Italy, Water Research Institute
Via Salaria Km 29,300, I-00015 Monterotondo, Rome;*

²*CEMUC, University of Coimbra, Coimbra, Portugal;*

³*Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal*

⁴*Department of Life Sciences, FCTUC, University of Coimbra, Coimbra, Portugal*

**barracaracciolo@irsa.cnr.it*

The knowledge of the high soil biodiversity is linked to the ability to integrate different levels of research, from the taxonomic/macrosopic one, directly observable, to the microscopic molecular one, in a holistic vision for recognizing and appreciating the importance of each organism and of their interactions.

At the microscopic level, the soil microbial community represents the biggest part of the existing biodiversity, but it is still largely undiscovered. The use and integration of molecular methods makes it possible to improve knowledge about it. In this context, the application to soil samples of two different molecular phylogenetic techniques, such as FISH (Fluorescence In Situ Hybridization) and DGGE (Denaturing Gradient Gel Electrophoresis) will be illustrated.

DGGE is very useful as a screening approach (fingerprint) after the entire DNA microbial community extraction. FISH technique is very sensitive for identifying *in situ* (without extracting nucleic acid from cells) more specific microbial taxa. However, it depends on the cell activity and on their content in rRNA. Indeed, the DGGE, based on the amplification of small fragments of genetic material, makes it possible to overcome the obstacle of low cellular activity, which limits the FISH analysis.

Both methods were used to assess the microbial community composition of a degraded soil, characterized by a low organic matter content and the presence of persistent organic pollutants from human activities (*polychlorinated biphenyls*). The results and information obtained are compared and discussed.

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19-eP

The P nutrition of Palestine oak under global change

Inga Dirks¹, Julia Köhler², Hila Gil¹, Shimon Rachmilevitch¹, Ina C. Meier²

¹*French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein
Institutes for Desert Research, Ben-Gurion University of the Negev,
84990 Midreshet Ben-Gurion, Israel;*

²*Plant Ecology, Albrecht von Haller Institute for Plant Sciences, University of Göttingen, Germany
inga.dirks@gmail.com*

The productivity of Temperate and Mediterranean forests may increasingly be limited by P shortage, as indicated by declining P concentrations and increasing N:P ratios in leaves and fine roots. Continued high atmospheric N deposition in combination with extended summer droughts will likely further alter the availability of P for Mediterranean forest trees in the next decades. In order to develop sound predictions on the P nutrition of Palestine oak (*Quercus calliprinos* Webb.) under global change we conducted three-factorial greenhouse experiments (2 soil moisture × 2 N supply × 3 P supply levels) to study the effect of soil moisture, N and P on growth, morphology, C allocation, P uptake and use efficiencies, and rhizodeposition in oak saplings. A focus was on the influence of the mycorrhizal community composition on *in situ* root exudation and P uptake capacity as measured by a $\text{H}_3^{33}\text{PO}_4$ feeding experiment. Main objectives were to determine (1) the influence of high drought and N on leaf and root P concentrations and associated N:P ratios, (2) the effect of decreasing P availability on P uptake and P use efficiencies, and (3) the relationship between low P or drought and root exudation. Our first results show that root exudation is stimulated by both low P and drought and by extreme (high and low) N:P ratios, which may function as adaptive response. Our poster will discuss the possible relationship between root morphology, root exudation, and ^{33}P uptake efficiencies of Palestine oak at variable P availabilities and with drought.

Keywords: *decreasing precipitation; essential plant nutrient; increasing N deposition; P acquisition strategies; rhizodeposition*

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20-eP

Microbial dehydrogenase activity under different types of soil and plant cover

Martina Horvat, Goran Palijan

*University of J. J. Strossmayer in Osijek, Department of Biology,
Sub Department of Quantitative Ecology, Cara Hadrijana 8/a, 31000 Osijek, Croatia*
gpalijan@biologija.unios.hr

This investigation was conducted in eastern Croatia, where lowland area is substantially changed by agriculture. Cultivated plants replaced stable plant community of mixed forest consisted mainly of *Quercus robur* and *Carpinus betulus*. Dehydrogenase activity (INT) and selected environmental variables (pH, moisture, electrical conductivity, soil organic matter) were determined in two types of soil (chernozem, luvisol), two soil horizons (5-10 cm, 55-60 cm) and three types of plant cover (corn, sunflower and forest soil). Seven random samples were collected from each combination of soil and plant cover and from both horizons during September 2013. Three-way permutational multivariate analysis of variance provided evidence about differences between tested factors. As expected, most significant difference emerged between soil horizons, then between soil types and between types of plant cover. By further investigation of interaction effects the differences between plant cover types at different soils became evident. The forest was always different from crops. Between-crop effects were not evident in chernozem at both horizons. Yet they were present in luvisol at both horizons, and sunflower had positive effects on all measured soil properties compared to corn. Crop effects compared to forest were higher at the surface horizon in chernozem, but in luvisol were higher at the deeper horizon. Results suggest that regarding measured soil properties, there was no difference between soil usage for crop production in chernozem, but it was better to use luvisol for sunflower production. Future investigations should examine are there any effects on aboveground biomass. These results could help planning agricultural activity in soils of different nutrient status, especially in soils of low quality.

Keywords: chernozem; luvisol; Zea mays L.; Helianthus annuus L.; forest



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Edge effect of expanding Great Cormorant colony on forest mycorrhizal fungi

*Audrius Kačergius¹, Jonas Kasparavičius², Reda Iršėnaitė², Gražina Adamonytė²,
Jurga Motiejūnaitė², Ričardas Taraškevičius²*

¹*Vokė Branch of Lithuanian Research Centre for Agriculture and Forestry, Žalioji Sq. 2, Trakų Vokė,
LT-02232 Vilnius, Lithuania;*

²*Nature Research Centre, Akademijos Str. 2, LT-08412 Vilnius, Lithuania
jurga.motiejunaite@botanika.lt*

Colonies of piscivorous birds are a source of huge loads of nutrients, much exceeding atmospheric depositions and including large amounts of N and P, as well as other elements. In forests, this results in tree death and total change of an ecosystem. Ectomycorrhizal (ECM) fungi were studied in a Scots pine forest affected by colony of great cormorants in western Lithuania. No ECM roots were found in the dead forest of the oldest parts of the colony, though several fruit bodies of ECM species appeared in sites where few new trees were establishing. In the active parts of the colony ECM fungi did not produce sporocarps, but several species still formed mycorrhiza, though numbers were low compared to pristine forest. To study changes in above- and below-ground ECM communities following expansion of the colony, three study zones were designated: D (colony edge in the yr. 2010), E (pristine forest in 2010, first bird nests in 2012), and G (established in 2012, pristine forest then) (see Kutorga et al. 2012). In D zone, steady increase of P and N in soil was noted during observation period, in zone E increase of nutrients was slow and fluctuating until autumn of 2013, in zone G decrease of N and fluctuation of P was observed until autumn of 2013 when amounts of both elements increased. Numbers of fructifying ECM species followed pattern of nutrients: no sporocarps in zone D, fluctuation and gradual decrease in zone E, no changes in zone G. Below-ground, total number of ECM roots and morphotypes remained stable in zone D, in zone E these indices almost doubled in 2012 (colony expansion) and remained higher in yr. 2013 than in zone D and pristine zone G. Number of species obtained by molecular identification of ECM roots showed same tendencies as sporocarps: lowest numbers in zone D and highest numbers in zone G. Note: in zone D, no true ECM fungi were isolated - only root endophytes *Phialocephala* spp. and *Meliniomyces* spp. or unidentified ascomycetes.

Keywords: piscivorous birds; eutrophication; ectomycorrhiza; phosphorus; nitrogen

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22-eP

**Recovery of degraded coniferous forests for environmental sustainability
restoration and climate change Mitigation - the LIFE-FoResMit project**

*Alessandra Lagomarsino¹, Ugo Chiavetta², Isabella De Meo¹, Paolo Cantian²,
Alessandro Paletto³, Kalliopi Radoglou⁴*

*¹Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria,
Research Centre for Agrobiological and Pedology (CREA-ABP);*

*²Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria,
Forestry Research Centre (CREA-SEL);*

*³Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Research Unit for Forest
Planning and Monitoring (CREA-MPF);*

*⁴Democritus University of Thrace, Dept. of Forestry and Management of
Environment and Natural Resources (DUTH)*

alessandra.lagomarsino@entecra.it

The LIFE-FoResMit project aims at testing and verifying the effectiveness of management options for the conversion of peri-urban degraded coniferous forests to restore ecological stability and climate change mitigation potential.

Forest degradation implies a long-term loss of productivity, which thereby lower the capacity to supply products and/or services. In this context, the conversion of degraded forest pine plantations to facilitate the introduction of late-successional native broadleaves species, means to help restoring natural functioning processes (e.g. natural regeneration, or more generally, self-organization), increasing their stability, resilience and self-perpetuating capacity.

Innovative silvicultural treatments based on the regulation of light penetration through the forest cover and the selection of more productive trees will be applied in selected peri-urban forests in Italy and Greece. Proposed methodology aims at creating small to moderate gaps in the tree crown layer (patch thinning) or at reducing regularly the crown coverage density (progressive thinning).

The project will provide data on vegetation structure, biomass increment, C accumulation in all relevant pools of vegetation and soil (above and belowground biomass, litter, dead wood and soil, IPCC 2003) and CO₂ and other greenhouse gas (GHG) emissions, thus giving a complete picture of mitigation potential of management practices.

Organic matter degradation and transformation processes driven by soil biological communities will be crucial for the success of the proposed intervention. In particular, biological activity will determine the fate of dead organic matter and its role as C pool, sink, or source. The magnitude and direction of decomposition processes leading to C loss through microbial respiration and/or to C accumulation through microbial immobilization and storage in soil pools will be assessed.

Keywords: peri-urban forests; thinning; green-house gas; C sequestration; decomposition

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23-eP

Application of autochthonous fungi and forest soil for seedling mycorrhization - trials in Montenegro

Jelena Lazarević

Biotechnical Faculty, University of Montenegro, Mihaila Lalića 1, Podgorica, Montenegro

ena.lazarevic@gmail.com

Plant survival and fast development of seedlings after afforestation depend on their quality. It is important in South-east Europe due to unfavourable, warm and dry environmental conditions during growing season. Seedling mycorrhization is important for achieving higher seedlings quality. Mycorrhizae improve water uptake, mineral nutrition, growth and adaptation of forest trees and provide protection of seedlings against pathogens.

Effect of inoculations on ectomycorrhizal formation and seedling growth characteristics were tested on *Pinus nigra* seedlings, inoculated with vegetative and spore inocula of ten native ectomycorrhizal fungi, or with soils from 4 unmanaged forests. Containerized seedlings were grown in open field. The utilisation of spore (10^6 , 10^7 , 10^8) and vegetative (1:4, 1:8, 1:16) inocula of *Pisolithus arhizus* and *Suillus granulatus* proved to be an effective method for obtaining containerized ectomycorrhizal *P. nigra* seedlings in open field conditions. According to ectomycorrhizal development on seedlings roots, even better effects were achieved after first growing season by using of unmanaged forest soils from *Abieti-Fagenion moesiaca* and from *Pinetum heldreichii* forests as ectomycorrhizal inocula (1:9; 1:19). After the inoculation with forest soil, we identified the same mycorrhizal morphotype prevailing in natural regeneration on the sites where the forest soils were collected. Forest soil could be recommended in targeted production of planting material on small to medium scale, while we can use spore inocula of *P. arhizus* and *S. granulatus* in large scale nursery production. Controlled mycorrhizal inoculation of seedlings is not a common practice in Montenegrin nurseries. A new technology with alteration in growing substrates, regimes of fertilization and pesticides applications could be adopted for achieving better quality of *P. nigra* seedlings and the other conifers.

Keywords: *Pisolithus arhizus*; *Suillus granulatus*; forest soil; inoculation; seedlings nurseries; *Pinus nigra*

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24-eP

New bioactive trichobrachin-like peptaibols detected in natural forest habitat-derived isolates from the *Longibrachiatum* section of the filamentous fungal genus *Trichoderma*

Marik Tamás¹, András Szekeres¹, Lea Atanasova², Irina S. Druzhinina², Maria A. Andersson³,
Mirja Salkinoja-Salonen³, Balázs Leitgeb⁴, Csaba Vágvolgyi¹, László Kredics¹

¹Department of Microbiology, Faculty of Science and Informatics, University of Szeged
Közép fasor 52, H-6726 Szeged, Hungary;

²Area Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, Vienna
University of Technology, Gumpendorfer Straße 1a/1665., 1060 Wien, Austria;

³Department of Food and Environmental Science, University of Helsinki,
P.O. Box 56., FI-00014 Helsinki, Finland;

⁴Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences,
Temesvári krt. 62., H-6726 Szeged, Hungary

kredics@bio.u-szeged.hu

Filamentous fungi are producers of a large number of secondary metabolites with wide spectra of biological effects. Among them, peptaibols represent a group of compounds produced mainly by fungal species from the filamentous fungal genus *Trichoderma*.

A simple peptaibol characterization strategy including purification and structural elucidation steps was applied to examine the peptaibol production of 3 strains from the *Longibrachiatum* section of genus *Trichoderma*, *T. aethiopicum* TUCIM 1817, *T. novae-zelandiae* TUCIM 4158 and *T. pseudokoningii* TUCIM 1277, all deriving from natural forest habitats (disturbed semiforest, native *Notophagus* forest and the bark of *Seilschmiedia tawa*, respectively). After the solid phase clean-up of culture extracts, mass spectrometric analysis of peptaibols produced by the examined strains was performed by on-line reversed-phase high performance liquid chromatography coupled to electrospray ionization ion trap mass spectrometry.

All 3 examined species produced 20-21-residue trichobrachin-like compounds, the sequences of which are different from any peptaibols reported in the literature so far. The spectra of the peptaibols produced by the 3 species were entirely different from each other. The largest amount of peptaibols consisting of 4 yet unknown compounds was produced by *T. aethiopicum* TUCIM 1817, while 7 and 6 new, trichobrachin-like compounds were detected from *T. pseudokoningii* TUCIM 1277 and *T. novae-zelandiae* TUCIM 4158, respectively. Lung cells proliferation inhibition tests and membrane damage bio-assay with boar sperm cells revealed that although *T. novae-zelandiae* TUCIM 4158 produced the smallest amount of peptaibols, its compounds were the most inhibitory to mammalian cells.

Keywords: *Trichoderma*; *Longibrachiatum*; peptaibol; trichobrachin

Acknowledgments: Research supported by grants OTKA K-105972, TÁMOP-4.2.2.D-15/1/KONV-2015-0010 and bilateral grant 90ÖU3 from the Austrian-Hungarian Action Fund.

25-eP

**Analysis of microbial communities in environmental samples:
from DGGE to Illumina MiSeq**

Tijana Martinović^{1,3}, Marko Bajc¹, Nejc Thaler², Miha Humar², Hojka Kraigher¹, Barbara Kraigher³

¹Department of Forest Physiology and Genetics, Slovenian Forestry Institute,
Večna pot 2, 1000 Ljubljana, Slovenia;

²Department of Wood Science and Technology, Biotechnical Faculty, University of Ljubljana,
Jamnikarjeva 101, 1000 Ljubljana, Slovenia;

³Chair of Microbiology, Department of Food Science and Technology, Biotechnical Faculty,
University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

tijana.martinovic@gozdis.si

The complexity of microbial communities in the environment in terms of species abundance and lack of culturing methods makes community structure difficult to evaluate. In our study we compared fungal and bacterial communities in different construction wood samples exposed to outdoor conditions. Industrial wood biodegradation is a constantly emerging topic due to the scarcity of more resistant tree species, at least in most of Europe. Norway spruce (*Picea abies*) is commonly used in commercial applications and was thus set as a control. The influence of different wood treatments on microbial communities was also analyzed in spruce samples. The first treatment was thermal wood modification and the second one was based on application of alkaline copper quaternary (ACQ, Silvanolin®) for limiting microbial growth. Furthermore, we included representatives of the more resistant tree species from North America: Douglas fir (*Pseudotsuga menziesii*) and western red cedar (*Thuja plicata*) in our study. The communities were profiled and analyzed using DGGE method and BioNumerics® software. Representative bands from fungal profiles were excised and sequenced by Sanger method. Preliminary sequencing results revealed that most of the sequences do not represent typical wood degrading fungi (e.g. *Phaeococcomyces*, *Tremella*, *Dioszegia*, *Coniochaeta*, *Aureobasidium*). Many of the sequences recovered in this study showed a low level of identity with sequences of known fungi, making determination of their identity and biological function within the community impossible. Such results prompted us to apply next generation sequencing (NGS) - Illumina MiSeq platform. Even though the data processing for the NGS might be much more complex, one of the main advantages of NGS approach is the possibility to use degenerate primers covering a wider range of taxa and, of course, a much improved sequencing depth compared to the DGGE-sequencing approach. With NGS we expect to gain more informative results for both fungi and bacteria and new insights into biological wood degradation processes in building wood and in nature.

Keywords: wood degradation; fungi; bacteria; community structure; Illumina MiSeq

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26-eP

From the forester's tool box: Using forest manipulation to separate the factors driving soil carbon dynamics

*Mathias Mayer¹, Bradley Matthews¹, Hans Göransson¹, Boris Rewald¹, Klaus Katzensteiner¹,
Harald Berger², Markus Gorfer², Douglas Godbold¹*

*¹Institute of Forest Ecology, Department of Forest and Soil Sciences,
University of Natural Resources and Life Sciences - BOKU, Vienna, Austria;*

*²Fungal Genetics and Genomics Unit - Health & Environment Department -
Austrian Institute of Technology GmbH - AIT, Tulln, Austria*

mathias.mayer@boku.ac.at

Trees strongly affect soil carbon (C) dynamics, both directly via belowground allocation of C from photosynthesis and substrate input from litterfall, and indirectly via influence on the soil microclimate (e.g. shading, water uptake). Microbial decomposition in soil for example has been shown to be sensitive to the availability of labile C inputs from rhizosphere (Subke et al. 2011), the chemical composition and quantity of plant substrate inputs (Brant et al. 2006) and changes in soil temperature (Davidson and Janssens, 2006). In order to study the influence of respective factors on soil C dynamics separately, we established a comprehensive manipulation experiment, including tree-girdling, clear-cutting and biomass addition treatments in August 2015. We presume tree-girdling affects belowground inputs of labile C and microbial community structure and functioning, and clear-cutting and biomass addition affect C inputs, microbial community as well as soil microclimate. To investigate consequences on soil C dynamics, we measure soil microclimate, soil CO₂ efflux, standard soil parameters (e.g. organic C, dissolved C, microbial biomass), root biomass and activity, enzymatic activity, microbial community (molecular based), and C mineralization rates. Here, we will present detailed information on the set up of our research site and on the different manipulation treatments, as well as preliminary results from the first months of the experiment.

Keywords: Girdling; clear-cut; microbial decomposition

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27-eP

Towards the fine root identification key of common tree species - progress after one year

Tanja Mrak, Jožica Gričar, Hojka Kraigher

Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana

tanja.mrak@gozdis.si

The pilot study that encompasses twelve common European tree species to develop the most useful approach for the fine root identification, is almost finished. The structure of the identification key should follow the rule from simple and quick towards complex and more time consuming. As simple and quick, morphological characters can be regarded and if these fail, anatomical characters must be considered, preferentially from cross sections. Besides the most terminal fine roots, also roots with diameter 1, 3 and 5 mm were involved in our study. Roots with diameter 1 mm in all investigated species possess enough anatomical features that can be identified with great certainty. Despite the fact, that bark forms a large proportion of tissues in investigated roots with secondary growth, secondary xylem is the part of the root where the most stable characters are located. In cross sections of broadleaved trees, arrangement of vessels, ray width and axial parenchyma are the most reliable features, while in longitudinal sections, ray-vessel pits should be observed. In bark, all the features, such as sclereids, fibres and crystals were found to be very variable and may be absent or not yet developed in 1 mm roots. The shape of primary xylem may be helpful in some cases. Roots of some tree species possess features that do not occur in stem tissues.

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28-eP

Potential role of pedofauna on seeding inoculation with ectomycorrhizal fungi

Yasmine Piñuela^{1,2}, Tine Grebenc², Hojka Kraigher²

¹*Technical University of Madrid (UPM), Campus Ciudad Universitaria,
Avenida de Ramiro de Maeztu s/n, 28040 Madrid, Spain;*

²*Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana, Slovenia
yasmin.pinuela@hotmail.com*

The maintenance of ecosystem services provided by soils, soil biodiversity and production in agroforestry systems is of vital importance to prevent degradation of soils and the whole ecosystem. The main focus of the study was to prepare the knowledge set-up and experimental design to determine the influence of selected pedofauna species (earthworms) on an early stage ectomycorrhizal (ECM) colonization and fine root development under pot conditions. The approach was targeted as a potential application in commercial seedling inoculation with ectomycorrhizal fungi for out planting and improving the production of natural habitats. The principal objective of the trial was to evaluate mycorrhizal colonization rates and plant quality of seedlings, previously inoculated with ectomycorrhiza (genus *Tuber*) interacting with earthworms (*Lumbricus* sp.) using an evaluation method developed by Fischer and Colinas (2014). Beside the known protocol we applied a WinRHIZO® software (Régent Instruments Inc., CA) for assessing root and ectomycorrhiza parameters which allowed us a standardized approach, and easy computing and data analysis. The following root parameters were analysed for each seedling: total root length, average diameter, diameter distributions of the total root system and specific root length. Using named parameters we evaluated the hypothesis on how earthworm's activity affected the root and ectomycorrhiza development of non-inoculated and ectomycorrhizal fungi inoculated seedlings. A detailed methodology and preliminary data will be presented.

Keywords: *earthworms; ectomycorrhiza; fine roots; WinRHIZO; Tuber*

Acknowledgments: Study supported by EUFORINNO, (European Forest Research and Innovation, FP7 EU CAPACITIES programme REGPOT, 2012-2015 N. 315982), by the Research Programme P4-0107 of the Slovenian Forestry Institute and by a Short Term Scientific Mission within the Cost Action FP1305.

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29-eP

Link between *Bursaphelenchus xylophilus*-associated bacteria and endophytic microbial community of *Pinus* with Pine Wilt Disease

*Diogo Neves Proença*¹, *Paula V. Morais*^{1,2}

¹CEMUC, University of Coimbra, Coimbra, Portugal;

²Department of Life Sciences, University of Coimbra, Coimbra, Portugal

diogo.proenca@gmail.com

Pine wilt disease (PWD), a major illness of several *Pinus* species, native to North-America, has spread into Asia and recently into Europe (Portugal and Spain). *Bursaphelenchus xylophilus*, the pinewood nematode (PWN), is considered the only causative agent of PWD. It has been proposed that PWD is a complex disease induced by both PWN and the bacteria it carries. This work aimed to assess the microbial community, associated with the PWN and within the host plant, in order to elucidate the link between both community structures. Cultivation methods were used for bacteria carried by PWN from Portugal and USA. Cultivation and molecular methods (DGGE, Illumina) were used to assess the endophytic microbial community of pine trees from Portugal. Both bacterial community structures, by cultivation and molecular methods, share the class *Gammaproteobacteria* as the most abundant. The major bacterial species associated with the nematodes differed according to the forest area and none of the isolated bacterial species was found in all different forest areas studied. However, strains from the genus *Pseudomonas* were found associated with PWN from all countries. Moreover, bacteria associated with the PWN were able to produce siderophores, proteases and lipases and 21 of these strains showed nematocidal activity towards PWN. Bacteria belonging to the classes *Gammaproteobacteria*, *Betaproteobacteria*, *Bacilli*, *Alphaproteobacteria* and *Flavobacteriia* were identified associated with PWN as well as part of the endophytic microbial community of *P. pinaster*. Furthermore, by using DGGE and Illumina was possible to detected 24 additional classes comparatively to cultivation methods. In conclusion, considering the diversity of the nematode-associated bacteria and their presence in endophytic microbial community we cannot suggest an active role of the bacteria in PWD.

Keywords: *diversity; bacteria; microbial community; Illumina; nematode*

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30-eP

Meta-analysis of ectomycorrhizal diversity in beech (*Fagus sylvatica* L.) dominated stands in Europe

Christoph Rosinger, Hans Göransson, Douglas Godbold

*Institute of Forest Ecology, Department of Forest- and Soil Sciences,
University of Natural Resources and Life Sciences, Peter-Jordan-Straße 82, 1190 Vienna, Austria
christoph.rosinger@boku.ac.at*

To investigate the abundance of ectomycorrhizal (EM) species in different pure beech and beech dominated stands across Central Europe, a literature study was carried out. Using the search words 'Beech/*Fagus sylvatica*' and 'ectomycorrhizal community/diversity/species' in Google Scholar, thirty-one publications were found. Fifteen of them reported the frequency of appearance of ectomycorrhizal fungi in different beech stands. Most papers, however, showed species lists with fragmentary or without information about abundance. Six papers with comprehensive abundance data about nine different sites were used for further analysis, ranging from Italy to Denmark and with a broad range of stand features (managed/unmanaged, 50-120 years). In total, 74 different EM species were found. Furthermore, the analysis revealed the following EM community patterns: (i) two multi-host species *Cenococcum geophilum* and *Russula ochroleuca* occurring at several sites with relatively high abundances; (ii) few species with narrow host range (*Lactarius subdulcis*, *L. pallidus*, *L. blennius* and *Laccaria amethystine*) showing low abundances on various sites; (iii) two EM species (*Hebeloma sinapizans*, *Lactarius salmonicolor*) appearing to be very dominant on one site; (iv) a long list of EM species with very low abundance on either one or two sites. Further connections, also to functioning of certain EM species, could not be drawn due to fragmentary information available. The factors determining the single appearance of most of the rare species and their function remain unexplained.

Keywords: *beech; Fagus sylvatica; ectomycorrhiza; diversity*

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31-eP

Microbial control of biogeochemistry during drying-rewetting: the legacy of drought

Johannes Rousk

Microbial Ecology, Department of Biology, Lund University, 22362 Lund, Sweden

johannes.rousik@biol.lu.se

Drought and drying-rewetting cycles are frequent stressors for soil microbial communities; a stress that is predicted to grow increasingly influential with climate change. Understanding how the microbial community controls biogeochemistry under these dynamic events is instrumental to enable predictive power for C and nutrient cycling in soils. We investigated the effect of drought and drying-rewetting cycles on bacterial and fungal growth, community size and composition, and on soil respiration, in laboratory microcosms. The microbial growth dynamics underlying the dramatic soil C pulse induced by rewetting a dry soil at high time-resolution in a series of week long studies in a range of soils. Our analysis by investigating how the legacy of drought modulated the actively growing microbial community and their biogeochemistry by assessing a continental set of long term drought experiments including soils from across all of Europe.

We determined two types of respiration responses induced by rewetting a dry soil: (i) an instant maximal peak followed by an exponential decline or (ii) an immediate peak maintained for almost 20 h, followed by a short period of exponential increase, reaching a maximal peak rate only after > 24 h, followed by an exponential decline. Microbial growth contrasted sharply with the respiration dynamics of both types of rewetting responses. (i) a linear increase starting at time zero, and convergence to similar rates as in a moist control soil, or (ii) zero growth for almost 20 h, followed by an exponential increase in growth to rates many-fold times higher than that of the moist control soils within 30 h after rewetting. The cross-continental study on drought effects in Europe showed that the legacy effect of drought was minimal on contemporary rates of microbial growth and functioning. However, during the perturbation event of rewetting dry soil, the microbial C-use efficiency was consistently increased by a legacy of drought.

Keywords: microbial ecology; bacterial and fungal growth; soil C cycle; drought; soil moisture dependence



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What lies beneath: root-associated bacteria to improve the growth and health of olive (*Olea europaea* L.)

*David Ruano-Rosa, Antonio Valverde-Corredor, Carmen Gómez-Lama Cabanás, Rafael Sesmero,
Jesús Mercado-Blanco*

*Departamento de Protección de Cultivos, Instituto Agricultura Sostenible (CSIC), Córdoba, Spain
jesus.mercado@ias.csic.es*

In recent decades we have witnessed growing public concern on the abuse/misuse of agrochemicals to control plant pathogens. Besides, the fact that some phytopathological agents (for instance, the soil-borne fungus *Verticillium dahliae* Kleb.) are very difficult to control by alternative methods to those based on chemical-based products, has urged researchers to seek effective measures within integrated disease management frameworks. Biological control, alone or in combination with other approaches, emerges as one of the most promising alternatives to confront plant pathogens in a sustainable, environment-friendly strategy. In this study, a collection of indigenous culturable bacteria (>300) from olive roots was generated. First selection was performed based on their antagonistic activity against *V. dahliae*. As a result, some 190 isolates were selected and further (i) tested against relevant plant pathogens (*Alternaria alternata*, *Colletotrichum godetiae*, *Colletotrichum nymphaeae*, *Fusarium oxysporum* f. sp. *dianthi*, *Phytophthora cinnamomi*, *Pseudomonas savastanoi* pv. *savastanoi*, and *Rosellinia necatrix*), (ii) identified molecularly (16S rDNA and *gyrB* genes), and (iii) screened for the presence of phenotypes usually associated to antagonism and/or plant growth promotion (PGP). Results showed prevalence of Proteobacteria (55.5 %) and Firmicutes (36.5 %) phyla, *Pseudomonas* (28.6 %) and *Bacillus* (33 %) being the most abundant genera. Although none of the strains showed effective against all tested pathogens, twelve of them belonging to genera *Paenibacillus*, *Rhizobium*, *Pseudomonas* or *Bacillus* presented a broad antagonistic activity, together with interesting phenotypic characteristics such as enzymatic activities associated to biocontrol and PGP (e.g. production of siderophore or chitinase activity). These results indicate that some of these bacteria could be good candidates to be used in future bioformulations aiming to improve health and growth of this tree crop.

Keywords: antagonism; biocontrol; plant growth promotion; rhizobacteria; Verticillium dahliae

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33-eP

Soil nitrogen mineralization profiles at the tree line

Lixia Wang, Douglas Godbold

Institute of Forest Ecology, Universität für Bodenkultur (BOKU).

Peter Jordan Straße 82, 1190 Vienna, Austria

lixia.wang@boku.ac.at

In the Austrian Alps, N availability and N mineralization in soils of different vegetation types was determined at the tree line (*Picea abies*, *Pinus mugo* and *Rhododendron ferrugineum*) and at a reference site of *Picea abies* at a lower elevation. Nitrogen mineralization was measured *in situ* using a buried PVC tube incubation method, and in laboratory incubation under controlled conditions. Ion-exchange resin capsules were installed at the interface of humus and mineral soil for estimating N in the soil solution.

Net nitrogen mineralization in the laboratory incubation was determined in different soil horizons (H, A, B Horizon). The results indicated that ammonification occurred in humus layer, and net nitrogen mineralization rate of humus layer was significant higher in the H layer than in the A and B horizons. Net nitrogen mineralization rate was markedly differed between different vegetation types. Soil under *Rhododendron ferrugineum* had a significant lower nitrogen mineralization rate than *Pinus mugo* and *Picea abies*. Magnitude of the *in situ* PVC tube incubation N-mineralization was similar to the laboratory incubation mineralization. The N-mineralization rate obtained for the different vegetation types from PVC tube incubation and ion-exchange resin capsule had same pattern as the laboratory incubation. The values decreased in order *Picea abies* < *Pinus mugo* < *Rhododendron ferrugineum*. Significant lower N-mineralization rate and soil N availability were found under *Rhododendron ferrugineum*.

Key words: N mineralization; soil N availability; tree line

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Lists of Meeting Participants

Iftekhhar U. Ahmed	University of Natural resources and life sciences (BOKU), Institute of Forest Ecology, Vienna, Austria
Valeria Ancona	CNR-IRSA, National Research Council of Italy, Water Research Institute, Bari, Italy
Algis Aučina	Vilnius University, Botanical Garden, Lithuania
Mark Bakker	Bordeaux Sciences Agro, Villenave d'Ornon, France
Petr Baldrian	Institute of Microbiology of the ASCR, Prague, Czech Republic
Raffaella Balestrini	CNR-IPSP, National Research Council of Italy, Institute for Sustainable Plant Protection, Torino, Italy
Tjasa Baloh	Slovenian Forestry Institute, Department of Forest Physiology and Genetics, Ljubljana, Slovenia
Anna Barra Caracciolo	CNR-IRSA, National Research Council of Italy, Water Research Institute, Rome, Italy
Anna Benedetti	CREA-RPS, Council for Agricultural Research and Analysis of Agricultural Economics, Research Center for the Study on Relationships between Plant and Soil, Rome, Italy
Luis Fernando Benito-Matías	CNRGF "El Serranillo" Ministerio de Agricultura, Alimentación y Medio Ambiente, Guadalajara, Spain
Annamaria Bevivino	ENEA Casaccia Research Center, Rome, Italy
Martin Bidartondo	Imperial College London & Royal Botanic Gardens, Kew, United Kingdom
Stefano Bisoffi	CREA, Council for Agricultural Research and Analysis of Agricultural Economics, IT
Ewa Blonska	University of Agriculture in Kraków, Faculty of Forestry, Department of Forest Soil Science, Krakow, Poland
Isabella Borja	NIBIO, Norwegian Institute of Bioeconomy Research, Oslo, Norway
Gergely Boros	MTA Centre for Ecological Research, Budapest, Hungary
Francis Q. Brearily	Manchester Metropolitan University, Manchester, UK
Ivano Brunner	Swiss Federal Research Institute WSL, Birmensdorf, Switzerland
Martina Cardoni	Università degli Studi di Roma "La Sapienza", Roma, Italy
Monique Carnol	University of Liège, Liège, Belgium
Aurelio Ciancio	CNR-IPSP, National Research Council of Italy, Institute for Sustainable Plant Protection, Bari, Italy
Aisha Coggan	University of Essex, Colchester, United Kingdom
Mariantonietta Colagiero	CNR-IPSP, National Research Council of Italy, Institute for Sustainable Plant Protection, Bari, Italy

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Jérôme Cortet	Université Paul Valéry - Montpellier III, France
Carla Cruz-Paredes	University of Copenhagen, Copenhagen, Denmark
Pavel Cudlin	CZECHGLOBE, Global Change Research Centre, Academy of Sciences of the Czech Republic
Jorge Curiel Yuste	CSIC-MNCN, Museo Nacional de Ciencias Naturales, Madrid, Spain
Claudia Dalmastri	ENEA Casaccia Research Center, Rome, Italy
Lise Dalsgaard	NIBIO, Norwegian Institute of Bioeconomy Research, As, Norway
Flavia De Bono	Università Campus Bio-Medico di Roma, Italy
Gaby Deckmyn	University of Antwerpen, Belgium
Yoseph Tewodros Delegn	University of Natural resources and life sciences (BOKU), Institute of Forest Ecology, Vienna, Austria
Belete Dessie Assefa	University of Natural resources and life sciences (BOKU), Institute of Forest Ecology, Vienna, Austria
Jovana Devetakovic	University of Belgrade, Faculty of Forestry, Belgrade, Serbia
Martina Di Lenola	CNR-IRSA, National Research Council of Italy, Water Research Institute, Rome, Italy
Lucian Dinca	Forest Research and Management Institute, Brasov, Romania
Maria Dinca	Forest Research and Management Institute, Brasov, Romania
Inga Dirks	French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Ben-Gurion, Israel
Cristian Mihai Enescu	University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Agriculture, Forestry Specialization, Bucharest, Romania
Amandine Erktan	INRA -UMR AMAP, Montpellier, France
Manuel Fernando Lopez	CSIC- Estacion Experimental Zaidin, Granada, Spain
Silvia Fineschi	CNR-IPSP, National Research Council of Italy, Institute for Sustainable Plant Protection, Sesto Fiorentino Firenze, Italy
Domen Finžgar	Slovenian Forestry Institute, Ljubljana, Slovenia
Alessandro Frandi	CNR-DiSBA, National Research Council of Italy, Department of Biology, Agriculture and Food Sciences, Rome, Italy
Luis Fonseca	Life Sciences Department-Coimbra University - Coimbra, Portugal
Oskar Franklin	International Institute for Applied Systems Analysis (IIASA), Laxenburg, Austria
Beat Frey	Swiss Federal Research Institute WSL, Birmensdorf, Switzerland
Mauro Gamboni	CNR-DiSBA, National Research Council of Italy, Department of Biology, Agriculture and Food Sciences, Rome, Italy



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Luis Gonzaga García-Montero	Technical University of Madrid, Spain
Maria Glushkova	Forest Research Institute – BAS, Sofia, Bulgaria
Douglas Godbold	University of Natural resources and life sciences (BOKU), Institute of Forest Ecology, Vienna, Austria
Hans Göransson	University of Natural resources and life sciences (BOKU), Institute of Forest Ecology, Vienna, Austria
Stefano Grego	World Agricultural Heritage Foundation (WAHF), Rome; University of Tuscia Viterbo, Italy
Giulia Gregori	Università Campus Bio-Medico di Roma, Italy
Paola Grenni	CNR-IRSA, National Research Council of Italy, Water Research Institute, Rome, Italy
Bryan Griffiths	SRUC, Crop and Soil Systems Research Group, Edinburgh, UK
Giorgio Guerrini	Università Campus Bio-Medico di Roma, Italy
Kutuzovic Branimir Hackenberger	University of Osijek, Croatia
Kutuzovic Davorka Hackenberger	University of Osijek, Croatia
Mona N. Högberg	Swedish University of Agricultural Sciences, Department of Forest Ecology and Management, Umeå, Sweden
Katarzyna Hryniewicz	Nicolaus Copernicus University, Torun, Poland
Emira Hukic	Faculty of Forestry, University of Sarajevo, Bosnia-Erzegovina
Massimo Iannetta	ENEA, Biotechnologies and Agro-Industry Division, Italy
Leszek Karlinski	Institute of Dendrology Polish Academy of Sciences, Kórnik, Poland
Rasmus Kjøller	University of Copenhagen, Denmark
Darta Klavina	LSFRI Silava, Salaspils, Latvia
Evsey Kosman	Tel Aviv University, Israel
Marta Kowalska	Institute of Dendrology, Polish Academy of Sciences (PAS), Poland
Laszlo Kredics	University of Szeged, Department of Microbiology, Faculty of Science and Informatics Hungary
Vladimir Kunca	Technical University in Zvolen, Slovakia
Alessandra Lagomarsino	CREA-ABP, Council for Agricultural Research and Analysis of Agricultural Economics, Agrobiology and Pedology Research Centre, Florence, Italy
Jelena Lazarević	University of Montenegro, Biotechnical faculty, Podgorica, Montenegro
Stela Lazarova	Institute of Biodiversity and Ecosystem Research, Sofia Bulgaria
Francesco Loreto	CNR-DiSBA, National Research Council of Italy, Department of Biology, Agriculture and Food Sciences, Rome, Italy

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Martin Lukac	University of Reading, UK
Erica Lumini	CNR-IPSP, National Research Council of Italy, Institute for Sustainable Plant Protection, Turin, IT
Irena Macek	University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
Tijana Martinović	Slovenian Forestry Institute, Ljubljana, Slovenia
Mathias Mayer	University of Natural resources and life sciences (BOKU), Institute of Forest Ecology, Vienna, Austria
Ina Christin Meier	University of Göttingen, Plant Ecology, Albrecht von Haller Institute for Plant Sciences, Germany
Jesús Mercado-Blanco	CSIC- Instituto Agricultura Sostenible, Departamento de Protección de Cultivos, Córdoba, Spain
Luis Merino-Martín	INRA, UMR AMAP, Montpellier, France; Centre for Ecology & Hydrology, Crowmarsh Gifford, Wallingford, Oxfordshire, United Kingdom
Juha Mikola	University Of Helsinki, Finland
Jelena Milovanovic	Futura Singidunum University, Faculty of Applied Ecology, Belgrade, Serbia
Mari Moora	University of Tartu, Estonia
Jurga Motiejūnaitė	Nature Research Centre, Vilnius, Lithuania
Tanja Mrak	Slovenian Forestry Institute, Ljubljana, Slovenia
Jerome Nespoulous	Université de Montpellier, France
Franco Nigro	Università degli Studi di Bari - Aldo Moro Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente, Bari, Italy
Edda Oddsdottir	Icelandic Forest Research, Reykjavik, Iceland
Michail Orfanoudakis	Democritus University of Thrace, Orestiada, Greece
Jose Julio Ortega-Calvo	CSIC- Instituto de Recursos Naturales y Agrobiología de Sevilla, Spain
Ivika Ostonen	University of Tartu, Estonia
Sofia Pagliarani	Università degli studi di Viterbo "La Tuscia", Viterbo, Italy
Goran Palijan	University of J. J. Strossmayer, Osijek, Croatia
Taina Pennanen	Natural Resources Institute Finland, Vantaa, Finland
Isabella Pentimone	CNR-IPSP, National Research Council of Italy, Institute for Sustainable Plant Protection, Bari, Italy
Tanita Pescatore	Università degli Studi di Roma "La Sapienza", Roma, Italy
Marcin Pietras	University of Gdańsk, Faculty of Biology, Poland
Yasmine Piñuela	Technical University of Madrid (UPM), Madrid, Spain; Slovenian Forestry Institute, Ljubljana, Slovenia



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Flavia Pizzabiocca	Università Campus Bio-Medico di Roma, Italy
Diogo Neves Proença	CEMUC, University of Coimbra, Coimbra, Portugal
Uros Radojevic	Futura Singidunum University, Faculty of Applied Ecology Serbia
Relena Rose Ribbons	University of Natural Resources and Life Sciences Vienna, Austria
Ludovica Rolando	Università degli Studi di Roma "La Sapienza", Roma, Italy
Christoph Rosinger	Institute of Forest Ecology, Department of Forest- and Soil Sciences, University of Natural Resources and Life Sciences, Vienna, Austria
Johannes Rousk	Lund University, Sweden
Maria Ludovica Saccà	CNR-IRSA, National Research Council of Italy, Water Research Institute, Rome, Italy
José Paulo Sampaio	UCIBIO, Universidade Nova de Lisboa, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Caparica, Portugal
Olaf Schmidt	University College Dublin, Ireland
Mirjana Sijacic Nikolic	University in Belgrade Faculty of Forestry, Belgrade, Serbia
Nadejda Soudzilovskaia	Leiden Univeristy, Netherlands
Mark Tibbett	University of Reading, UK
Elena Vanguelova	Forest Research, Alice Holt Research Station, Farnham, Surrey, UK
Hakan Wallander	Lund University, Sweden
Lixia Wang	University of Natural resources and life sciences (BOKU), Institute of Forest Ecology, Vienna, Austria
Oktay Yildiz	Duzce University, Forestry Faculty, Duzce, Turkey
Andrey Yurkov	Leibniz-Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
Miglena Zhiyanski	Forest Research Institute – BAS, Sofia, Bulgaria
Michał Zloch	Nicolaus Copernicus University, Torun, Poland

