



# Soil biodiversity and European woody agroecosystem



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# **Soil biodiversity and European woody agroecosystem**

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**COST Action FP1305 BioLink**

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

**Proceedings of the 2018 Annual Meeting**

**Granada, 14-16 March 2018**

Edited by  
Paola Grenni, Manuel Fernández-López and  
Jesús Mercado-Blanco



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## **WELCOME**

*On behalf of the Organizing and Scientific Committee we are honoured to announce the organization of the COST Action FP1305 meeting (“BioLink 2018: Soil Biodiversity and European Woody Agroecosystems”) in Granada (Spain) from 14<sup>th</sup> to 16<sup>th</sup> March 2018. After the previous and successful meetings held in Reading (UK), Krakow (Poland), Rome (Italy), Sofia (Bulgaria), Prague (Czech Republic) and Tartu (Estonia) we are delighted to host the final meeting. We will address the objectives of the COST Action “Linking Belowground Biodiversity and Ecosystem Function in European Forests”, focus on forests and tree crops, ecosystem services, climate change etc. It will include plenary talks by international experts as well as oral and poster presentations chosen from submitted abstracts.*

*Granada is located at the foot of the Sierra Nevada and 30 minutes from the Mediterranean Sea. This historical city is home to the Alhambra, the great Arabic castle complex constructed over many centuries, considered to be one of the 10 wonders of the world, Granada is synonymous with culture, heritage, nature and beauty. We are delighted to invite you to the BioLink 2018 meeting and look forward to welcoming you in Granada.*



**Manuel Fernández-López and Jesús Mercado-Blanco**  
*Chairs of the Meeting*



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## INTRODUCTION

*The role of belowground biodiversity in promoting health and resilience of European woody agro-ecosystems was the main focus of the Final Annual Meeting of the COST Action BioLink ‘**Soil biodiversity and European woody agroecosystems**, which took place in Granada, from the 14<sup>th</sup> to 16<sup>th</sup> March 2018.*

*This Final Meeting gathered the ‘BioLink community’, a solid network of researchers devoted to investigations on the current state of knowledge on the connection between soil function, as mediated by soil biota, and aboveground ecosystem productivity, thereby supporting a wide range of human activities and, more importantly, life in our planet. The meeting particularly focused on tree agro-ecosystems (both forests and tree plantations) to explore the role of soil biodiversity in the health and productivity of systems dominated by vegetal species displaying peculiarities such as large biomass, complicated anatomy, large root systems, longevity and perennial nature. Since trees play important roles in providing key ecosystem services, increasing our understanding of the links between belowground biota and trees is instrumental to generate novel and more effective and sustainable management strategies for forest and tree crops. We strongly believe that the Meeting, as well as its precedents along the ‘BioLink’ Action, successfully contributed to a lively, timely and motivating debate. More than 100 scientists from many European countries attended the Meeting, presenting nearly 70 communications, and sharing a unique occasion to exchange their knowledge and experiences on the captivating topic of the functional belowground biodiversity in European woody agro-ecosystems.*

*Manuel Fernández-López and Jesús Mercado-Blanco*





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## Meeting Program

### Wednesday 14.03.2018

8:00 – 9:00 Registration

9:00 – 9:30 Opening ceremony

9:30 – 11:00 Plenary session chaired by **Martin Lukac**.

9:30 – 10:10 **Gabriele Berg**, TU Graz, Austria (COST Invited Speaker)  
*Linking microbial belowground diversity and plant health.*

10:10 – 10:30 **Mauro Lanfranchi**, University of Cumbria, Ambleside, U.K.  
*The effects of forest management on the carbon quality of soil.*

10:30 – 10:50 **Carmen Gómez-Lama Cabanás**, IAS-CSIC, Córdoba, Spain.  
*Indigenous olive rhizosphere Bacillales members as effective biocontrol agents against Verticillium dahliae.*

11:00 – 11:30 Coffee-break

11:30 – 13:30 Plenary session chaired by **Martin Lukac**.

11:30 – 12:00 **Aurelio Ciancio**, CNR, Italy (Invited Speaker)  
*Valorizing complexity of soil food webs and related microbiome-based services for sustainable crop production*

12:00 – 12:30 **Jorge Curiel**, BC3-Basque Centre for Climate Change, Spain (Invited Speaker)  
*Alterations of C allocation patterns associated with climate-change induced vegetation die-off: Effects over soil pools, soil biodiversity and functions, and Controls of soil respiration*

12:30 – 12:50 **Rodica Pena**, University of Goettingen, Germany  
*Temporal turnover of ectomycorrhizal fungal communities in response to new habitats created by tree harvesting*

12:50 – 13:10 **Douglas Godbold**, Universität für Bodenkultur (BOKU), Vienna, Austria  
*Winter activity of ectomycorrhizas of Pinus sylvestris and Pinus sibirica in Mongolia.*

13:10 – 13:30 **Carla Cruz-Paredes**, University of Copenhagen, Denmark  
*Bacterial and fungal community responses over a steep wood-ash gradient in a spruce plantation.*

13:30 – 15:30 Lunch

15:30 – 17:00 Plenary session chaired by **Mark Bakker**.

15:30 – 16:00 **Maria Teresa Cervera**, INIA, Spain (Invited Speaker)  
*Dissection of Pinus pinaster response to water deficit: Integration of complementary strategies to improve drought response*



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16:00 – 16:20 **Vicent de Leijster**, Utrecht University, The Netherlands

*The impact of soil and vegetation management on nutrient cycling and understory vegetation diversity in European almond orchards*

16:20 – 16:40 **María Eugenia Ramos-Font**, EEZ - CSIC, Granada, Spain

*Grazed fuelbreaks in Andalusia as a tool for wildfire prevention: a new name for an old practice.*

16:40 – 17:00 **Luis Merino-Martín**, Université de Montpellier, France

*Soil microbial diversity in open and closed forest patches along a soil temperature gradient: the importance of root properties for bacterial community coalescence in the rhizosphere.*

17:00 – 17:30 Coffee-break

17:30 – 19:00 Plenary session chaired by **Katarzyna Hryniewicz**

17:30 – 18:00 **László Kredics**, University Szeged, Hungary (Invited Speaker)

*Devastating forest pathogens from the genus Armillaria: from genomics to biocontrol*

18:00 – 18:20 **Lur Moragues-Saitua**, NEIKER-Tecnalia, Derio, Spain

*Wood ash and biochar addition to forests do not shift soil microbial functional diversity in the short term.*

18:20 – 18:40 **Diogo Neves Proença**, University of Coimbra, Portugal

*Link between genomic identification and biochemical characterization of novel siderophores produced by endophytic bacteria*

18:40 – 19:00 **Lars Vesterdal**, University of Copenhagen, Denmark

*Tree species effects on stocks and vertical distribution of soil carbon: Links to mycorrhizal association, microbial characteristics and soil fauna*

20:00 – 22:30 Welcome reception at Quinta Alegre Palace

**Thursday 15.03.2018**

9:00 – 11:00 Working groups meeting

11:00 – 11:30 Coffee-break

11:30 – 13:30 Plenary session chaired by **Mari Moora**.

11:30 – 12:00 **Jaco Vangronsveld**, Hasselt University, Belgium (COST Invited Speaker)

*The impacts of soil contamination on tree rhizosphere bacterial communities in a forest on an old military site*

12:00 – 12:20 **Elena Vanguelova**, Forest Research, Alice Holt Lodge, Surrey, UK

*Links between soil biochemistry and biodiversity with oak health status*

12:20 – 12:40 **Gaby Deckmyn**, University of Antwerp, Belgium

*KEYLINK: a new soil model including soil structure and soil fauna as key drivers.*



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12:40 – 13:00 **Omar Flores**, MNCN-CSIC, Madrid, Spain

*KEYLINK model: a short introduction to its use and usefulness*

13:00 – 13:30 **Rut Aspizua Cantón**, Agencia de Medio Ambiente y Agua, Granada, Spain (Invited Speaker)

*LIFE ADAPTAMED - Protection of key ecosystem services by adaptive management of Climate Change endangered Mediterranean socioecosystems*

13:30 – 15:30 Lunch

15:30 – 17:20 Plenary session chaired by **Elena Vanguelova**

15:30 – 16:00 **Johannes Rousk**, Lund University, Sweden (Invited Speaker)

*Adapted temperature relationships of microbial communities in warming soils enhance carbon-use efficiencies*

16:00 – 16:20 **Antonio J. Fernández-González**, EEZ-CSIC, Granada, Spain

*Linking belowground bacterial communities to healthy olive trees*

16:20 – 16:40 **Alessandra Lagomarsino**, CREA, Firenze, Italy

*Microbial communities and activities of forest floor fractions in degraded pine forests after thinning treatments.*

16:40 – 17:00 **Maria K. Sakka**, University of Thessaly, Greece

*Quantifying the power of awareness of belowground biota: perceptions differ among European countries*

17:00 – 17:20 **Daniel García-Angulo**, MNCN-CSIC, Madrid, Spain

*Drought-induced dieback altered soil biogeochemical cycles and soil microbial functioning along Quercus ilex historical land use and climatic gradient*

17:20 – 19:30 Coffee-break and Poster session

17:30 – 19:00 Parallel workshop on KEYLINK chaired by Gaby Deckmyn

*(the attendees need to install phyton-xy, <https://python-xy.github.io>)*

20:00 Bus to Conference dinner at La Chumbera

23:00 Return to the Hotel by bus

20:00 Bus to Conference dinner at La Chumbera

23:00 Return to the Hotel by bus

## **Friday 16.03.2018**

9:30 – 11:30 Plenary session chaired by **Annamaria Bevivino**

9:30 – 9:50 **Frank Ashwood**, Forest Research, Alice Holt Lodge, Surrey, UK

*Developing a systematic protocol for deadwood earthworm surveys: a trial using an unmanaged oak woodland chronosequence.*

9:50 – 10:10 **Masudur Rahman**, University of Liège, Belgium

*Effect of drought legacy and tree species admixing on bacterial growth and soil respiration upon drying-rewetting in a young tree plantation.*



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10:10 – 10:30 **Laura M. Suz**, Royal Botanic Gardens, Kew, UK

*Plant-fungal interactions in Alpine ecosystems.*

10:30 – 10:50 **Taina Pennanen**, LUKE, Helsinki, Finland

*Ectomycorrhizal fungi increase the vitality of Norway spruce seedlings under the pressure of Heterobasidion root rot in vitro but may increase susceptibility to foliar necrotrophs.*

10:50 – 11:10 **Jelena Lazarević**, University of Montenegro, Podgorica, Montenegro

*Fungal biodiversity associated with fire-disturbed Pinus heldreichii forest soils in Montenegro*

11:10 – 11:30 **Rasmus Kjøller**, University of Copenhagen, Denmark

*Wood ash in forestry and effects on ECM fungal activity and community structure*

11:30 – 12:00 Coffee-break

12:00 – 13:30 MC meeting and closing section chair by **Martin Lukac**

12:00 – 13:00 Final BioLink MC meeting

13:00 – 13:30 Closing session by **Martin Lukac**

13:30 – 15:30 Lunch and departure





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## **2018 Annual Meeting**

**“Soil biodiversity and European woody agroecosystem”**

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**COST Action FP1305 BioLink**

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

**Abstracts for Oral Presentations**



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## **PLENARY SESSION, Part 1 and 2**

Chair:

*Martin Lukac.*, School of Agriculture, Policy and Development,  
University of Reading, UK; BioLink Coordinator



*Soil biodiversity and European woody agroecosystem  
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1-OP

**Linking microbial belowground diversity and plant health**

Gabriele Berg (COST invited speaker)

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**ABSTRACT**

The microbiome of soil and plants plays a crucial role in plant and ecosystem health (Berg et al., 2017). Plants and their associated microorganisms form a holobiont and have to be considered as co-evolved species assemblages. The microbiomes associated with plants form tight networks, which revealed strong species and niche specialization. Soil-borne diseases are microbiome diseases; they are the result of a microbial imbalance in soil and consequently in the rhizosphere and endosphere of plants. Structural and especially functional microbial diversity is suggested as a key factor in preventing plant diseases. Here, examples will be presented from soil - seed – rhizosphere microbiomes (Berg et al., 2018). Interestingly, antibiotic resistances (resistome), the most urgent drug resistance trend according to the global action plan on antimicrobial resistance (WHO 2015) is strongly connected with microbiome diversity. The plant-associated microbial diversity is interconnected with other microbiomes, e.g. indoors. It can be also transferred to the gut microbiome because fruits and vegetables are the major component of a healthy diet. Increasing chronic diseases in children can be explained by the “theory of disappearing microbiota” published by Blaser (2017). These novel insights will lead to a paradigm shift regarding the microbiome and health issues.

**References**

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Berg G, Raaijmakers JM. 2018. Saving seed microbiomes. *ISME J*. doi:10.1038/s41396-017-0028-2.  
Blaser MJ. 2017. The theory of disappearing microbiota and the epidemics of chronic diseases. *Nat Rev Immunol* 17(8):461-463.

2-OP

**The effects of forest management on the carbon quality of soil**

M. Lanfranchi<sup>1,2</sup>, A. Weatherall<sup>1</sup>, A. Arias-González<sup>3</sup>, N. Gartzia-Bengoetxea<sup>3</sup>, E. Vanguelova<sup>2</sup>  
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**ABSTRACT**

Globally, soils are the largest pool of actively cycling organic carbon (C) (Castellano et al., 2015), and climate change mitigation strategies strongly rely on the ability to improve soil organic carbon (SOC) stabilization, whose underpinning dynamics are still poorly understood. Forests have a major influence upon the world's climate, and their role in combating climate change through carbon capture is increasingly important. Carbon storage in forest soils makes 75% of total forest carbon in the UK (Vanguelova et al., 2013). Woody biomass from forest residues has the potential to make a significant contribution to Carbon dioxide emission reduction through fossil fuel substitution.

Whilst recognizing the importance of the multifunctionality and a wide range of ecosystem services forests provide, it is their role in carbon sequestration in trees and especially soils, as well as carbon substitution by wood that remain of utmost importance for climate change mitigation (Morison et al., 2012).

Methods of quantifying belowground C storage are improving, but little is known about C quality in soil organic surface horizons, or the influence of forest management (Varik et al., 2013). Current techniques for measuring SOC often do not account for the quality of C; yet SOC can be partitioned with mean residence time into pools of differing stabilities (Muller and Linhares-Juvenal, 2016).

This experiment compares the effects of three forest management practices (treatments): whole-tree harvest (where brash was removed from the sites), clearfell (conventional clearfelling) and clearfell with fertilization. The experiment investigates the effect of treatments on the C quality of well-developed litter, fermentation and humified soil organic surface horizons in second rotation Sitka spruce (*Picea sitchensis*) plantations on peaty gley soils in Scotland.

Organic samples from each plot were ground and analysed for carbon fractions (Van Soest et al., 1991) on an ANKOM200 Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Neutral and Acid detergent fibres (NDF and ADF) and acid detergent lignin (ADL) were used to calculate the amount of cell solubles (as the difference between the initial weight and NDF), hemicellulose (as the difference between NDF and ADF), cellulose (as the difference between ADF and ADL), and lignin on an ash-free dry mass basis (Gartzia-Bengoetxea et al., 2009; Olarieta et al., 2017).

By using C chemical fractionation methods to assess the quality of C in forest soils, this experiment extends the work of a previous study (Vanguelova et al., 2010) on the effect of forest management practices on the soil total C, N and nutrients at the same sites. This found



that whole-tree harvest significantly increased soil C and N concentration and content due to less mineralisation in the organic soil horizons where brash was not present compared to clearfell managed stands.

Our preliminary results suggest that there are differences in the amount of lignin between soil organic horizons, with fermentation layers holding an average >39% C in the form of lignin, compared to <29% in the litter and <24% in the humified layer, which may be due to the highest amount of tree fine roots in the fermentation layer. However, the quality of soil organic matter in terms of fibre analysis seems not to be affected by forest management although forest management clearly affects the C stocks of the organic soil horizons and particularly the fermentation and humified horizon.

*Keywords: organic surface horizons, chemical fractionation, forest management, soil carbon quality, whole-tree harvest.*

**Acknowledgments:** A special thanks to Neiker Tecnalia during the Short Term Scientific Mission (STSM) EU COST action FP1305 BIOLINK for support with the equipment and consumables, and the dedicated and passionate staff. Thanks to Forest Research and University of Cumbria for the invaluable contribution through funding and expertise.

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3-OP

**Indigenous olive rhizosphere *Bacillales* members as effective biocontrol agents against *Verticillium dahliae***

Carmen Gómez-Lama Cabanás<sup>1</sup>, Garikoitz Legarda Cristobal<sup>2</sup>,  
David Ruano-Rosa<sup>1</sup>, Paloma Pizarro-Tobías<sup>3</sup>, Antonio Valverde-Corredor<sup>1</sup>,  
José Luis Niqui Arroyo<sup>3</sup>, Juan Carlos Trivino<sup>2</sup>, Amalia Roca Hernández<sup>3</sup>,  
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**ABSTRACT**

The use of biocontrol agents (BCAs) against the soilborne fungus *Verticillium dahliae* Kleb., the causal agent of Verticillium wilt of olive (VWO), is an appealing and environmentally-friendly control measure to protect nursery-produced olive plants (López-Escudero and Mercado-Blanco, 2011). Previous studies showed that the root/rhizosphere of healthy olive (*Olea europaea* L.) plants is an important source of BCAs (Gómez-Lama Cabanás et al., 2018). These BCAs tend to be more efficient since they are adapted to the target niche where they will exert their antagonistic activity. In this study, three *Bacillales* members from healthy olive planting material (cv. Picual) were isolated and selected based on their in vitro antagonistic capability against *V. dahliae* and other olive pathogens. The three bacteria were in-depth characterized using a multifaceted approach based on: in planta assays, morphological, biochemical, and metabolic characteristics, chemical sensitivities, whole-genome analyses, genotypic and phenotypic identification of traits commonly involved in plant-bacteria interactions, multi-locus sequence analyses (MLSA), and assessment of root colonization ability. Strains PIC28, PIC73 and PIC167 showed effective in planta control against the defoliating pathotype of *V. dahliae*, strain PIC73 being the rhizobacteria showing the best performance as BCA against VWO. MLSA of conserved genes enabled the identification of two of these strains as *Paenibacillus polymyxa* (PIC73) and *P. terrae* (PIC167). However, strain PIC28 still remained as *incertae sedis* within the *Bacillus* genus, *B. cereus* and *B. thuringiensis* being the closest species. Strain PIC73 exhibited the best root colonization ability. This holistic strategy provided a comprehensive understanding of novel olive-BCA-*V. dahliae* tripartite interactions. Information generated will be useful for the future development of effective formulations against this pathogen.

**Keywords:** *Olea europaea*, *Verticillium wilt*, *Biocontrol*, *Paenibacillus spp.*, *Rhizobacteria*

**References**

- Carmen Gómez-Lama Cabanás, Garikoitz Legarda Cristóbal, David Ruano-Rosa, Paloma Pizarro-Tobías, Antonio Valverde-Corredor, Jose Luis Niqui Arroyo, Juan Carlos Triviño, Amalia Roca Hernández, Jesús Mercado-Blanco. 2018. Indigenous *Pseudomonas* spp. strains from the olive (*Olea europaea* L.) rhizosphere as effective biocontrol agents against *Verticillium dahliae*: from the host roots to the bacterial genomes. *Frontiers in Microbiology* 9:277. doi: 10.3389/fmicb.2018.00277
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4-OP

## **Valorizing complexity of soil food webs and related microbiome-based services for sustainable crop production**

Aurelio Ciancio (*Invited Speaker*), Laura C. Rosso, M. Colagiero, I. Pentimone

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### **ABSTRACT**

The data produced on soil microbiota by high-throughput technologies may yield detailed knowledge on the biological processes and services that many belowground trophic groups provide. However, given agricultural diversification, well defined experimental approaches are needed to valorize and exploit specific soil taxa or microbial groups. Our studies focussed on interactions of phytonematodes with rhizosphere soil microorganisms, and on the potential of some species for biocontrol. Regulation of nematode pests in soil food webs is often the result of a few microbial antagonists, often active through density-dependent mechanisms. External drivers, such as pesticide applications, may directly or indirectly affect belowground densities and species composition.

Traditional methods, such as time series and population dynamics or modelling studies, applied to soil or rhizosphere, cannot account for the complex interactions or functional redundancies often observed (Itoh et al. 2014; Liu et al. 2016). Furthermore, although informative in the long-term for tree crops, they are demanding in terms of labour or time. To study the interactions linking nematocides and nematodes to soil bacteria we tested a metagenomic NGS approach, combining the introduction of root knot nematodes (RKN) followed by a nematocide (fenamifos), on tomato in controlled conditions.

The drivers of such diversity shifts, RKN and fenamifos, overall induced an enrichment in the soil metabolic capacities in the varying conditions tested. Data showed that the effects related to treatments mostly occurred at finest taxonomic levels, involving several unclassified OTUs whose clusterings mirrored the soil conditions applied. Network analyses showed a dominant clustering of  $\beta$ -proteobacteria and a few out-of-network species with negative correlations, including members of Proteobacteria, Actinobacteria, Gemm-2 and Bacilli. Although present in low numbers, they occupied specific trophic niches, likely involved in antagonistic activities. PIECRUST analysis applied to the NGS data showed, however, that few metabolic pathways were differentially represented among the conditions tested. They mainly concerned antibiosis and aminoacid metabolism, suggesting a microbiome functional redundancy, balancing the changes induced by the treatments.

Further studies on the hyphomycete *Pochonia chlamydosporia* highlighted the multiple role played in the rhizosphere by this fungus as a saprotroph, a biocontrol agent and/or an endophyte. The fungus consistently showed a high endophytic capacity coupled with RKN parasitism. Previous surveys showed that *P. chlamydosporia* was widespread in agricultural soils in Italy on annual and tree crops, with an average prevalence around 75% of samples. Recent genomic data showed that it is highly enriched in genes encoding for hydrolytic enzymes, as reported from colonized barley roots (Larriba et al., 2014; Lin et al., 2018). Given its potential also as a growth promoter, we studied the changes induced by isolate DSM26985 when endophytic in tomato roots, by transcriptome analyses. Data showed that *P. chlamydosporia* induced differential gene expression and root re-programming. In a

saprotrophic-to-parasitic transition the fungus showed differences in the expression of genes involved in cellular signals, transport, or DNA repair (Rosso et al., 2011). The metabolism of *P. chlamydosporia* as an egg consuming biocontrol agent contrasts indeed with its activity as a root sentinel. Eliciting an early plant defense response may in fact reduce the number of nematode eggs produced on roots, and hence limit the fungus own food source. A better understanding of the *P. chlamydosporia* behaviour in the rhizosphere is hence needed, to improve either its formulation and use in the field, as well as for comparison of isolates, based on known genes and/or root or nematode interactions.

More specialized biocontrol agents such as the fastidious bacteria of the genus *Pasteuria* are known for their efficiency in nematodes regulation, whereas antagonistic generalists appear less prone to exploitation in nematode regulation and root protection. In field conditions, it is questionable whether complex relationships occurring in soil depend on a restrict number of species, that may be functionally significant for pest regulation and management. Linking drivers affecting the agro-ecosystem evolution to sustainability has also implications for biodiversity conservation and farm productivity (Ciancio and Gamboni, 2017). The latter goals have been often in conflict in intensive cropping systems. Selecting which drivers is important and what can be neglected represent an a priori assumption whose fairness may affect subsequent crop management and food production sustainability

*Keywords: Biocontrol, Endophytism, Soil Metagenomics, Nematode, Root*

*Acknowledgments: Research partially funded by Eureka! Eurostars E!7364 (Poch\_art) and MIPAF (Rome, Italy), project BIOMED.*

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## 5-OP

### **Alterations of C allocation patterns associated with climate-change induced vegetation die-off: Effects over soil pools, soil biodiversity and functions, and Controls of soil respiration**

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## **ABSTRACT**

The number of episodes of forest defoliation and mortality associated with drought events has increased substantially during the last decades, and they are further expected to increase even more given the projected climate change scenarios. These increase in episodes of tree mortality suggest that many tree species worldwide are experiencing their survival limits under the currently warmer and increasingly drier conditions (especially during summer). For instance, in the past years, extended tree mortality has been observed in the temperate-continental climate region of Romania (Eastern Europe), where due to the decreases in summer precipitation many populations of Scots pine (*Pinus sylvestris* L.), Black pine (*Pinus Nigra* subs. *nigra*), and Silver fir (*Abies alba* Mill.) have suffered substantial damages. This phenomenon is extended also in the Mediterranean basin, where, many tree species have started to show increased rates of crown defoliation, reduced growth, and mortality during the last decades. Here, even drought-tolerant species, such as *Quercus ilex* L. subsp. *ballota* [Desf.] (Holm oak) have started to show acute signs of decline, extended areas from the Iberian Peninsula being affected. Less is known, however, about how and at which extent climate-change induced vegetation mortality may alter patterns of carbon (C) allocation to e.g. vegetative growth and plant allocation to soil organs and soil organic matter (SOM). The loss of health and subsequent death of the vegetation is responsible for both modifying abiotic conditions (e.g. radiation incidence and soil moisture) and limiting the capacity of ecosystems to capture carbon (GPP) and hence supply the energy (carbohydrates specially) demanded by soil biological communities. This may result in irreversible losses of the soil biodiversity that sustain soil functions (e.g. nitrogen fixation, mineralization of essential nutrients or C stabilization) and hence on the capacity of soils to store carbon (C) and retain/provide essential nutrients like nitrogen (N) or phosphorous (P) to further sustain ecosystem growth and health.





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We here want to summarize the results obtained from a large latitudinal study consisting on a total of more than 30 permanent plots distributed throughout the Iberian Peninsula (Spain) and the Carpathians mountains (Romania) to investigate the effects of tree defoliation and mortality over historical growth patterns (dendrochronology, NDVI), leaf/root functional traits, soil C and nutrient pools, soil biodiversity and soil functioning. Our results show strong decadal-time scale impacts of climate-change related tree mortality over vegetation growth and C allocation patterns, alterations of the vegetation functioning (leaf and fine roots functional traits) and dynamics (secondary sucesional processes) causing chronic effects over the diversity and capacity of soil biota to provide essential functions for ecosystem survival and resilience. Alterations of the controls and magnitude of key ecosystem processes such as soil respiration, and the larger outgoing flux of CO<sub>2</sub> from terrestrial ecosystems, further suggests that models should take into account tree health and mortality in order to predict future terrestrial emissions in scenarios of climate change.

*Keywords: Forest die-off, dendrochronology, plant functional traits, soil biodiversity, soil functioning, soil respiration*

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6-OP

**Temporal turnover of ectomycorrhizal fungal communities in response to new habitats created by tree harvesting**

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**ABSTRACT**

Ectomycorrhizal (ECM) fungi dominate temperate and boreal forest soils, playing an essential role in plant growth, nutrient cycling, and food web dynamics. ECM fungi depend on root carbohydrates supply. The common forestry practice of whole-tree harvesting leaves the tree roots on the felling site, giving rise to a soil compartment, where root competition is altered. Thus, massively root ingrowth takes place alongside root degradation. In this study, we investigated EM fungal community turnover and stability in a soil habitat, with roots detached from the tree. We hypothesized that ECM community dynamics boosts in response to root severing compared to intact roots. We further hypothesized that some ECM taxa declined by energy deprivation due to the root disruption and other taxa proliferating by colonizing the new ingrowth roots. We found out that community asynchrony, an indicator of stability, decreased within six month of root severing, and recovered in the next 15 months. Total community turnover indicated that, in addition to differences in species richness, there were also greater fluctuations in the species present in the control, root ingrowth, and no-root ingrowth samples. There was a large reordering in the relative abundance of dominant species in ingrowth samples. For example, *Lactarius subdulcis* decreased substantially in samples over time but remained stable and dominant in the control samples.

Temporal functional diversity of ECM communities indicated a clustering of functionally similar ECM taxa. Habitat filtering and not competition were the driving force of the ECM fungal community structure in new habitats, created by the tree roots left in the soil by tree harvesting.

*Keywords: Forest management, Disturbance, ecosystem, microorganisms*

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7-OP

## **Winter activity of ectomycorrhizas of *Pinus sylvestris* and *Pinus sibirica* in Mongolia**

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### **ABSTRACT**

Ectomycorrhizal fungi are pivotal drivers of ecosystem functioning in boreal forests. They are an important pathway of plant-derived carbon into the soil and facilitate nitrogen and phosphorous acquisition. In forests in Mongolia, trees and ectomycorrhizas must survive several months of temperatures below freezing in winter and several months of low rainfall in summer. Fine roots of Scots pine (*Pinus sylvestris*) and Siberian pine (*Pinus sibirica*) and associated soil were collected from Nukht in Bogd-Khan National Reserve near Ulaanbaatar Mongolia at an elevation of 1600-1700 m in April 2016 at the end of winter. At the time the samples were taken the soil was still frozen, and the soil had been frozen for at least 5 months. Soil temperatures of down to -11 °C were measured for several weeks. For each tree species, 11-13 morphotypes of ectomycorrhizas were determined on root tips, and 12 identified to taxa. On the ectomycorrhizas the activity of a range of extracellular enzymes was determined. On some taxa of ectomycorrhizas had high activity of N-acetyl-glucosaminidase, acid phosphatase and laccase, and contributed greatly to total community enzyme activity. Our investigation shows that even in frozen soil taken at the end of winter ectomycorrhizas are active. This suggests that either the ectomycorrhizas have a high tolerance to freezing or that they develop in still frozen soil.

*Key words: Ectomycorrhizas, enzyme activity, soil freezing, Pinus sibirica, Pinus sylvestris*

### **MATERIALS AND METHODS**

Ectomycorrhizal root samples of Scots pine (*Pinus sylvestris* L) and Siberian pine (*Pinus sibirica*) and surrounding soil were collected from Nukht in Bogd-Khan National Reserve (107°06'65" E and 47°44'970" N at an elevation of 1700-1800 m) of Mongolia in 22nd of April, 2016. Study site is located in the vicinity of Ulaanbaatar city. When the samples were taken the soil was frozen, the samples were then stored at -16 °C until analysis.

### **Soil analysis**

Soil pH was determined on field moist soil using a 1:2 soil suspension in distilled water and  $\text{CaCl}_2$ . Soil moisture content was determined gravimetrically, by measuring the moisture loss after drying at 80°C for 24 hours.

For analysis of total C and total N, soil dried at 80°C was finely ground in a mortar, and total C and N were measured in 100-150 mg samples using automated dry combustion in a TruSpec CN analyser (Leco, St. Joseph, USA).

### **Ectomycorrhizal morphotyping**

In order to assess the ectomycorrhizal community structure, fine roots were removed from each soil core taken in 2016 to give a sample with approximately 150-300 root tips per core. The samples were then washed carefully, placed into petri-dishes filled with clean tap water, and stored at 4 °C (analyzed within three weeks). All clearly definable ectomycorrhizal root tips from each sample were sorted into morphotypes based on the method described by Agerer (1997), using a ZEISS (Stemi 2000-CS) dissecting microscope which was connected with an AxioCam ERc5s camera. The final identification to genus or species level (where possible) was carried out by sequencing of DNA (see below). The total number of root tips colonized by each of the morphotype was counted under the dissecting microscope. Between 1 and 10 ectomycorrhizal root tips of each morphotype were placed into micro-centrifuge tubes. The number of root tips varied from one to ten depending on the abundance of the morphotype. The samples were then stored at -20°C until DNA extraction.

### **DNA extraction and PCR amplification**

The 1.5 ml micro-centrifuge tubes containing the ectomycorrhizal root tips were placed in liquid N for 5-10 minutes, and the tips were ground with a sterilized glass bar. DNA from the crushed ectomycorrhizal root tips was extracted by using DNeasy Plant Mini kits (QIAGEN), and the extracted DNA was stored at -20 °C until the PCR reactions were run. For the PCR reactions, 1µl DNA template was mixed with 12.5µl MyTaq mix (BIOLINE), 0.5µl ITS1F (20µM) primer (CTTGGTCATTTAGAGGAAGTAA forward), 0.5µl ITS4 (20µM) primer (TCCTCCGCTTATTGATATGC reverse), and diluted with 10.5µl distilled deionized H<sub>2</sub>O. For the PCR, the Thermocycler (TProfessional Basic) cycling parameters were an initial denaturation at 95°C for 1min, a second denaturation at 94°C for 30 seconds, annealing at 50°C for 40 seconds, and extension at 72°C for 30 seconds, followed by a final auto-extension step at 72°C for 4.5 minutes. The step from the second denaturation to extension was run for 35 cycles. To check the success of the PCR amplification, electrophoresis was carried out using 1% regular agarose gel stained with SERVA DNA Stain G in a 1% Tris-EDTA buffer solution. The gel was then visualized under UV light. If a clear single band was visible on the gel, the PCR products were sent for sequencing. Sequencing was done by Macrogen Inc., Seoul, Korea. Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using a ABI PRISM® BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using an ITS4 primer. The fluorescent-labelled fragments were purified from the unincorporated terminators using the BigDye® XTerminator™ purification protocol. The samples were re-suspended in distilled water and subjected to electrophoresis in an ABI PRISM® 3730XL sequencer (Applied Biosystems). The sequences obtained were manually checked and edited using Finch TV\_1\_4\_0. Query sequences were compared with sequences on the UNITE and NCBI databases to identify the species of ectomycorrhiza; all but one of the sequences had a similarity of over 97% (Table S1). The sequences were deposited in GenBank with Accession No. MG859940 - MG859966. Morphotypes could not be identified using the DNA analysis and were labelled as unknown.

### **Ectomycorrhiza exoenzyme analysis**

Potential exoenzyme activities were determined using the high-throughput photometric and fluorimetric 96-well black microplate assays described by Pritsch and Garbaye (2011). The enzymes activities were expressed as  $\text{pmol mm}^{-2} \text{ min}^{-1}$  of total surface area of root tips. The total surface area of the root tips was determined after scanning and image analysis using the PC program WinRhizo 2012b Pro (Regent Instr., Quebec, Canada).

### **Soil temperature**

Temperature sensor (model DS1922L-F5, precision:  $0.5^{\circ}\text{C}$ , accuracy:  $\pm 1^{\circ}\text{C}$ ) were set to record temperatures every 60 minutes. For installation, the sensors were wrapped in plastic bags to prevent corrosion, and buried to 5 cm beneath soil surface on 28th August 2016. The data were read using a one wire viewer on 6th May 2017 and on 7th October 2017. Means were calculated from the 12 months 9 days readings.

## **RESULTS AND DISCUSSION**

During winter 2015-2016 air temperatures were below  $0^{\circ}\text{C}$  from the beginning of November 2015 until mid-March 2016. The lowest temperature reached was  $-40^{\circ}\text{C}$  in January 2016. Soil temperatures were not measured during the winter 2015-2016, however measurements of soil temperature over the winter 2016-2017 showed that the soil was below  $0^{\circ}\text{C}$  from 25 October 2016 to 1 May 2017, and the lowest temperature reached was  $-12.5^{\circ}\text{C}$ .

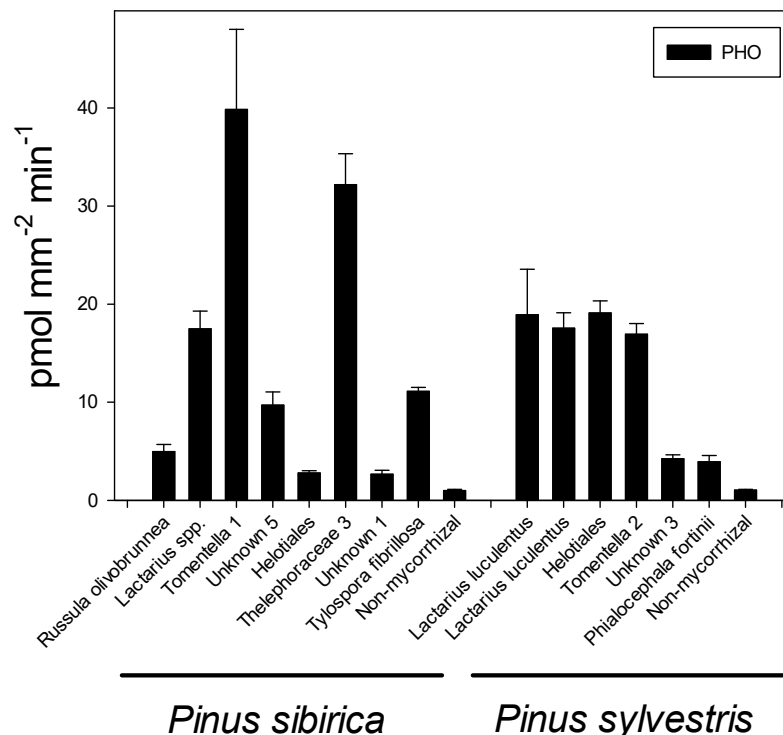
Fine roots of *Pinus sylvestris* and *Pinus sibirica* collected in April 2016 had 11 and 13 different morphotypes of ectomycorrhizas, respectively (Table 1). On *Pinus sylvestris* the dominant ectomycorrhizal taxa were *Lactarius luculentus* and *Helotiales*, whereas on *Pinus sibirica* the dominant ectomycorrhizal taxa were *Russula olivobrunnea* and a *Lactarius*. All of the taxa of ectomycorrhizas were turgescient and vital.

**Table 1.** Fungal taxa identified on root tips of *Pinus sylvestris* and *Pinus sibirica* at Nukht in Bogd-Khan National Reserve, Mongolia.

No	Closest Genbank/Unite Match	Similarity (%)	<i>Pinus sibirica</i>	<i>Pinus sylvestris</i>
1	<i>Russula olivobrunnea</i>	95-99%	+	
2	<i>Russula puellaris</i>	99%	+	+
3	<i>Helotiales</i>	95-99%	+	+
4	<i>Lactarius luculentus</i>	95-99%		+
5	<i>Lactarius</i>	82%	+	
6	<i>Tricholoma bonii</i>	99%	+	
7	<i>Russula nauseosa</i>	97%-99%	+	
8	<i>Tylospora fibrillosa</i>	99%	+	
9	<i>Tomentella</i>	98%-99%	+	+
10	<i>Thelephoraceae</i>	98%-99%	+	+
11	<i>Clavulina</i>	99%		+
12	<i>Phialocephala fortinii</i>	98%		+
13	<i>Suillus granulatus</i>	74%		+
14	Unknown 1		+	
15	Unknown 2		+	
16	Unknown 3			+
17	Unknown 4		+	
18	Unknown 5		+	
19	Unknown 6			+
20	Unknown 7		+	
21	Unknown 8			+



To assess the activity of the ectomycorrhiza taxa, exoenzyme activity of  $\beta$ -xylosidase,  $\beta$ -glucuronidase, cellobiohydrolase,  $\beta$ -glucosidase, leucine amino peptidase, N-acetylglucosaminidase, acid phosphatase and laccase were determined in an assay run at 20 °C. Two enzymes involved in degradation of more recalcitrant compounds, the chitinase N-acetyl-glucosaminidase as well as laccase showed activity on several of the ectomycorrhizal taxa (data not shown). Similarly, high of acid phosphatase was also shown (Fig 1). However, the enzymes involved in degradation of cellulose;  $\beta$ -xylosidase,  $\beta$ -glucuronidase, cellobiohydrolase,  $\beta$ -glucosidase had only low activity (data not shown). Similarly, leucine amino peptidase involved in peptide degradation also only had a very low activity. The levels of activity of acid phosphatase varied strongly between the ectomycorrhizal taxa (Fig 1). In both *Pinus sylvestris* and *Pinus sibirica* the dominate taxa *Lactarius* contributed 23 to 47% of the total ectomycorrhizal community activity.



**Figure 1.** The activity of acid phosphatase of ectomycorrhizal taxa and non-mycorrhizal root tips of *Pinus sylvestris* and *Pinus sibirica* collected at Nukht in Bogd-Khan National Reserve, Mongolia at the end of winter. Bars show mean  $\pm$  SE.

This work shows that in Mongolian forests even in frozen soil *Pinus sylvestris* and *Pinus sibirica* support viable and active ectomycorrhizas. As the soils have been frozen for up to 6 months, this suggest that either the ectomycorrhizas are tolerant to soil temperature of below -10 °C, or that ectomycorrhizas can develop at low soil temperatures in spring.

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8-OP

## **Bacterial and fungal community responses over a steep wood-ash gradient in a spruce plantation**

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### **ABSTRACT**

Wood ash, the waste product from the incineration of forest biomass, contains a high concentration of macronutrients. To counteract the loss of nutrients, recycling wood ash back to the forest has been proposed. Wood ash is also a liming agent with a pH of about 12-13. Thus, besides the obvious benefits, there are also concerns that need to be addressed before wood ash can be applied at large scale. Increases in soil pH stimulate microbial activity and can lead to changes in microbial community composition. Since microorganisms control important ecosystem functions such as decomposition and mineralization, any changes in their activity or community composition can have major effects in terrestrial ecosystems. Therefore, it is of high importance to study the ecological consequences of wood ash application to forest soils before it can be used at a larger scale. Current legislation allows dosages of 3 t ha<sup>-1</sup> to be applied on forest soil. The aim of this study was to evaluate the effects that different dosages of wood ash can have in microbial communities and to try to find a threshold value that could allow higher dosages to be applied. With this study and other experiments performed in the same study site, we concluded that higher amounts of wood could be applied.

### **MATERIALS AND METHODS**

A reduced scale experiment with extreme wood ash application rates was established on a Norway spruce monoculture stand in Denmark. A control treatment (0 t ha<sup>-1</sup>) and wood ash at five different ash application rates 3, 9, 15, 30, 90 t ha<sup>-1</sup> were established in 2x2 plots. Wood ash was spread manually with a spade evenly over the soil surface, in a single addition. The wood ash used in this experiment was a wood chip fuel mixed ash with a pH of 13.

One year after wood ash application, soil was sampled and DNA extracted. To study bacterial communities, amplification of 16S rRNA gene was done using the primers 354f and 806r. For fungal communities ITS2 was amplified using primers gITS7 and ITS4. Sequencing from both amplicons was performed on the MiSeq (Illumina) platform.

### **RESULTS AND DISCUSSION**

The results showed that wood ash did not impose any adverse effects in terms of richness or diversity, even at extreme amounts, for bacterial or fungal communities. Yet, significant changes in community composition were found, but only after adding 9 t ha<sup>-1</sup> or more for bacterial communities, and 30 t ha<sup>-1</sup> or more for fungal communities. This is in line with



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previous finding from the same experiment field were Cruz-Paredes et al. (2017) found that bacterial growth increased significantly only after 15 t ha<sup>-1</sup> and fungal growth was only affected after 30 t ha<sup>-1</sup>. In the same experiment, changes in community composition evaluated through microbial PLFAs were found only with 30 and 90 t ha<sup>-1</sup>. Likewise, Vestegård et al. (2017) reported that changes in the bacterial-based compartment of the soil food web were stronger than in the fungal-feeding organisms.

Overall, there are no negative effects of wood ash application on microbial richness or diversity, and changes in the community composition are only found with high dosages (9 t ha<sup>-1</sup>). Therefore, we do not foresee negative implications for microbial communities, if legislative limits for wood ash dosages are slightly raised.

*Keywords: wood ash, bacterial communities, fungal communities, microbial diversity*

Acknowledgment: This work was part of the ASHBACK project ([www.ashback.dk](http://www.ashback.dk)) running from 2014-2018. We thank all participants of this project and the Danish Council for Strategic Research for funding.

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## **PLENARY SESSION, Part 3**

Chair:

*Mark Bakker*, INRA - Centre de Bordeaux Aquitaine // Bordeaux  
Sciences Agro, Villenave d'Ornon (France)



9-OP

**Dissection of *Pinus pinaster* response to water deficit: Integration of complementary strategies to improve drought response**

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**ABSTRACT**

Water deficit is considered one of the most important stresses limiting forest tree growth and survival. Water scarcity, which is endemic in the Mediterranean region, will progressively increase as a consequence of climate change (IPCC Fifth Assessment Report. 2014. WGII AR5; <http://www.ipcc.ch/report/ar5/wg2/>). Maritime pine (*Pinus pinaster* Ait.), the main source of softwood in south-western Europe, is an autochthonous species with a relatively small geographical distribution found along a rainfall cline. Although it is characterized by a significant genetic and adaptive diversity, the mechanisms regulating pine adaptive responses to environment are still largely unknown.

During the last years, we have been studying the molecular mechanisms underlying maritime pine response to water stress and drought tolerance. We have analyzed different populations in the frame of provenance-progeny tests (Eveno et al. 2008, Cabezas et al. 2015), selecting genotypes with contrasting responses to be used as progenitors of a reference progeny that segregates for this trait (de Miguel et al. 2012). Following the phenotypic characterization of this reference progeny we constructed a genetic map and performed QTL analysis to identify regions involved in drought response (de Miguel et al. 2014, 16). These analyses allowed us to select F<sub>1</sub> genotypes showing contrasted responses to drought to analyze the physiological changes from a genome-wide perspective using a comparative transcriptomic and metabolic profiling approach at the organ level comparing plants exposed to moderate water stress with well-watered plants. We identified organ-specific transcripts, constitutively accumulated transcripts as well as transcripts differentially represented in sensitive or / and tolerant genotypes in response to water stress (de María et al. 2017).

Additionally, we are exploring alternative strategies to modulate *Pinus pinaster* drought response: i) the use of rootstocks showing contrasted drought response to study how they may affect drought response of the grafted scions. Although grafting is an extended practice in fruit tree and herbaceous species to improved scion biotic or abiotic stress tolerance and nutrient uptake, it has been rarely used in conifer species. ii) The effect of rhizospheric microbiome and root-inhabiting (endophytes) microbial communities on pine drought stress tolerance.

**Keywords:** *Pinus pinaster*, *abiotic stress*, *transcriptomics*, *candidate genes*, *epigenetic control*

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10-OP

**The impact of soil and vegetation management on nutrient cycling and understory vegetation diversity in European almond orchards**

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**ABSTRACT**

The conventional management, which includes intensive tillage and ground cover removal, in woody fruit-crop systems contributes to the widespread land degradation in Mediterranean Europe. This research assesses if alternative soil and vegetation management practices could enhance nutrient cycling, agricultural provisioning and ground cover vegetation complexity. Therefore, a field experiment was conducted in five almond farms where the following treatments were implemented: no-tillage (NT), reduced tillage with green manure (GM) and compost (CM) and as a control, conventional tillage (CT). Phosphatase was positively affected by all the alternative treatments. NT was most effective in improving the vegetation cover diversity, but GM turned out to be just as effective to enhance understory vegetation biomass and coverage. The impact on almond production varied strongly between plantation and year, therefore additional research is needed. To conclude, the implementation of green manure, no-tillage and compost on a monoculture almond farm could be effective strategies to improve or and rehabilitate services provided on the farm, such as nutrient cycling and vegetation species conservation.

**INTRODUCTION**

The European Mediterranean area is not only highly susceptible to land degradation, due to the local biophysical and climatic conditions, but also due to anthropogenic land management activities (Parras-Alcántara et al. 2016). Currently, woody fruit-crop systems (almonds, olives, vineyards) play an important role in this as a result of the widespread conventional management, which includes frequent tillage, and ground cover vegetation removal. Land management practices related to agro-ecological principles are gaining more attention because of the balance in ecosystem service delivery they provide, and even the rehabilitating effect they may have on ecosystem services (Tittonell 2014; Bommarco et al. 2013; Hainzelin et al. 2014; Bennett & Garry 2009). As early as now, little is known about the effect that agroecology-based management practices could have on the rehabilitation of ecosystem services.

This research is a field experiment where four soil management strategies that were implemented in five almond orchards to investigate the impact on nutrient cycling, agricultural provisioning and habitat provisioning.

## METHODS

We conducted a full factorial design in five almond plantations, where the following treatments were implemented: 1) Conventional tillage (CT), 2) No-tillage (NT), 3) Reduced tillage with green manure (GM), and 4) Compost (CM).

*Soil measurements:* In each plot three soil samples of 1-2 kg were taken, each consisting of ten sub-samples randomly taken from the 0-20 cm soil layer of three different areas in the plot.

(a) *Enzymatic activity:* Dehydrogenase activity was measured according to the methodology described by García et al. (1997). To assess the Phosphatase activity and  $\beta$ -glucosidase we used the methodology of Ramos et al. (2011). Urease activity was determined according to Nannipieri et al. (1982).

(b) *Soil organic matter and carbon and chemical soil assessment:* Organ Matter content (OM), total N (N), available P (P), extractable K (K) were estimated by using the official methods described in Ministerio de Agricultura, Pesca y Alimentación (1994).

*Vegetation measurements:* The ground cover was assessed according to the point-intercept method, modified as proposed by Ruiz-Mirazo & Belén (2012). In each treatment plot, six 10 m long transects were randomly laid out, and each consisted of hundred points with a distance of 10 cm. On each point the plant species was identified, or when there was no plant, the point was measured as bare soil.

*Almond productivity:* Trees were harvested with the vibrating harvesting machine, with a minimum of 4 trees per sample, or manually per tree, both are common harvesting techniques in the area. The machine collects the fruits and removes the peel, but not the shell. The machine was emptied after the selected sample group was harvested and fruits were collected and weighted; this was repeated multiple times. In the two farms where the harvest was done manually, fruits were collected by hitting the tree with a stick and collecting all the falling fruits by nets that were placed below the trees. In each manually harvested plot, the fruits of 15 trees were taken.

*Statistical analysis:* Data was analysed with a generalized linear mixed model, by using the *lme4* package in R. In this analyse the treatments were taken as a fixed factor and the farms were taken as a random factor.

## RESULTS & DISCUSSION

The activity of the soil enzymes, Phosphatase and Glucosidase, were found to be affected ( $P < 0.1$ , Table 1) by the treatments. There was a lower activity in Phosphatase on the CT treatment (conventional tillage) compared to the three other treatments, NT (no tillage), GM (green manure) and CM (compost). The activity of the enzyme Glucosidase showed a comparable strong trend, however this was not significant ( $P < 0.1$ ). The activity of the other two enzymes, Dehydrogenase and Urease, were not influenced by the treatments. The indicators for soil nutrient status (N, P, K, OM and pH) did not differ between treatments.

In the vegetation assessment, a total number of 8310 observations of plants were recorded. The most dominant species were found to be *Vicia ervilia*, *Lolium rigidum* and *Vicia sativa*. Both NT and GM treatments contained a higher vegetation cover than CT and CM ( $P < 1 \text{ E-}06$ ), and NT also contained a more species diverse vegetation cover than CT and CM ( $P < 1 \text{ E-}06$ ). Moreover, the understory vegetation covers in the GM treatment contained on average 35 tonnes more vegetal carbon than CT, which was a significant difference ( $P < 0.05$ ).

In 2016 GM resulted in the higher productivity (kg tree<sup>-1</sup>) than CT and NT ( $P < 0.001$ , Table 2). On the contrary, in 2017 CT had on average the highest productivity, this was, however, not significant. Production differed strongly between years and between farms. The nut weight was found to be significantly higher in CM than NT.

To conclude, the implementation of green manure, no-tillage and compost application on a monoculture almond farm could be effective strategies to improve or and rehabilitate services provided on the farm, such as nutrient cycling and vegetation species conservation.



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11-OP

**Grazed fuelbreaks in Andalusia as a tool for wildfire prevention: a new name for an old practice**

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**ABSTRACT**

The abandonment of many traditional agricultural practices over the last few decades in southern European countries has led to an expansion of homogeneous fire-prone vegetation communities and, as consequence, of wildfires. Mediterranean silvopastoral practices have been traditionally used by shepherds to remove the combustible load, as some researchers indicate: silvopastoralism is as a new name for an old practice (Etienne, 1996). In this context, in the 1980's France was the front-runner in using grazing as a complementary tool for the maintenance of fuelbreaks in Europe (Travaud, 2009). Grazed fuelbreaks are low combustibility areas, strategically located in the forest, which are grazed by livestock to reduce the growth of vegetation and therefore, they perform as a tool in wildfire prevention. Moreover, this practice contributes to maintain the population in rural areas and to channel agrienvironmental aids from the CAP. Grazed fuelbreaks were introduced in Andalusia (Spain) in 2003. The Andalusian Government (Regional Ministry of Environment and regional planning, CMAOT) requested the scientific support of the Group of Mediterranean Pastures and Silvopastoral Systems from the CSIC (Spanish National Research Council) to develop a research in grazed fuelbreaks in a pilot area of 39 ha, with one shepherd and 500 sheep. As a result of this research, in 2005 the Grazed Fuelbreak Network in Andalusia (RAPCA) program was created (Ruiz-Mirazo et al., 2011). In this program the Andalusian Forest Service (INFOCA) defines every year the different fuelbreaks areas, then, one shepherd is assigned to each area under the commitment of grazing their livestock intensively in order to remove a significant amount of vegetal biomass. Finally, shepherds receive a payment for the service they provide to the society (Regulating Ecosystem Services) whose quantity depends, amongst other parameters, on the degree of accomplishment achieved according to an expert evaluation before and after the grazing period.

The aim of this communication is to show the applied research which has been developed in this collaborative framework (CSIC-Andalusian Government) in order to address very specific issues of management, always linked to the integration of livestock grazing with wildfire prevention. The research group has focused on the development of different tools for the management of wildfire preventive silviculture: 1) Management of a *Pinus halepensis* Miller reforestation for the establishment of a fuelbreak (different thinning regimes) (Ruiz-Mirazo and González-Rebollar, 2013), 2) seedings with wild (*Vicia peregrine* L., *Trigonella polyceratia* L., *Cynodon dactylon* (L.) Pers. etc.) and cultivated species (oats, barley, vetch and bitter vetch), 3) design of two monitoring methodologies (accurate and semi-accurate monitoring) applied in the RAPCA to evaluate the effectiveness of grazing through the assessment of the impact of livestock in vegetation, i.e., the degree of accomplishment by the shepherds (Ruiz-Mirazo et al. 2011 Ruiz-Mirazo and Robles, 2012); and 4) development of a formula for the calculation of the payment for the services provided by shepherds, which is a function of the grazed



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surface, distance to the sheepfold, slope, type of vegetation, together with the degree of accomplishment achieved by the shepherd.

The Grazed Fuelbreak Network in Andalusia (RAPCA) program is a successful example of silvopastoralism and is completely integrated in the management plans of the Andalusian Government. Furthermore, it has inspired to other regions of Spain and, as a consequence, they are beginning to develop similar programs. At national level RAPCA was awarded with the “Batefuego de Oro 2012” as a management tool for wildfire prevention. Currently this network covers more than 6,300 ha of fuelbreak, involves around 110,100 animals (sheep, goats and some cattle) and more than 220 farmers that receive money for the service they provide to the society. Finally, a joint meeting between shepherds, technicians and scientists is being organized in order to analyse the RAPCA trajectory 15 years after its birth; and to address the future challenges to which this program can and must respond: i) in the ecological framework, to promote studies to increase the knowledge in the relationships pasture-herbivore, ii) in the management framework, to develop innovative techniques to facilitate the technical assessment in the RAPCA, and to enhance other traditional silvopastoral practices (i.e. “redileo”, fencing in Spanish), iii) in the social framework, to promote studies to evaluate the ecosystem services provided by grazing in fuelbreaks.

*Keywords: Preventive silviculture, fire, Mediterranean monte, livestock*

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12-OP

**Soil microbial diversity in open and closed forest patches along a soil temperature gradient: the importance of root properties for bacterial community coalescence in the rhizosphere**

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**ABSTRACT**

The elevational patterns of plant and animal diversity have been extensively described in ecological theory. However, how soil-temperature gradients and spatial heterogeneity of the forest and its associated root, soil and litter properties affect the diversity of soil microbial communities remains unclear. We studied the effects of a soil-temperature gradient and spatial heterogeneity of forests on taxonomic and phylogenetic patterns of bacterial and fungal communities in plant roots, rhizosphere and bulk soil.

Soil samples were collected in closed (tree islands) and open (gaps) forest at three altitudes in the French Alps (1400, 1700 and 2000 m). Samples were separated into three fractions: plant roots (at the community level, as roots were not sorted by species), rhizosphere and bulk soil. Bacterial (16S) and fungal (ITS) diversity was determined, along with soil aggregate stability, litter and root traits.

Phylogenetic clustering processes were observed for bacterial but not for fungal communities. Forest spatial heterogeneity significantly influenced taxonomic and phylogenetic diversities and divergence (phylogenetic relatedness) for bacterial communities. The soil temperature gradient and forest spatial heterogeneity affected fungal phylogenetic divergence, even though no over-dispersion or clustering processes were found. Soil aggregate stability was a significant factor explaining the bacterial and fungal communities' structure and was correlated with bacterial taxonomic diversity and fungal divergence. Bacterial communities' composition was better explained by root traits than in fungal communities, particularly for rhizosphere and root communities.

Our findings highlight the importance of root traits describing the taxonomic and phylogenetic diversity of bacterial communities. Conversely, fungal communities did not show clustering processes and the variance of their structure was not significantly explained by the root or litter traits measured. Additionally, our results highlight the importance of spatial heterogeneity of forests to study the soil biodiversity of forests.

*Key words: bacteria, fungi, 16S, ITS, root traits, soil aggregate stability, phylogenetic clustering.*

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## **PLENARY SESSION, Part 4**

Chair:

*Katarzyna Hryniewicz*, Nicolaus Copernicus University  
Torun, Poland

13-OP

**Devastating forest pathogens from the genus *Armillaria*:  
from genomics to biocontrol**

László Kredics<sup>1</sup>(*Invited Speaker*), Neha Sahu<sup>2</sup>, Thu Huynh<sup>1,3</sup>, Orsolya Kedves<sup>1</sup>, Zsolt Merényi<sup>2</sup>, Gábor Kovács<sup>3</sup>, Liqiong Chen<sup>1</sup>, Bettina Bóka<sup>1</sup>, Zoltán Patocskai<sup>4</sup>, Martin Münsterkötter<sup>3</sup>, Csaba Vágvolgyi<sup>1</sup>, László G. Nagy<sup>2</sup>, György Sipos<sup>3</sup>

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**ABSTRACT**

Fungi from the genus *Armillaria* (Basidiomycota) are among the most devastating soil-borne forest pathogens causing root rot in nut crops as well as fruit and timber trees in both hemispheres of the world. Their vegetative diploids (genets) are long-lived regular decay drivers of the native forest ecosystems, and their immensely enlarged subterranean colonies are among the largest known terrestrial organisms on Earth. The complex lifestyle of *Armillaria* colonies is supported by an abundant spread of unique soil-borne rhizomorphs: root-like structures which create the potential for *Armillaria* species to encompass multiple hosts and to form extensive ecological networks (Sipos et al. 2018).

The genomes of *Armillaria* species are under extensive investigations, six genomes have already been sequenced and published so far. Recent genomic studies highlighted the significant expansion of the genomes based on the coding genes rather than transposable elements (Sipos et al. 2017). The *Armillaria* genomes harbour unique repertoire of cell-wall-degrading enzymes and numerous lineage-specific genes expressed in rhizomorphs and developing fruiting bodies.

Current surveys of damaged and healthy forests in Hungary and Austria led us to identify conifer- and oak-specific species. Among them we found *A. gallica* with an unexpected genetic diversity, which may indicate a so far unidentified clade with multiple species. Metagenomic studies of the freshly collected fruiting body tissues predicted potential bacterial endosymbionts and an *Armillaria*-related surface- or microenvironment-associated microbiome.

Among the biocontrol agents of soilborne fungi, bacteria from the genera *Pseudomonas* and *Bacillus* produce biologically active metabolites inhibiting fungal growth, while *Trichoderma* species (Ascomycota) apply competition, antibiosis and/or mycoparasitism against a wide range of fungal plant pathogens. Representatives of these genera may be promising alternatives to chemical pesticides for controlling *Armillaria* root rot. Therefore, a large number of *Trichoderma*, *Pseudomonas* and *Bacillus* strains were isolated from *Armillaria*-infested forest soil samples. *In vitro* antagonism and antibiosis experiments revealed the potential of certain *Pseudomonas* and *Trichoderma* isolates to control *Armillaria* species. Some of the selected biocontrol candidates proved to be capable of siderophore and indole-3-acetic acid



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production as well as phosphorous mobilization – plant growth promoting properties which could also be potentially exploited during the biological control of *Armillaria* root rot.

**Keywords:** *Armillaria, genome analysis, transcriptomics, metagenomics biocontrol*

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14-OP

## **Wood ash and biochar addition to forests do not shift soil microbial functional diversity in the short term**

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### **ABSTRACT**

Community level physiological profiles (CLPP) performed by the MicroResp method are reliable ecological indicators to assess soil microbial functional diversity. Functional diversity provides a great insight to microbial roles in ecosystems, and it is regulated among others, by soil physicochemical properties. The application of biochar or wood ash to forest soils is often employed to improve soil structure and soil hydraulic properties, increase soil organic matter stocks, neutralise acidity and restore nutrients. We hypothesized that addition of new carbon sources (biochar and wood ashes) may induce a change in the microbial functional diversity. Two experimental sites were established on acidic soils: one on a loamy soil (SOC% 3.9; pH: 4.8) and another on a sandy loam soil (SOC% 10.8; pH: 3.8). CLPP were performed using MicroResp system in surface (0-5 cm) soil samples. The carbon sources used were selected on the basis of their ecological relevance to the soil microbial community. CLPP data of each experimental site was ordinated by principal component analysis. The first two ordination axes (explaining more than 70% of the variance) were selected and their relation with soil hydraulic and nutritional properties, soil structure and microbial biomass carbon was analysed. The major findings of this study may be summarised as follows: a) In the short term, a shift in the microbial substrate use profile for wood ash and biochar additions was not observed, b) the soil microbial community responded most strongly to the addition of carboxylic acids and carbohydrates in both experimental sites, and c) in the loamy soil the first axis of the PCA was related with pH and the second one to the mean weight diameter of soil aggregates, and in the sandy-loam site, the first axis was related to the available water content and the second one to the mean pore diameter.

*Keywords: forest soils soil functional properties, functional diversity, wood ash, biochar*

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15-OP

## **Link between Genomic Identification and Biochemical Characterization of Novel Siderophores Produced by Endophytic Bacteria**

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### **ABSTRACT**

The massive use of chemicals has impacted farmer's economy and has also decreased environmental quality. Besides chemical compounds, the use of plant growth promoting bacteria (PGPB) has been suggested as an alternative to the classical strategies. *Pinus pinaster* trees affected by Pine Wilt Disease, caused by pinewood nematode (PWN), had an incredible endophytic diversity. Endophytes may act as PGPB by fixing atmospheric nitrogen, synthesizing phytohormones and enzymes, and sequestering iron from soil.

277 bacterial strains isolated as endophytes of *P. pinaster* trees from Portugal (Proença et al. 2017), 61 bacterial strains carried by PWN from Portugal (Proença et al. 2010) and 38 bacterial strains carried by PWN from USA (Proença et al. 2014) were evaluated for their production of siderophores on Iron-CAS agar plates (Schwyn and Neilands 1987). The DNA of the best strains *Rouxiiella* sp. Arv20#4.1 and *Pantoea* sp. A41C3 was extracted and their genomes were sequenced by Illumina (MiSeq), followed by assembly and annotation. Culture supernatants were assessed for siderophore activities by a modified CAS assay (Esuola et al. 2016), to determine Fe, Ga, Cu, V and Al chelating compounds. Catecholate and hydroxamate siderophore production was performed (Arnow 1937; Atkin, Neilands, and Phaff 1970). Siderophores in supernatants were obtained by ion exchange based solid phase extraction followed by HPLC enrichment. The resulting 30 fractions were characterized for metal chelation and siderophore type. The best candidates were identified by LC-MS and MS/MS.

The genomes of both strains showed genes involved in the production of siderophores as well as several genes that are involved in plant growth promotion. Siderophores production by both strains is dependent on days of growth. Strain Arv20#4.1 produced hydroxamate-type siderophores (25 µM) while strain A41C3 produced catecholate-type siderophores (70 µM) and showed more affinity to iron or copper, respectively. However, best supernatant fraction of strain Arv20#4.1 (92 µM of NH<sub>2</sub>OH equivalents) and of strain A41C3 (309 µM of DHBA equivalents) showed more affinity to chelation of Cu and Fe, respectively. That may suggest that strains produced different siderophores since other fractions showed different metal chelation ratios. The results from LC-MS together with genomic information highlighted the possibility of novel siderophores.

Strains Arv20#4.1 and A41C3 showed (novel) siderophores with different metal chelation and different structures, hydroxamate and catecholate, respectively. Together with genomic characteristics, these strains need to be explored as PGPB potential.

**Keywords:** Siderophores, Genome, Pine Wilt Disease, Endophytes

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16-OP

**Tree species effects on stocks and vertical distribution of soil carbon: Links to mycorrhizal association, microbial characteristics and soil fauna**

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**ABSTRACT**

Tree species selection is one of the relevant forest management options in the context of mitigating increasing atmospheric CO<sub>2</sub> by soil organic carbon (SOC) sequestration. Information on tree species effects is also needed to assess the influence of tree species change with predicted climate warming. Tree species with leaf litter traits driving slow rates of leaf litter decomposition have traditionally been associated with accumulation of higher SOC stocks than tree species with fast litter decomposition rates. This hypothesis has mainly been based on observations of thick C-rich forest floors under tree species associated with ectomycorrhizae (ECM). However, a recent hypothesis has suggested that tree species with foliar litter traits conducive to fast decomposition will lead to more pronounced microbial transformation and stabilization of litter C (Cotrufo et al. 2013). The latter tree species are often associated with arbuscular mycorrhizae (AM) and may enhance deeper incorporation of C by more active soil fauna communities and by higher belowground rates of litter input.

The Danish common garden tree species experiments include both ECM and AM tree species that differ widely in traits such as foliar litter chemistry. Soil C stocks in six common European tree species formed distinct groups that largely reflected their mycorrhizal association, but the pattern was also associated with other biotic drivers such as the abundance of burrowing earthworms. Forest floor C stocks were consistent with the traditional perception of slowly decomposing ECM species being conducive to high SOC stocks, but an intriguing pattern of more C in the mineral soil in AM tree species supported the recent microbial stabilization hypothesis and suggested deeper incorporation of C in more stable forms.

Based on new results on microbial, macro- and mesofauna communities, fine root dynamics and soil information, this talk will revisit Danish common garden tree species experiments for a synthesis of processes and patterns in organic matter formation that may explain observed patterns in SOC stocks and vertical SOC distribution.

*Keywords: soil carbon stock, soil carbon vertical distribution, soil fauna, mycorrhizal association, microbial community*



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## **PLENARY SESSION, Part 5**

Chair:

*Mari Moora*, Institute of Ecology and Earth Sciences; University of Tartu, Tartu, Estonia



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17-OP

**The impacts of soil contamination on tree rhizosphere bacterial communities in a forest on an old military site**

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**ABSTRACT**

Soils contaminated with explosives due to ordnance decommissioning, manufacturing and use, is a common issue worldwide. Trinitroaromatic explosives are of concern due to their toxicity, mutagenicity and carcinogenicity. An effective, sustainable and economic remediation alternative for 'dig and dump' remediation is phytoremediation, the use of plants and bacteria to remove pollutants from soil. Trinitrotoluene (TNT), is recalcitrant to degradation, and advanced tools are required to mine soils for novel degradative bacteria and better exploit phytoremediation at explosives contaminated sites. Genomic, physiological and analytical data will be presented characterizing the microbial communities at a 100-year old TNT-contaminated site, a site where we went to search for bacteria with interesting genes for TNT-degradation. Further, the construction of a collection of genotyped bacteria with degradative and plant-growth promoting properties will be discussed. Field tests showed a significant effect of TNT on soil microbiota diversity and composition, both in the non-vegetated bulk soil and in the rhizosphere of young sycamore samplings. Key-species enriched in TNT polluted soil were identified, and a draft genome for *Raoultella ornithinolytica* strain TNT was prepared, from which a homolog of the Old Yellow Enzymes (OYE), an enzyme able to liberate nitrite from TNT, was identified and characterized. Metagenome libraries are being used to search for additional OYE homologues. Mesocosm experiments showed a positive effect of inoculating OYE-containing microbes in the rhizosphere of grasses, with significant effects on TNT-transformation rate, plant biomass and health. Genomics-enabled microbial enrichments is paving the way for microbe-stimulated phytoremediation for military site restoration.



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18-OP

## **Links between soil biochemistry and biodiversity with Oak health status.**

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### **ABSTRACT**

Acute Oak Decline (AOD) affects both native oak species (*Quercus robur* and *Q. petraea*) in England and Wales and is of great concern as Oaks represent the largest component of native broadleaf woodland in the United Kingdom. Affected trees have characteristic stem symptoms, dark coloured liquid runs out from cracks between the bark plates and necrotic lesions are present in the phloem tissue. The symptoms are found in conjunction with galleries of the two-spotted oak bupresid (*Agrilus biguttatus*) and specific necrogenic bacterial species isolated from lesion areas. Similar symptoms have been described across Europe and form part of a wider oak decline complex which can establish following exposure to environmental predisposition factors, such as drought.

In order to understand the occurrence of oak decline it is necessary to not only investigate the impact of biotic agents and their interactions, but rather consider the whole system beginning with the links to environmental factors. A survey with more than 500 locations has been used to map AOD occurrence with soil type, climatic factors and deposition (nitrogen, sulphur and base cations) using GAM models. The presence of AOD in England and Wales is significantly influenced by rainfall, air temperature, and elevation, as well as nitrogen, sulphur and base cation deposition. Preliminary analysis highlighted differences between soil types and soil moisture, however these need to be investigated at smaller scales, e.g. at site and tree level. This knowledge underpins risk mapping and will help develop best practice management advice.

This spatial study reemphasised the importance of predisposition factors in the Oak decline syndrome and led to the site/tree specific investigation of the links between soil biogeochemical indicators and soil fungal and microbial diversity and the relationships of these with Oak health and nutrient status. Preliminary results suggest strong links between belowground traits and tree health, but proof of cause and effect is required.

19-OP

**KEYLINK: a new soil model including soil structure and soil fauna as key drivers**

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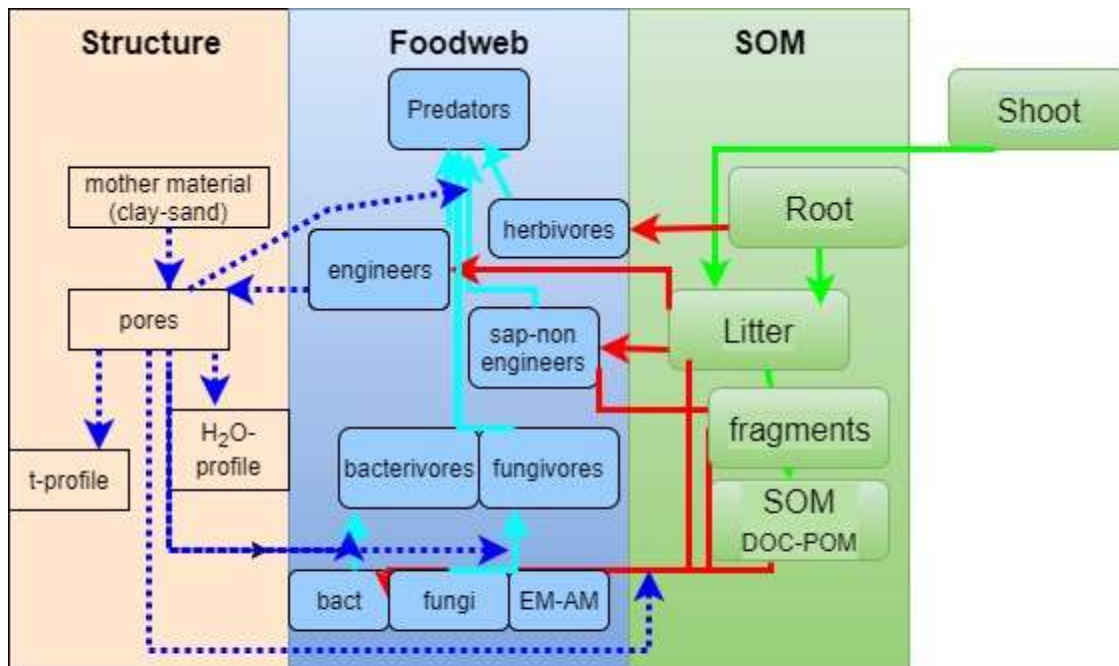
**ABSTRACT**

Recent improvements in understanding and modelling of the soil and SOM are not yet implemented into existing ecosystem models (review: Vereecken et al. 2016). Many ecosystem models do not represent any structure of the soil (with only one organic and one mineral layer), although it is clear that humidity and temperature profile are of high importance, and cannot be simulated without soil structure. Moreover, physical accessibility of SOM is an important determinant of its stability (besides chemical recalcitrance). Although the importance of soil fauna as ‘engineers’ on soil structure is well-documented, this is seldom represented in ecosystem level models (Barot et al. 2007). The important role of other faunal foodweb agents (Bengtsson et al. 1996) is completely ignored in ecosystem models, although functional diversity of the foodweb is of major importance for a well-functioning soil. It is also clear that the importance of EM fungi was not recognized in the older soil models, while recent research suggests their importance both as main components of older SOM and as networks between trees that exchange both C and nutrients (Averill & Hawkes 2016).

At the same time, many very detailed models exist that require too many data for implementation into ecosystem models or are difficult to parameterize but offer interesting insights into soil functioning. In ecosystem models the representation of the soil needs to be relatively simple, fast in calculations and requiring only limited input.

Within COST actions KEYSOM and BIOLINK a new integrative soil model ‘KEYLINK’ was developed that is simple enough for inclusion into existing ecosystem models, yet includes the newest insights concerning:

- Main soil fauna ‘agents’ divided into functional groups: engineers, fungivores, predators....
- Main drivers: water – t – structure –pH – litter quality
- Main processes: SOM stabilization, decay, nutrient & water uptake



#### Text

*Keywords: soil model, soil fauna, structure, foodweb*

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20-OP

**KEYLINK model: a short introduction to its use and usefulness**

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**ABSTRACT**

Most existing ecosystem models do not represent properly the structure of the soil (Vereecken *et al.*, 2016), which is indispensable for connecting the food web with its role controlling soil carbon (C) biogeochemical cycle. KEYLINK is a new model which integrates knowledge about soil structure and how this affects soil organic matter (SOM) dynamics (transformations, mineralization and stabilization), hidrology and the structure of the soil food web.

Using quite affordable parameters about climate, initial soil carbon pools and rates of key biotic processes (e.g. aerobic respiration and death rates of organisms), this model allows us to simulate, on a daily scale (and from any date), the changes in the biomass (g C m<sup>-2</sup>) of the different functional group populations in the food web, as well as changes in litter, SOM, CO<sub>2</sub> emissions and soil water content. It also gives, under different scenarios of main drivers (pH, climate, litter quality), a good representation of the evolution of fungal-to-bacterial ratios, which are crucial for the C storage potential of soils (Bailey *et al.*, 2002).

We will show how to use KEYLINK model and the output resulting from it, mainly graphs of the ecosystem responses to different input scenarios, as for example the resilience of ecosystems to the exclusion of key soil functional groups as engineers or predators, the effects of changes in the litter input quality, the relevance of different types of soil structure, or how climate change (through changes in temperature and water availability) will affect soil fauna, C sequestration and soil CO<sub>2</sub> emissions.

*Keywords: soil model, structure, soil fauna, food web, C biogeochemical cycle*

**Acknowledgments:** This work was supported by the KEYSOM (ES1406) and BioLink (FP1305) COST Actions, the STSM program of BioLink, and the FPU grant program of the Spanish Ministry of Education. We also acknowledge to all people that have contributed to the background of this model.

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21-OP

**LIFE ADAPTAMED - Protection of key ecosystem services by adaptive management of Climate Change endangered Mediterranean socioecosystems**

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**ABSTRACT**

Natural protected areas provide essential ecosystem services that are currently being affected by the negative effects of climate change. At the same time, natural protected areas are ecological laboratories where researchers and managers can investigate about natural processes and generate knowledge that can be applicable in other similar ecological situations. That is the main objective of Life ADAPTAMED project, which aims to mitigate the negative effects of climate change on key ecosystem services that three iconic and representative Mediterranean natural protected areas provide to local inhabitants and their socioeconomic sectors. This 5 year project, co-funded by the European Union, is led by the Ministry of Environment of the Junta de Andalucía (CMAOT). It includes partners such as the University of Granada, through the Interuniversity Institute for Earth System Research in Andalusia (IISTA); the Biological Station of Doñana, under the Superior Council for Scientific Research (CSIC); the University of Almería, through the Andalusian Center for Evaluation and Monitoring of Global Change (CAESCG); the International Union for Conservation of Nature (IUCN) and the Parque de las Ciencias of Andalusia. It is co-funded by Aguas de Lanjarón.

**INTRODUCTION**

Climate change currently affects 80% of the ecological processes on Earth to a greater or lesser extent (Scheffers *et al.*, 2016). Natural protected areas provide a valuable amount of ecosystem services that are currently being affected by the negative effects of climate change. Protection of these ecosystem services represents an innovative and essential approach for the conservation of natural areas (Turner *et al.*, 2007 and Jose, 2009). In this way, forests, coastal areas, mountains, scrublands and river ecosystems provide a wide variety of services directly linked to human health and well-being. However, these services are currently being affected by the deterioration that imply the temperature increase, the unpredictability of rainfalls, the arrival of invasive species and other phenomena that are rapidly changing the world (IPCC, 2013).

These services such as water provision, soil formation and retention, the regulation of climate, carbon storage and reservoirs, oxygen production, provision of wood, honey, etc. are specially affected in the Mediterranean region, which is confronted with a considerable increase in longer and more frequent drought and heat waves. And, on the basis of current data, this

affection is expected to increase significantly in the future (IPCC, 2014).

Life ADAPTAMED project aims to mitigate the negative effects of climate change on key ecosystem services that three iconic and representative Mediterranean natural protected areas provide to local inhabitants and their socioeconomic sectors. It will also generate valuable knowledge to be applied in other similar ecosystems along the Mediterranean basin.

## **MATERIAL AND METHODS**

The project is developed in three Andalusian Natural Protected Areas:

- Doñana National and Natural Park. It is probably, the most important wetland in Europe, and one of the best representatives of original Mediterranean coastal forest and scrubland.
- Sierra Nevada National and Natural Park. Representing high mountain ecosystems, with one of the highest biodiversity values within the Mediterranean hotspot.
- Cabo de Gata Natural Park. A desert coastal area at the south-western edge of the Iberian Peninsula that holds one of the most singular ecosystems in Europe.

The ADAPTAMED project focuses on developing, implementing, monitoring, evaluating and disseminating adaptive management measures addressed to those socio-ecosystems identified as key for the provision of, among others, soil retention, polinization, pastures, C retention, primary production, water provision, prevention of forest fires and of desertification. To achieve this objective, adaptive management actions are tested in a pilot scale, exploring different management options, all of them aimed to improve resilience in the targeted socioecosystems under a changing scenario. The results in terms of ecological services conservation are monitored in order to assess the most effective practices. This complex target can only be achieved through transdisciplinary work teams where scientists and managers share common goals.

## **DISCUSSION**

The main active management actions carried out within the project in the target ecosystems are:

- Pine selective cuttings.
- Cork oaks (*Quercus suber*) seedlings and plantations.
- Oaks (*Quercus pyrenaica*) and holm oaks (*Quercus ilex*) pruning and selective cutting.
- Restoration of pre-desert ecosystems (*Ziziphus lotus*).
- Soil conservation practices.
- Restoration of high mountain scrublands (*Junniperus communis*).

Pine plantations are present at the three natural protected areas involved and in many other sites around the Mediterranean basin. Furthermore, these planted forests hold many environmental problems, because of their current excessive density and homogeneous structure. They need a deep adaptation process to improve their resilience and resistance to climate change effects. The actions included in the project involve several activities aimed to improve forest heterogeneity, natural vegetation regeneration, dose of competence between trees and recovery of the ecological function characteristic of a natural forest.

In a similar way, the lessons learnt when managing oak forests (cork oaks, holm oaks and Pyrenean oaks) within the project will be useful in a wide range of conditions where they currently grow. Best management practices must necessarily combine goods production (i.e. cork, wood and acorns for cattle) with the tree structures most resilient to droughts, higher temperatures and the increased influence of forest pests and illnesses.

Restoration of some very peculiar ecosystems, such as *Ziziphus lotus* scrublands, with a very high potential in biodiversity conservation in dry areas, has a double effect in both nature conservation and awareness rise in local population in relation to the benefits that they offer to the surrounding economy, for example as a reservoir for entomofauna species which are

beneficial for agriculture.

To assess the most successful implemented practices, a temporal and spatial multiscale indicator system is being carried out through monitoring the impact of the project actions by combining fieldwork with satellite images analysis. At the same time, in order to quickly transform the information gathered into accessible useful knowledge, a specific information System is being developed for the project with the collaboration of the Andalusian Network for Environmental Information (Red de Información Ambiental de Andalucía), in coordination with the most important environmental data networks at the regional, national and international scale. Finally, several communication and dissemination actions ensure that the project results are effectively spread along the Mediterranean basin. More information: [www.lifeadaptamed.eu](http://www.lifeadaptamed.eu)



*Keywords: Ecosystem services, climate change adaptation, active management, Natural Protected Areas, research-practice partnership.*

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## **PLENARY SESSION, Part 6**

Chair:

*Elena Vanguelova*, Centre for Ecosystem, Society and Biosecurity, Forest Research, Alice Holt Lodge, Farnham, Surrey, UK



22-OP

**Adapted temperature relationships of microbial communities in warming soils  
enhance carbon-use efficiencies**

Johannes Rousk<sup>1</sup>(*Invited Speaker*), Kevin Geyer<sup>2</sup>, Melissa A. Knorr<sup>2</sup>, Serita D. Frey<sup>2</sup>

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**ABSTRACT**

Ecosystem models predict that climate warming will stimulate microbial decomposition of soil carbon (C), resulting in a positive feedback to increasing temperatures. The current generation of models assume that the temperature sensitivities of microbial processes do not respond to warming. However, recent studies have suggested that the ability of microbial communities to adapt to warming can lead both strengthened and weakened feedbacks. A further complication is that the balance between microbial C used for growth to that used for respiration – the microbial carbon-use efficiency (CUE) – also has been shown through both modelling and empirical study to respond to warming. In our study, we set out to assess how chronic warming (+5° C during 9 years) of a temperate hardwood forest floor (Harvard Forest LTER, USA) affected temperature sensitivities of microbial processes in soil. To do this, we first determined the temperature relationships for bacterial growth, fungal growth, and respiration in plots exposed to warmed or ambient conditions. Secondly, we parametrised the established temperature functions microbial growth and respiration with plot-specific measured soil temperature data at a hourly time-resolution from the forest floor to estimate the real-time variation of in situ microbial C production and respiration. To estimate the microbial CUE, we also divided the microbial C production with the sum of microbial C production and respiration as a proxy for substrate use.

We found that warm-adapted bacterial and fungal communities both shifted their temperature relationships to grow at higher rates in warm conditions which coincided with reduced rates at cool conditions. As such, their optimal temperature ( $T_{opt}$ ), minimum temperature ( $T_{min}$ ) and temperature sensitivity ( $Q_{10}$ ) were all increased. The temperature relationship for respiration, in contrast, showed a much smaller response to warming, with smaller changes in  $T_{opt}$ ,  $T_{min}$  and  $Q_{10}$ . When these physiological changes were scaled with soil temperature data to estimate real-time variation of microbial respiration and growth rates *in situ*, the warm-adaptation resulted in elevated microbial CUEs during summer temperatures in warm-adapted communities and reduced CUEs during winter temperatures. By comparing simulated microbial CUEs in cold-adapted communities exposed to warmed conditions to microbial CUEs in the warm-adapted communities exposed to those temperatures, we demonstrated that the shifts towards warm-adapted microbial communities had selected for elevated microbial CUEs for the full range of *in situ* soil temperatures recorded at the site. Our results suggest that adaptation of microbial temperature relationships to warming will enhance microbial CUEs, shifting their balance of C-use from respiration to biomass production. If our estimates scale to ecosystem level, this would imply that warm-adapted microbial communities will ultimately



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have the potential to store more C in soil than their cold-adapted counter parts could when exposed to warmer temperatures.

*Keywords: Simulated global change, warming experiment, microbial ecology, soil carbon cycle, carbon sequestration in forest soils.*

23-OP

**Linking belowground bacterial communities to healthy olive trees**

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**ABSTRACT**

The importance that cultivated olive (*Olea europaea* L. subsp. *europaea* var. *europaea*) has in the Mediterranean Basin is outstanding. In some countries such as Spain, olive crop has undisputable social, economic and agro-ecological relevance. Nevertheless, its future as a strategic commodity within the Mediterranean Agriculture is challenged by a range of threats. Verticillium wilt of olive (VWO), caused by the soil-borne fungus *Verticillium dahliae* Kleb., constitutes one of the most serious biotic constraints in many areas where this tree is cultivated. The increasing spread of the disease, particularly infections caused by highly-virulent isolates of the pathogen, the so-called defoliating pathotype, has generated great and justified concern among olive farmers and in the olive oil industry. Moreover, VWO is very difficult to control, and there is no single effective measure when applied individually (López-Escudero & Mercado-Blanco 2011). Plants live in close association with an enormous diversity of microorganisms, and their composite genome is considered by some authors as the “the plant’s second genome” (Berendsen *et al.* 2012). It is therefore not surprising that members of these plant-associated microbial communities may play an antagonistic role against phytopathogens. Within this framework, we aimed to implement a strategy to reduce *V. dahliae* infection based on the whole characterization of the microbiome associated with olive roots (rhizosphere and root endosphere) of a range of olive cultivars growing in a single location (Olive World Germplasm Bank, Córdoba, Spain). These olive varieties, originating from 9 different olive-growing countries, also have different susceptibility/tolerance level to VWO. Our goals are to (i) identify the core and accessory microbiomes present in olive roots, (ii) characterize specific components of these microbiomes which could be associated with tolerance to this disease, and (iii) identify single microorganisms or consortia with potential to be used as biocontrol tools against VWO.

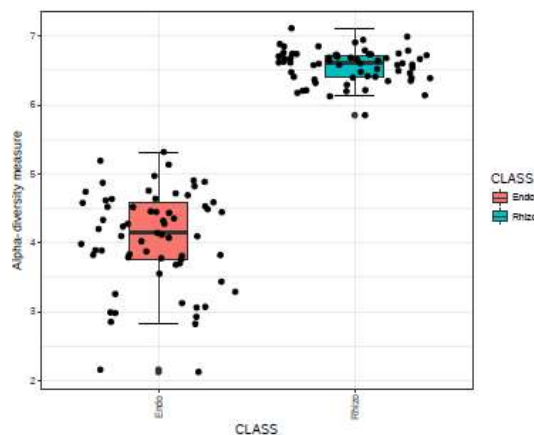
**MATERIAL AND METHODS**

Soil and root samples were collected from the Olive World Germplasm Bank (IFAPA, Córdoba, Spain). The 22 olive cultivars surveyed are grown in the same orchard to avoid differences related to the physicochemical profile of the soil. The first 5 cm of soil were removed and the collection of soil from 5 to 20 cm was performed following the main roots of each plant until finding metabolically active roots. Root samples were also collected from the same plant to assess the root endophytic communities as well. Three soil and root samples from 3 different

trees of each cultivar were collected. The soil DNA was obtained with the Power Soil DNA Isolation kit (MoBio, Laboratories Inc., CA) within the 24 hours of samples collection. The root DNA was obtained, after root surface sterilization and grinding, with 'Illustra DNA extraction kit Phytopure' (GE Healthcare, Little Chalfont, UK). The DNA was sequenced with Illumina MiSeq in a commercial sequencing service (The Institute of Parasitology and Biomedicine "López Neyra", Granada, Spain). A library was constructed amplifying the hyper-variable regions V3-V4 from 16S rRNA gene according to Takahashi et al. (2014) and sequenced with a PE 300 strategy. Raw sequences were overlapped with fastq-join and classified with MOTHUR (<https://www.mothur.org/>) to remove reads belonging to mitochondria and chloroplast. Then, using the software SEED2 (<http://www.biomed.cas.cz/mbu/lbwrf/seed/>) the prokaryotic sequences were trimmed and clustered. Further analyses were performed using STAMP (<http://kiwi.cs.dal.ca/Software/STAMP>), to obtain the communities profile at phylum/class level, and MicrobiomeAnalyst (<http://www.microbiomeanalyst.ca/faces/home.xhtml>) to obtain alpha and beta diversity indexes and statistical analyses.

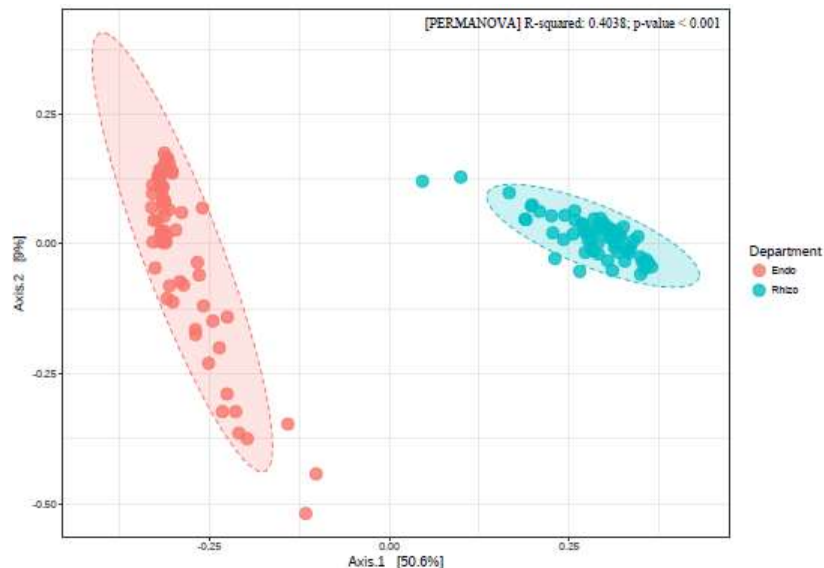
## RESULTS AND DISCUSSION

A total of 9,345,384 and 5,123,404 raw reads were obtained from root endosphere and rhizosphere, respectively. After overlapping and removing non-prokaryotic, wrong (with at least one ambiguity), short (less than 400 bp) and chimeric (USEARCH v8 implemented in SEED2) reads, only 886,472 and 3,195,960 good quality reads were retained from root endosphere and rhizosphere, respectively. The smallest sample had 237 reads and the largest reached 90,580 reads both coming from root endosphere samples. After relative log expression data normalization, the alpha diversity (Shannon index) showed statistically significant differences (Mann-Whitney p-value:  $5.51e^{-23}$ ) between rhizosphere soil and root endosphere samples, the former being significantly higher (Figure 1).



**Figure 1.** Boxplot of Shannon indexes of samples from endosphere versus samples from rhizosphere.

Concerning the beta diversity, a clear difference was observed between bacterial communities from the rhizosphere soil and those inhabiting within the roots (Figure 2). These results highlight that the olive genotype exert a much stronger selective effect on bacterial communities able to live inside the roots than that on the communities colonizing the root surrounding region.



**Figure 2.** PcoA with Bray-Curtis dissimilarities of samples from the olive root endosphere vs. samples from the olive rhizosphere.

These results show that both compartments (inside and outside the root) have clearly different 'bacteriome' profiles. Furthermore, bacterial communities from the rhizosphere are more homogeneous than those from the root endosphere. The reason(s) for this endophyte heterogeneity remains to be investigated, but influence of the olive genotype and/or its geographical origin are most likely involved. Linking these differences to the susceptibility/tolerance to VWO is one of the aims in our future analyses.

*Keywords: Verticillium wilt of olive (VWO), rhizosphere, root endosphere.*

**Acknowledgments:** This work was funded by the project OVerMan: "Omics-assisted strategies for Verticillium wilt of olive management" (AGL2016-75729-C2-1-R), from the Spanish Ministerio de Economía, Industria y Competitividad/Agencia Estatal de Investigación, co-financed by the European Regional Development Fund (ERDF).

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24-OP

**Microbial communities and activities of forest floor fractions in degraded pine forests after thinning treatments**

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**ABSTRACT**

Litter decomposition is a key process of biogeochemical cycling in forest ecosystems, which has an impact on biodiversity, nutrient availability, green-house gas emissions, soil organic matter and carbon sequestration. The objective of the study was the evaluation of forest floor composition at three decomposition stage, considering: i) C and N amount; ii) the abundance and composition of microbial communities (bacteria and fungi); iii) the hydrolytic enzyme activities; iv) the CO<sub>2</sub> emissions. We hypothesized changes of structural and functional diversity during the decomposition processes, affecting in turn carbon and nitrogen cycling. The study has been conducted in a degraded peri-urban pine forest of central Italy in the frame of the FoResMit LiFe project. Forest floor has been collected under three management regimes: i) traditional thinning, ii) selective thinning and iii) no thinning. Leaf (L), fragmented (F) and humified (H) litter fractions have been separated considering the different decomposition stage.

A significant decrease of carbon content and C/N ratio has been observed along decomposition process. This matched with changes in bacterial community composition, as well as with the increase of fungal richness and diversity, and enzyme activity. Forest floor contributed between 18 and 37% of total CO<sub>2</sub> efflux from soil, on average. Thinning increased forest floor pool without affecting microbial composition, diversity and activity.

**Material and methods**

The study area is the peri-urban forest of Monte Morello (43°51'20"N; 11°14'23"E), located in the Sesto Fiorentino municipality and close to the urban area of Florence in Tuscany Region. This forest is the result of the reforestations activities realized from 1909 to 1980; specifically experimental plots are 50 to 60 years old. The main tree species used are *Pinus nigra* J.F. Arnold, *Pinus brutia* Ten. subsp. *brutia*, *Cupressus sempervirens*, *Fraxinus ornus* L., *Quercus cerris* L. and *Quercus pubescens* L.. After reforestation the stands have been abandoned with consequences on trees stability, mortality, absence of regeneration, marked susceptibility to adversities and increase of fire risk (Cenni et al., 1998). Between September and December 2016, three silvicultural treatments have been tested in replicated plots of 1.5 ha approx. with the objective of restoring the ecological stability and enhance the resistance and resilience of the forest: traditional (negative selection) and selective (positive selection) thinning, *plus* three control plots without intervention (Paletto et al., 2017).

Forest floor has been collected in January 2017, after thinning operations, by pressing a 600-700 cm<sup>2</sup> steel sheet sampling frame 10 cm deep (or similar) into the forest floor and collecting all litter material above the soil. After sampling, forest floor has been separated into three components: leaf – L (fresh or slightly discoloured, with no or weak breaking up, material), fragmented – F (medium to strongly fragmented material with many mycelia and thin roots), humified – H (completely decomposed amorphous material). Samples have been dried and homogenized at 0.5 mm with a cut-mill for carbon and nitrogen content determination with an elemental analyzer (Flash 2000, Thermo Fisher).

CO<sub>2</sub> emissions were measured in two replicates for each of the 9 plots with a portable IRGA (EGM4, PP system) from collars with a without forest floor from April to December 2017.

Enzyme activity was measured according to the methods of Marx et al. (2001) and Vepsäläinen et al. (2001), based on the use of fluorogenic methylumbelliferyl (MUF)-substrates. Soil was analysed for N-acetyl-β-glucosaminidase, β-glucosidase, butyrate esterase, acid phosphatase, arylsulphatase, β-xylosidase, cellulose and acetate esterase activity.

The composition and structure of the bacterial and fungal communities were estimated by PCR-DGGE exploiting the 16S rDNA of bacteria and 18S rDNA of fungi. Bacterial and fungal community diversity was characterized through two indicators: richness (R, number of bands) and Shannon-Weiner index (H). nMDS was used to represent the similarity distance between each DGGE profile in a two-dimensional space.

## Results and discussion

The three components of forest floor showed well distinguished characteristics, with a decrease of C content along the litter decompositions level (Table 1). A correspondent increase of N % determined a decrease of C/N ratio. C is lost first during decomposition process and the concentration of N increases consequently. This pattern occurred independent of treatments and remained stable along time. Differently, both thinning treatments increased the amount of forest floor (significantly for L and F fractions).

**Table 1.** Biomass, C and N percentage and C/N ratio of forest floor

Forest floor contribution to CO<sub>2</sub> emissions was highly variable (between 2 and 72 %) and did not follow temperature trend. A lower contribution, on average, was observed in thinned plots.

	L				F				H			
	biomass kg m <sup>-2</sup>	N %	C %	C/N ratio	biomass kg m <sup>-2</sup>	N %	C %	C/N ratio	biomass kg m <sup>-2</sup>	N %	C %	C/N ratio
control	0.32 <sup>a</sup>	0.77	46	64	1.42 <sup>a</sup>	0.97	36	48	0.96	1.14 <sup>a</sup>	26	23
traditional	0.68 <sup>b</sup>	0.82	45	60	2.19 <sup>b</sup>	1.00	36	56	1.34	1.24 <sup>a</sup>	27	22
selective	0.43 <sup>b</sup>	0.75	46	64	1.93 <sup>b</sup>	1.12	40	57	1.17	1.39 <sup>a</sup>	29	21

**Table 2.** Percentage contribution of forest floor to CO<sub>2</sub> emissions from soil

Enzyme activities and functional diversity increased along decomposition stage, whereas non significant differences due to thinning treatments were observed. Among enzymes, arylsulphatase was the most represented in H fraction (80 %).

	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Average
control	4	48	37	24	26	39	21	25	63	37
traditional	12	21	23	30	17	19	4	6	20	18
selective	7	19	18	33	25	23	5	12	42	21

**Table 3.** Enzyme activity (mean of 8 enzymes) and functional diversity in the forest floor  
Bacterial communities did not change significantly in abundance and diversity, whereas the different decomposition stage alters significantly their composition. Differently, fungal community richness and diversity increased significantly along decomposition process, confirming results of arylsulphatase. Thinning treatments did not affect microbial community composition and diversity.

	L		F		H	
	Enzyme activity	Simpson-Yule index	Enzyme activity	Simpson-Yule index	Enzyme activity	Simpson-Yule index
control	50.81	6.63	54.29	7.10	59.81	7.42
traditional	46.11	6.62	57.68	7.06	51.16	7.47
selective	42.72	6.49	50.54	6.95	56.08	7.39

**Table 4.** Richness (R) and Shannon index (H) calculated on bacterial and fungal DGGE profiles.

	Bacteria						Fungi					
	R			H			R			H		
	L	F	H	L	F	H	L	F	H	L	F	H
control	23.7	20.7	20.3	3.13	2.91	2.90	8.3 <sup>c</sup>	11.3 <sup>bc</sup>	14.7 <sup>abc</sup>	2.08 <sup>b</sup>	2.38 <sup>ab</sup>	2.64 <sup>a</sup>
traditional	24.3	17.3	27.0	3.14	2.83	3.25	8.3 <sup>bc</sup>	12.3 <sup>abc</sup>	18.7 <sup>a</sup>	2.10 <sup>b</sup>	2.48 <sup>ab</sup>	2.87 <sup>a</sup>
selective	17.0	22.7	19.7	2.75	3.09	2.87	11.0 <sup>bc</sup>	12.7 <sup>abc</sup>	15.7 <sup>ab</sup>	2.37 <sup>ab</sup>	2.47 <sup>ab</sup>	2.69 <sup>a</sup>

Overall, decomposition process was characterized by changes in chemical and microbiological composition, driving higher enzyme activities in more decomposed fractions.

Thinning reduced microbial respiration, possibly as a consequence of the enrichment of fresh, low decomposed, organic matter (L and F litter fractions), without affecting microbial diversity and composition.

Acknowledgments: The work was financially supported by the LIFE program, in the context of FoResMit project (LIFE14/CCM/IT/905) "recovery of degraded coniferous Forests for environmental sustainability Restoration and climate change Mitigation".

**Keywords:** pine forest management, forest floor decomposition, CO<sub>2</sub> emissions, enzymes, microbial diversity

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25-OP

## **Quantifying the power of awareness of belowground biota: perceptions differ among European countries**

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### **ABSTRACT**

Belowground biodiversity plays a crucial role in agriculture and is directly related to farm practices. In the present study, we tested the awareness level of farmers on the importance of soil biodiversity in agro-ecosystems. At this aim, data were collected using questionnaires addressed mainly to tree crop farmers from five European countries, namely Greece, Hungary, Italy, Portugal and Serbia. More than 380 farmers joined the survey. In most of the involved countries, the majority of farmers stated to know the meaning of the term “biodiversity” and to enhance biodiversity with a variety of practices, such as crop rotation, tillage or integrated pest management. Moreover, they believe that enhancing and maintaining soil fertility increase productivity and being it considered as an environmentally sound practice, therefore deserving to receive financial support by EU Commission. Despite the fact that there were similarities among different countries, Serbia showed major differences on a number of aspects of the survey, including knowledge, monitoring and enhancing belowground biodiversity, implementing practices for pest management. Basically, a general farmers’ awareness on the relevance in adoption practices to promote belowground biodiversity in EU and non EU countries has been registered. These preliminary results call for elaborating useful guidelines destined to farmers in order to enlarge the knowledge and encourage the use of agricultural practices towards soil biodiversity maintenance and improvement.

*Keywords: Soil belowground biodiversity; Questionnaire, Tree crops; Sustainable Agricultural practices.*



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26-OP

**Drought-induced dieback altered soil biogeochemical cycles and soil microbial functioning along *Quercus ilex* historical land use and climatic gradient**

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**ABSTRACT**

Summer droughts induced by climate change in the Mediterranean basin are, in part, responsible for the observed decline of tree species so adapted to drought as holm oak (*Quercus ilex* subsp. *ballota*). Together with the intensive use at which *Q. ilex* has been historically subjected, tree defoliation and mortality might be responsible for limiting the capacity of soils to function and to retain nutrients. Here, we collected soil samples from 13 sites along the Iberian Peninsula distribution range of *Q. ilex* where holm oak showed symptoms of dieback (healthy, affected or dead trees) among three different types of land use: forest (> 60% crown coverage\*ha<sup>-1</sup>), open woodland (60% < crown coverage \*ha<sup>-1</sup> < 30%) and dehesa (> 30% crown coverage \*ha<sup>-1</sup>; Mediterranean open woodland produced by human land-use mainly for livestock grazing). Our results showed that at the rhizosphere level increase in drought-induced dieback reduced the capacity of soil communities to mineralize SOM (ammonification and soil heterotrophic respiration), further leading also to substantial losses in essential oligonutrients for tree growth (Sodium and Magnesium) but accumulation of mobile forms on nitrogen and phosphorous in soils not uptaken by plants.

*Keywords: holm oak, climate change, drought-induced dieback, soil biogeochemical cycles, soil microbial functioning*

**Acknowledgments:** This study was supported by the Spanish Ministry of Economy and Competitiveness grant VERONICA (CGL2013-42271-P) and FPI grant (BES-2014-067971)





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27-OP

**Developing a systematic protocol for deadwood earthworm surveys: a trial using an unmanaged oak woodland chronosequence**

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**ABSTRACT**

Decaying wood (or Coarse Woody Debris 'CWD') serves a key functional role in forest ecosystems; acting as a substrate and shelter for fungi and invertebrates, and improving forest soils through its decay into humic substances (Harmon *et al.*, 1986; Van Lear, 1996; Nisbet *et al.*, 1997). Whilst CWD colonisation processes have been described, little is known about earthworm: CWD interactions and the effects of CWD management on woodland earthworm populations (Hendrix, 1996). More information on the ecological impacts of CWD to forest health may help to promote a balance between intensive and low-impact forest management practices (Van Lear, 1996). However, much earthworm-related research and surveys have focused on agricultural land; and, where forest habitats are sampled, important microhabitats (e.g. CWD) are often overlooked by standard sampling methods (Schmidt *et al.*, 2015). This is because at present, no current methodology exists for surveying these habitats in a systematic manner. Undertaking systematic micro-habitat surveying alongside traditional soil sampling in may reveal a wealth of new information on earthworm ecology and species distribution. This research project aimed to: i) develop a systematic method for surveying deadwood-dwelling earthworms, and ii) determine the effect of woodland age and CDW decay class on woodland earthworm populations. Modified earthworm surveys were conducted across a chronosequence of oak forest stands in Alice Holt forest, Surrey, UK (Benham *et al.*, 2012). Results include a number of effects of woodland age and CWD decay class on earthworm populations, as well as three new records of the UK's rarest earthworm species *Denbrobaena pygmaea*. The novel sampling methodology appears to have been successful, revealing earthworm distribution data which would not have been obtained by traditional soil sampling methods alone. This research demonstrates that through increased woodland sampling and the inclusion of micro-habitats such as CWD, we may fundamentally improve our understanding of earthworm ecology and species distribution.

*Keywords: Coarse Woody Debris, Microhabitat, Deadwood, Deciduous Woodland,*

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28-OP

**Effect of drought legacy and tree species admixing on bacterial growth and soil respiration upon drying-rewetting in a young tree plantation**

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**ABSTRACT**

Drought events are expected to increase as a consequence of climate change, which will most likely lead to increased frequency of drying-rewetting events in soils. Further, the establishment of mixed species forests may be an option to mitigate drought stress to plants. Bacterial growth and respiration rates exhibit two kinds of response upon drying-rewetting. The influence of drought legacy and tree species mixing on the microbial responses upon drying-rewetting remains unknown, but could play important role in terrestrial carbon balance in the context of climate change. Using soils from a young experimental plantation in Belgium, we investigated whether mixed planting (oak monoculture, and oak in combination with 1-3 other tree species) under simulated drought (~50 % precipitation reduction for 2 years) influenced soil microbial activity, biomass, community composition (PLFA) and microbial response upon drying-rewetting. Bacterial growth and respiration rates were lower in drought exposed soils, but fungal growth was unaffected. Drought legacy resulted in a higher fungal-to-bacterial growth ratio in the one and two tree species mixtures, but no difference in the three and four tree species mixtures. Microbial biomass was consistently lower in drought-exposed soils compared to ambient controls with no effect of tree species admixing. Upon drying-rewetting, all soils exhibited similar growth and respiration patterns with lower bacterial growth and respiration rates in drought legacy soils. Tree species mixing did not influence bacterial growth rates, but the respiration rates were higher in four tree species mixtures between 18-30 hours after drying-rewetting.

Overall, our findings demonstrate that drought can have lasting effects on microbial biomass, with consequences for microbial function. Results suggest that tree species admixing to oak may alleviate the drought legacy effect on fungal:bacterial ratio, but does not modulate the bacterial growth and respiration rates upon drying-rewetting.

*Keywords: tree species diversity; FORBIO; bacterial and fungal growth; precipitation manipulation; temperate forest ecosystem, leucine incorporation*

**Acknowledgements:** We thank the Belgium Science Policy Office (BELSPO) for supporting MMR and this study under the project FORBIO Climate- Adaptation potential of biodiverse forests in the face of climate change via the BRAIN-be programme. The stay in Lund of MMR was supported by a short-term scientific mission (STSM) of the COST Action FP1305 BioLink- Linking belowground biodiversity and ecosystem function in European forests. This work was also supported by grants from the Crafoord Foundation (grant no. 20150561), the Swedish Research Council Formas (grant no. 941-2015-270) and the Swedish Research Council Vetenskapsrådet (grant no. 2015-04942). We thank A. Degueldre for help during soil sampling and E. Berglund for laboratory analyses.

29-OP

**Plant-fungal interactions in Alpine ecosystems**

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**ABSTRACT**

Alpine plant and fungal communities are models for understanding pioneer communities, the primary components of resilient ecosystems. Climate change is one of the biggest threats to organismal and functional diversity, yet little is known about its potential impacts on plant-fungal interactions. In alpine habitats, ectomycorrhizal (ECM) fungi are an essential functional guild as they are the main mutualists of the dominant woody perennial vegetation and play a fundamental role in carbon and nutrient cycling. Ectomycorrhizal fungi are required for the establishment of pioneer trees after environmental perturbations and therefore perform a key function in ecosystem resilience. Baseline diversity data on these fungal communities and their environmental drivers are needed in the face of future climate changes (Brunner et al. 2017).

We aim to: 1) study the ECM fungal communities associated with different alpine plants across the Alps, 2) elucidate the biotic and abiotic factors that affect their diversity and distribution and, 3) study the ECM communities that associate to closely related species and their hybrids in alpine ecosystems.

We sampled mycorrhizal roots, fruitbodies and soil associated with *Dryas octopetala*, *Bistorta vivipara* and *Salix herbacea* across five countries in the Alps. We also collected mycorrhizal communities from individuals of *Salix purpurea*, *S. helvetica* and their hybrids in an alpine glacier forefield in Switzerland (Gramlich et al. 2016).

We expect to: 1) identify a set of alpine ECM species or species assemblages unique to alpine ecosystems, 2) reveal their environmental drivers and threats, 3) generate a DNA database of alpine fungi for barcoding and phylogenetic analyses and, 4) get a better insight into the ECM communities that associate to sister plant species and their intermediate individuals.

**Keywords:** *fungi, ectomycorrhizas, Alps, environmental drivers, hybrids*

**Acknowledgments:** Winton (Harding) Alpine Plant Conservation & Research Programme. Collaborators, MSc, MRs and undergraduate students, volunteers and colleagues at RBG Kew and elsewhere.

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30-OP

**Ectomycorrhizal fungi increase the vitality of Norway spruce seedlings under the pressure of *Heterobasidion* root rot *in vitro* but may increase susceptibility to foliar necrotrophs**

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**ABSTRACT**

We tested if root colonisation by ectomycorrhizal fungi (EMF) could alter the susceptibility of Norway spruce (*Picea abies*) seedlings to root rot infection or necrotic foliar pathogens. Firstly, spruce seedlings were inoculated by various EMF and challenged with root pathogenic *Heterobasidion* isolates in triaxenic cultures. The ascomycete EMF *Meliniomyces bicolor*, that had previously shown strong antagonistic properties towards root rot causing *Heterobasidion in vitro* (Hyder et al. 2013), protected spruce seedlings effectively against root rot. In the second experiment we studied the effects of *M. bicolor* or forest humus EMF community on spruce growth allocation and susceptibility of distant organs towards necrotic foliar pathogens (*Botrytis cinerea* and *Gremminiella abietina*) in conventional forest nursery conditions. *B. cinerea* infection after winter was mild and the level of needle damage was independent of growth substrate and EMF colonisation. Needle damage severity caused by *G. abietina* was high in seedlings grown in substrates with high nutrient availability as well as in seedlings with well-established EMF communities in their roots (Velmala et al. 2018). Hence, on the contrary to its effectiveness towards root pathogens, *M. bicolor* was not able to provide protection from the foliar *G. abietina* infection. *G. abietina* infection changed resource allocation of the seedlings towards roots as the decrease in growth was stronger in shoots than in roots. The addition of forest soil microbes along with humus inoculum increased the EMF diversity, N contents and biomass of the seedlings but did not provide benefits against foliar pathogens either. Instead, the addition of fresh unsterilized humus seemed to be a slight risk factor probably due to the exposure of seedlings to wild needle damage caused by the microbes of the soil.

We found also that foliar infection of pathogen *G. abietina* seemed to reflect in ectomycorrhizal formation as the colonisation degree of these seedlings was the lowest in all substrates except for the sterilized humus substrate. Similarly, we have found that severe exposure to the needle pathogen *Gibberella avenacea* has a slight negative effect on the EMF richness on Norway spruce seedlings showing slow long-term growth performance (Velmala et al. 2014). Regardless of these indications of top-down movement of pathogen induced systemic signals we found no signs of EMF induced systemic effects in aboveground part of the host seedlings. This is in line with our recent observations showing that the impact of soil and its microbes is greater in the roots than shoots of spruce seedlings, and in general root traits show larger plasticity than shoots (Salmela et al., manuscript). Moreover, our results show that the positive effect of high EMF colonization and diversity seems to be due to either direct fungal antagonism or root mediated improved nutritional status.

Thus, it seems that the host-microbe interactions in spruce trees are highly species and even strain-specific, and no general responses can be expected without throughout knowledge on the identity of the organisms. It appears very characteristic to EMF-host interactions that the

within-species variations of effects on host performance are as high as the variation among different EMF species (Pennanen *et al.*, unpublished). Altogether our results show that albeit *M. bicolor* is able to protect spruce seedlings against *Heterobasidion* root rot in axenic cultures it fails to induce systemic protection against foliar pathogens. Unsterile inoculum sources, such as the forest humus layer, should not be considered for use in greenhouse conditions as they might predispose seedlings to unintended needle damages.

**Keywords:** *Picea abies*, *Meliniomyces bicolor*, *ectomycorrhiza*, *Botrytis cinerea*, *Gremminiella abietina*

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31-OP

**Fungal biodiversity associated with fire-disturbed *Pinus heldreichii* forest soils in Montenegro**

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**ABSTRACT**

Forest fires are among the most devastating events in forest ecosystems, but little is known about the recovery and succession of soil fungi following forest fires in high altitude habitats. We investigated fungal communities in fire-disturbed *Pinus heldreichii* H. Christ forest soils in south-eastern part of Montenegro. *P. heldreichii* is a montane to subalpine species of the Mediterranean basin occurring at the altitudes between 1200 and 2000 m and primary on calcareous soils in typical tree line habitats, often on steep ridges, mountainsides and screes, that are dry, exposed and nutrient poor. Being tertiary relict and sub-endemic, *P. heldreichii* fulfils an important ecological role. However, the remaining forests are threatened due to limited area of natural distribution, high fragmentation, and low natural regeneration. The major threats to stands of *P. heldreichii* include forest fires that during the last 15 years have devastated ca. 25% of the stands in SE Montenegro. Forest fires often result in the loss of a forest stand, but may also affect associated biodiversity including ectomycorrhizal (ECM) fungi that are important symbionts for successful regeneration, establishment and growth of *P. heldreichii*.

Soil samples were collected in 2016 from 4 localities that were affected by forest fires in 2010, 2012, 2014 and 2015, and from 2 non-disturbed control sites. *P. heldreichii* roots were also collected from control sites. Soil samples were divided into upper organic horizon consisting largely of burned needles, and lower horizon, representing early development organic soil (kalkomelanosol-A soil horizon). Resulting samples were subjected to extended chemical analysis and direct isolation of genomic DNA. Fungal ITS rRNA was amplified using barcoded fungal specific primers and subjected to high-throughput PacBio sequencing.

Sequencing resulted in 10,105 high quality sequences. Assembly showed the presence of 848 non-singleton contigs (at 98% similarity level representing different taxa). The number of high quality sequences obtained from fire-disturbed sites was on average 2.25 times lower than from non-disturbed sites. With further sequence analysis, we will investigate fungi present in forest soils in order to be able to evaluate changes in community structure over time, what could be essential for the regeneration of *P. heldreichii* on post-fire sites.

**Keywords:** *Pinus heldreichii*, forest fire, post-fire soil fungal community structure, mountain forest, high-throughput sequencing.

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BioLink), through a Short Term Scientific Mission at the Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

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32-OP

**Wood ash in forestry and effects on ECM fungal activity and community structure**

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**ABSTRACT**

Wood ash is a waste product from power plants fueled by forest products. Wood ash contains a number of nutrients (Ca, K, Mg, P etc.) and is in addition a potent liming agent, owing to its content of carbonates, hydroxides and oxides. When applied, wood ash typically increases pH 1-2 units in the top forest floor (Kjøller et al. 2017). An increase in pH may stimulate microbial activity (Cruz-Paredes et al. 2017), potentially leading to higher net nitrification and thus increasing the risk of N leaching. One of the key players closing the N cycle in temperate and boreal forests are ectomycorrhizal (ECM) fungi. The aim of this talk is therefore to highlight potential effects of wood ash application on ECM fungal mycelial production, their capacity to take up and retain N, and their diversity and community composition. This was achieved by 1) conducting a meta-analysis compiling past wood ash and liming experiments and 2) conducting two adjacent field experiments with wood ash application to a Danish spruce forest, one with moderate (3 - 6 t ha<sup>-1</sup>) and one with extreme (3 - 90 t ha<sup>-1</sup>) application dosages.

The systematic literature search precluding the meta-analysis retrieved 32 studies where the response of ECM fungi was recorded (Kjøller et al. 2017). For the experimental studies, in-growth mesh bags were installed in plots amended with 3 - 6 t ha<sup>-1</sup> wood ash. After mycelial colonization of the mesh bags, <sup>15</sup>N labelled ammonium and nitrate were applied. After a 24 h labelling period, extramatrical mycelia (EMM) was extracted and analyzed for production, <sup>15</sup>N incorporation (proxy for fungal activity), diversity and community composition. Also, soil samples were analyzed in parallel for community composition. The overall N-status of the site was assessed by measuring forest floor C/N ratio and leaching below the rooting layer using installed suction cups.

The meta-analysis indicated no effects of wood ash or liming applications on ECM abundance or species richness (Kjøller et al. 2017). In contrast, applications produced strong either positive or negative effects on particulate fungal taxa i.e. changing the community composition. In the field experiment with the moderate application rates (3 - 6 t ha<sup>-1</sup>), wood ash had effects on neither EMM in-growth nor their capacity to retain N. Still, there were significant seasonally differences in the amount of EMM produced and a preference for ammonium over nitrate. In addition, it was shown, that ECM mycelial production was positively correlated with N retention and that very little N passed through the mycelial "filter" in the mesh bags. In contrast to the meta-analysis we could not detect any effects on the ECM fungal community composition using the moderate application dosages, but when dosages were increased above 9 t ha<sup>-1</sup> effects became evident. Corroborating this last



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finding, nitrate and ammonium did not accumulate in the forest floor before applying these higher dosages of wood ash (Vestergård et al. 2017, Mette Hansen pers. Com). These results together demonstrate that applying up to 6 t ha<sup>-1</sup> of wood ash in plantation forests is a safe forest management practice that does not increase N leaching or negatively affect ECM fungi.

*Keywords: ectomycorrhizal (ECM) fungi , wood ash, nitrogen, mycelia, community composition*

Acknowledgements: This work was part of the ASHBACK project ([www.ashback.dk](http://www.ashback.dk)) running from 2014-2018. We thank all participants of this project and in acknowledge data gathered multiple people during this project.

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## **2018 Annual Meeting**

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**COST Action FP1305 BioLink**  
***Linking belowground biodiversity and ecosystem function  
in European forests***

**Abstracts for Poster Presentations**

1-P

**Analysis of microbial diversity and green-house gases production of pine decaying logs**

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**ABSTRACT**

In Sustainable Forest Management decaying wood plays an important role in biodiversity conservation, carbon balance, nutrient cycling and bioenergy production. The management of this important component of forest ecosystems is limited by the fact that little is known about relationship between substrate quality and community structure of wood-inhabiting microorganisms. During decomposition carbon stored in deadwood is lost either in the atmosphere or in the soil, but to our knowledge few information on the quantity of CO<sub>2</sub> and other green-house gases emitted are available. In the present research was investigated the correlation between the decay of logs (lying deadwood), the decomposer microorganisms and their activities, in terms of greenhouse gas production and enzymes, in a black pine degraded forest. The decomposition of deadwood was visually assessed using a five decay-class system and for each decay class four wood samples were collected. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O potential production from each decay class was measured in closed systems by means of gas-chromatography. Enzyme activities related to carbon, nitrogen, sulphur and phosphorus cycling were measured fluorometrically. The composition of decomposer microbial communities (fungi, bacteria and actinobacteria) was assessed by using PCR-DGGE fingerprinting. CO<sub>2</sub> production and enzyme activities were significantly higher in the last decay classes of deadwood. The molecular approach highlighted differences in microbial community structure both at species and abundance level, depending on the rate of decay.

*Keywords: pine deadwood decomposition, green-house gas emissions, hydrolytic enzyme activities, microbial communities, biogeochemical cycling*

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2-P

## **The *Quercus pyrenaica* rhizospheric microbiome**

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### **ABSTRACT**

Sierra Nevada National and Natural Park (South-East of the Iberian Peninsula) is a hotspot of biodiversity, where thousands of endemic and hundreds of threatened plant species can be found. Melojo-oak (*Quercus pyrenaica* Willd.) is an example of the retrogression process some species are undergoing there, since this species is considered as relict in this mountainous region. Global change, wildfires, the intensive timber industry, silvopastoral management of the forests as well as natural factors have led to a fragmentation of melojo-oak habitat, being the state of deterioration of these forests quite worrying nowadays (Camacho-Olmedo *et al.*, 2002).

Plant rhizosphere is characterised by the high diversity of microorganisms dwelling in this environment, in terms of taxonomy and functionality. In this ecosystem, microorganisms play important roles in plant nutrition and in stress adaptation, having significant consequences in their host's fitness which could be used to improve plants' health. Due to the importance of the dynamism and interactions found in the rhizosphere, we studied the diversity and structure of prokaryotic communities inhabiting *Q. pyrenaica* rhizosphere as a first step in microbiome-based restoration biotechnology. To achieve this goal, we focused on both total and potentially active communities by 16S rDNA and rRNA-based amplicon sequencing, respectively, of three different types of melojo-oak forests located in Sierra Nevada. Results showed that there were almost no differences in the diversity and composition of the prokaryotic populations inhabiting the three sites studied. However, although total and rRNA-based communities were composed by almost the same taxa, the community structure was quite different at different taxonomic levels.

### **Material and methods**

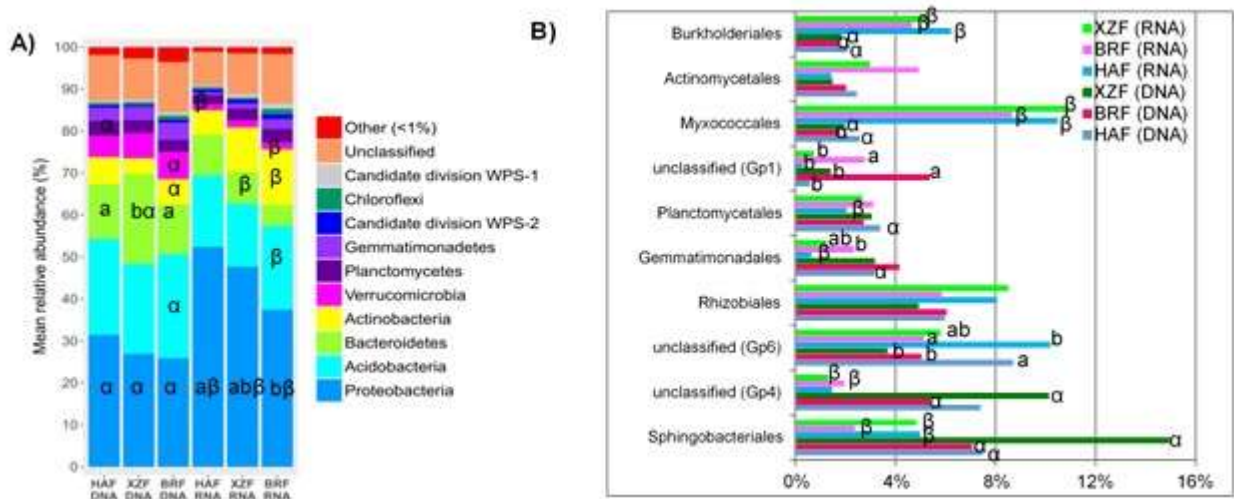
Three areas were selected in Sierra Nevada National Park from where samples were taken: a) HAF, a site covered by a mature old forest, b) XZF, where isolated single trees were found, and c) BRF, an area where low density forest was located. In each site three plots were delimited and three different trees were sampled in each plot. Rhizospheric soil was taken at a depth from 5 to 25 cm after the removal of the upper litter layer, and soil samples coming from the same plot were pooled. 2 g of each soil sample were mixed with 5 ml of LifeWard™ Soil Preservation Solution (MoBio Laboratories Inc.) and RNA PowerSoil® Total RNA Isolation Kit and the RNA PowerSoil® DNA Elution kits (MoBio Laboratories Inc.) were used to co-extract soil RNA and DNA, respectively, from each sample. cDNA was synthesised from the extracted RNA by retrotranscribing it after removing contaminant DNA. The hypervariable regions V3-V5 of the 16S rRNA gene were amplified with universal U341 and U926R primers (Baker *et al.*, 2003) as described by Calleja-Cervantes *et al.*, (2016). PCR products were purified and the total amount of 16S rDNA and rRNA-derived amplicons was measured with Qubit 3.0 (Thermo Fisher). Finally, amplicons were submitted to 454 GS FLX pyrosequencing

platform at Estación Experimental del Zaidín (Granada, Spain). The processing of the sequences was conducted with Mothur 1.33.3 software so that we could obtain high-quality sequences. Briefly, we removed those sequences shorter than 390 bp, with homopolymers > 8 nucleotides, as well as primers and MID. Afterwards, sequences were aligned against SILVA database (<https://www.arb-silva.de/>), potential chimeras were detected with UCHIME (Edgar *et al.*, 2011), and the remaining prokaryotic sequences were clustered into Operational Taxonomic Units (OTUs) at 97% similarity level. Singletons and virtual OTUs were discarded from the dataset and the taxonomic assignment was performed using the database from Ribosomal Database Project. Alpha diversity and richness indices were calculated from rarefied samples (2505 sequences). The taxonomic profiles of prokaryotic communities were analysed with STAMP (Parks *et al.*, 2014) and all the statistical tests (ANOVA followed by Tukey's HSD post-hoc test for multiple samples, Student's two sample t-test and PERMANOVA) were performed in R software (<http://www.R-project.org>).

#### Results and discussion

In terms of alpha diversity, the value of Shannon index was close to 6 in all samples, which indicated the high diversity of all communities. Although there were statistically significant differences between the Shannon indices calculated for HAF site ( $H'_{rDNA}$  6.05;  $H'_{rRNA}$  5.82), potentially active communities were as rich and even as DNA populations. No significant differences were detected between the sites sampled.

We could observe a clear separation of rDNA and rRNA-based samples in an NMDS plot (stress: 0.063). PERMANOVA suggested that although the library and the site had a significant influence on prokaryotic communities ( $p_{\text{library type}}$  0.01;  $p_{\text{site}}$  0.02), the type of nucleic acid played the strongest role ( $R^2_{\text{library type}}$  0.443;  $R^2_{\text{site}}$  0.19).



**Figure 1.** Relative abundance of main phyla (>1%) (A) and orders (B) in *Q.pyrenaica*'s rhizosphere. Different Latin letters indicate statistically significant differences between sampled sites (ANOVA test,  $p$ -value < 0.05 followed by Tukey's HSD test), while different Greek letters show significant differences between DNA and RNA-based libraries (two sample student's t-Test,  $p$ -value < 0.05).

At *phylum* level, we observed significant differences in the relative abundance of the main phyla (Fig. 1) just between HAF and BRF (Proteobacteria), and among XZF and HAF and BRF in DNA library (Bacteroidetes). The highest differences were found between DNA and RNA-based libraries, being Proteobacteria quite more abundant in the RNA-derived population (Fig. 1). This trend was also observed at order level, where we did not observe significant differences in the relative abundance of the main classified orders between sites but between



rDNA and rRNA-based communities. Myxococcales and Burkholderiales were significantly more abundant in the total population of all sites sampled, whereas less rDNA than rRNA-derived sequences related to Sphingobacteriales were detected in all cases (Fig. 1). Overall, all the main taxa were found in both libraries, however, the different relative abundance patterns observed for some of them highlight that active communities are just a subset of total prokaryotic population and point out the differential metabolic status of *Q. pyrenaica* rhizospheric communities. Although further studies are needed to define the composition of true active populations, the potentially active taxa here identified should be taken into account for future restoration tasks.

**Keywords:** *Quercus pyrenaica*, *prokaryotic diversity*, *16S rRNA and rRNA*, *next generation sequencing*, *active communities*

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3-P

**Biochar addition to a soil with low metal contamination and its effects on microbial community structure and plant growth.**

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**ABSTRACT**

Biochar induces changes in soil properties and crop biomass (Kammann et al., 2015; Kolton et al., 2017). Nevertheless, the mechanisms associated with improved plant biomass and soil microbial community structure in low metal contaminated agricultural soils are not well understood (Seneviratne et al., 2017). Here, we conducted a greenhouse experiment using two kinds of biochar from different feedstocks [wood chips (B1, from Carbon Terra, Germany) and wheat straw pellets (B2, from UK Biochar Research Center, Edinburgh University)]. Two Italian durum wheat (*Triticum durum*) varieties, Duilio and Marco Aurelio, that we had previously phenotypically selected for their contrasting growth performance in presence of biochar, were considered. Pots were filled with a gleyic fluvisol coming from the alluvium of the Litavka river (Láz close to Příbram City, Czech Republic), containing a negligible amount of nutrients (9% SOM) and a very little amount of Pb and Zn (total content of both metals up to 100 ppm). Four different treatments were performed for the two wheat varieties: only soil control (C), soil amended with 3% (w/w) woody biochar equilibrated with nutrient solution (B1+) and non-activated (B1-), and soil amended with 3% (w/w) wheat straw biochar non-activated (B2-).

The overall main objectives of this study, still in progress, are to evaluate the effect of the two biochars on plant growth and on the root-associated bacterial community, as well as to identify the relationship among them and soil structure (Noyce et al., 2016) and nutrient composition. Seven weeks after seed germination, fresh (FW) and dry weight (DW) of aboveground plant tissues and roots were evaluated. Bulk soil and rhizosphere samples were collected and DNA was extracted twice for subsequent metagenomics 16S rRNA analysis on illumine MiSeq platform. Soil, biochars and mixtures are being assessed through the main soil chemical-physical analysis. Here, we report our preliminary results that can answer the question if biochar exhibits a significant effect on soil traits, and/or wheat growth.

*Keywords: Biochar, low metal contaminated soil, durum wheat, soil microorganism community structure, metagenomics 16S rRNA analysis.*

*Acknowledgments: This experiment has been planned and performed thanks to the granting of a COST FP1305 BIOLINK STSM to Arianna Latini.*



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4-P

***Musa acuminata* (cv. Pequeña Enana) roots: an important reservoir of endophytes with potential as biocontrol agents against Panama disease**

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**ABSTRACT**

Banana (*Musa* spp. AAA) is a widely cultivated cash crop in tropical and subtropical climatic regions. Fusarium wilt of banana (Panama disease; PD), caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense*, poses a great risk to global banana production (Xue *et al.*, 2015). Pesticides no longer represent a sustainable option for its control, and many have been progressively withdrawn from use or are highly restricted. More environmentally friendly alternatives, such as microbial antagonists to control this pathogen, are thus now gaining growing interest (Raza *et al.*, 2016). The fact that endophytes are able to colonize a similar niche to that of vascular wilt pathogens favors them as potential biocontrol agents (BCAs) against PD. The aim of this study is to isolate, identify and characterize root endophytes from banana plants that can potentially be used as BCAs within an integrated management strategy of PD. Root plants originated from five farms representative of different banana orchards at Tenerife Island. To prevent contamination by microorganisms attached to the rhizoplane, a thorough root surface sterilization protocol was implemented. Then, individual colonies of bacteria and fungi showing distinctive morphology when growing in different culturing media were obtained from ground fresh root tissues. A collection of indigenous culturable bacteria and fungi (>800) from banana roots was thus generated (696 single/pure bacteria and 162 single/pure fungi cultures). Subsequently, *in vitro* antagonism tests against *F. oxysporum* f. sp. *cubense* subtropical race 4 (strain Foc CAV-095) were conducted. More than 100 strains showed antagonism against Foc CAV-095, pointing to the fact that banana roots are a good source of beneficial endophytic antagonists against Fusarium wilt. *In planta* assays will be conducted to assess the effectiveness of selected endophytes to control this relevant banana pathogen.

**Keywords:** *Fusarium oxysporum* f. sp. *cubense*, *Biocontrol*, *Musa acuminata*, *Panama disease*, *Root endophytes*.

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5-P

## **Seasonal patterns and species associations of earthworms in grassland habitat**

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### **ABSTRACT**

The spatial and temporal variability of earthworm populations is very high, both within and between differently managed types of land. Earthworms show clumped, patchy distribution (Rossi and Lavelle, 1998, Jiménez et al., 2001, Decaëns et al., 2003). This horizontal distribution is mainly a response to a spatial heterogeneity primarily determined with physico-chemical parameters of soil and food availability on various scales. In a given plot, the earthworm density depends on a group of different factors and which factor is the most limiting varies between ecosystems and earthworm species (Decaëns et al., 1997). One approach in the description and explanation of variation in their distribution is a geostatistical analysis. The aim of this study was to characterize the spatial variability of earthworm communities in grassland habitat in two consecutive seasons in relation to the habitat microtopography in order to facilitate earthworm sampling design. The variability was analyzed according to the earthworm species, ecological category (epigeic, endogeic or anecic), age (adult or juvenile) and habitat microtopography. Earthworms were sampled along a vertically stratified transect at 7 locations, at each location a 49 sampling points were arranged in a regular grid. To obtain high resolution microtopographic profile, elevation on each site was measured with the optical leveling instrument on multiple points. Data were used to create a digital elevation model (DEM) of each site revealing very subtle depressions and mounds. To test the spatial distribution of a single species a Morisita index of intraspecific aggregation ( $I\delta$ ) was calculated, spatial autocorrelation was tested with Geary's C and Moran's I index and semivariograms were calculated for each species, season and location. The results of this research show a patchy distribution of majority of adult individuals, and almost all juveniles. The Morisita index values from the literature are mostly higher, i.e. indicate a stronger aggregation from the values obtained within this research. However, the differences in index values are probably the consequence of earthworm population seasonal dynamics. Namely, as it is evident from our results that the earthworm patches change their size and position over time, i.e. the strength of aggregation varies. The index values for a particular species changed independently of season and location. In this research the range of semivariograms for all species and life stages was 8.67 m. For adult earthworms a range was somewhat smaller (8.37 m), while for juveniles it was bigger (9.2 m). The semivariogram range for a particular species in this research were lower than the ones in the literature. With this research a structure on small distances were detected which confirms that earthworm populations are structured, including autocorrelation on spatial distance of <5-10 m (Rossi, 2003). From the kriged sampling areas it is visible that the earthworm populations are not static and that the position of ceratin species, as well as a total area, that they covered on the sampled location, were changing. These comparisons could be many for several more species and the conclusion is tha the spatial distribution of adults and juveniles of the same sepcies is very similar. Sometimes the spatial distribution of juveniles and adults correspond within season, but sometimes a temporal shift





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is noticeable and the spatial distribution of juveniles in the summer corresponds to a spatial distribution of adults of the same species in the spring.

*Keywords: spatial distribution, geostatistics, microtopography, species assemblages*

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6-P

### **Linking the rhizosphere microbiome and acute oak decline**

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#### **ABSTRACT**

In Great Britain, the native oak species are affected by a complex decline-disease known as acute oak decline (AOD). The primary symptoms observed are necrotic lesions, stem bleeds and larval galleries of the bark-boring beetle, *Agilus biguttatus* (Denman *et al.* 2014). *Brenneria goodwinii*, *Gibbsiella quercinecans*, *Rahnella Victoriana* and potentially other bacteria were identified as key members of the AOD lesion microbiome (Broberg *et al.* 2018, Denman *et al.* 2017, Brady *et al.* 2017). Recent advances in human and plant diseases demonstrated and emphasised the impact of the composition of microbiomes in host health and resilience. Particularly, the rhizosphere microbiome plays an essential role in health, growth and development of their hosts (Hacquard and Schadt, 2015). These organisms participate in the biogeochemical cycles, nutrition and health of the trees, and can be beneficial, neutral or pathogenic (Uroz *et al.* 2016). However, little is known about the microbial communities that inhabit the rhizosphere of oak and how these consortia are correlated with AOD.

This study aims to characterize the microbial communities of English oak by high-throughput sequencing and explore the rhizosphere microbiome in the context of AOD. Rhizosphere soil samples were collected from healthy and AOD trees as well as bulk soil samples in three sites: Eastnor, Hatchlands and Richmond.

Results showed that bacterial and fungal communities are significantly impacted by forest location (Eastnor vs. Hatchlands vs. Richmond), forest type (parkland vs. woodland) and soil type (rhizosphere vs. bulk soil). Soil physicochemical parameters are the main drivers of microbial composition. No correlation has been found between tree health status and rhizosphere microbiome composition.

Further studies will be done and the ongoing project will provide new insights to understand how the bacterial and fungal members of rhizosphere and oak health status are linked.

*Keywords: Rhizosphere microbiome, Acute oak decline, High-throughput sequencing, Quercus robur*

**Acknowledgments:** We thank the site managers for their assistance and co-operation in allowing us access to the trees. We thank the Forestry Commission, Defra and Biocant for their contribution. Diogo Pinho was supported by FCT under the PhD grant SFRH/BD/100665/2014 and COST Action FP1305 under the STSM grant COST-STSM-ECOST-STSM-FP1305-071116-080698.



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7-P

## **The effect of of postdisturbance management and fire at the windthrow plots on soil microorganisms**

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### **ABSTRACT**

Wind disturbance as a major natural driver of forest dynamics in a large part of Europe can affect soil properties in different ways and for different time. In November 2004, over 12,000 ha of forest stands in the Tatra Mts. (Slovakia, Central Europe) were broken or uprooted by strong wind. In 2005, a part of disturbed plot was burnt by wildfire. After these events, monitoring of changes in soil properties started at the long-term research plots with different post-disturbance management (salvage cutting, no interference), the plot affected by fire and the plot not affected by disturbance (reference plot). In May 2014, the forest stand which served as a reference plot until that time was destroyed by another strong wind event. The objective of this study was evaluating the responses of soil microorganisms to disturbances 10-years after the event and finding out whether different post-disturbance management regimes on the windthrow area are reflected in different responses of soil microbiota after a decade. At the same time, the newly disturbed forest would add useful information about windthrow effects directly after a disturbance event and about the differences in soil microbial responses between “new” and “old” windthrow plots.

Ten years after the disturbance we observed still significant differences in microbial characteristics between burnt plot and the other plots with higher microbial activity at the burnt plot, while no significant differences were found among the other plots. Microbial characteristics at the “new” windthrow plot did not differ from the plots with different management except of the burnt plot. The results indicate that at a higher altitude the effect of fire on soil microorganisms is more distinct than removing or not removing of fallen trees and persists even over a decade.

### **MATERIAL AND METHODS**

The study was performed at the research plots established in 2005 in the windthrow area of the Tatra National Park situated at an altitude of 1040–1260 m a.s.l. with SE-S aspect and slope of 5–10%. The dominant soil type is Dystric Cambisol formed mostly from moraine material. Four research plots with area of 100 ha each and differing in their management regime were used for study:

- 1/ REX – former reference plot established in an intact forest stand and destroyed by strong wind in May 2014. Canopy was reduced to ca 15%. At the time of soil sampling, most of fallen trees were not removed from the plot yet.
- 2/ EXT – plot with salvage logging, fallen trees were removed from the plot after the windthrow in 2004.
- 3/ FIR – fire plot, fallen trees were extracted from the plot; however, in summer 2005 the plot was destroyed by a wildfire and surface organic layer was mostly burnt.
- 4/ NEX – not extracted, windthrow plot without any intervention, left to undergo the spontaneous succession;

Ten soil samples were taken from the mineral A-horizon (the depth of 0–10 cm) at each plot in autumn 2014. In soil samples, soil chemical as well as microbial characteristics (microbial biomass C, basal and substrate-induced respiration, N-mineralisation, catalase activity, richness and diversity of soil microbial functional groups based on the Biolog approach) were determined.

## **RESULTS**

Ten years after the disturbance significant differences in some soil properties between plots were confirmed. Among soil physico-chemical properties, distinct differences were found in soil acidity and especially C/N ratio. REX plot exhibited the highest acidity (pH-KCl 3.19) whereas at the other plots the average values did not drop below pH-KCl 3.49. The lowest C/N ratio was found at the EXT while the other plots exhibited higher C/N ratio (average C/N about 19). Neither C nor N concentration differed significantly between plots due to high intra-plot variability.

The differences in microbial characteristics between plots are not consistent. Among the measured characteristics, only basal respiration, N-mineralisation and richness of microbial functional groups differed significantly between plots. Generally, the FIR plot exhibited higher microbial activity while no significant differences were found in characteristics of microbial activity among the other plots. Microbial biomass differed between REX and EXT plots with higher microbial biomass at the REX plot. While the diversity of soil microbial functional groups did not differ between plots, richness of functional groups was significantly lower at the REX plot in comparison to the others. Among the other microbial indices, qCO<sub>2</sub> and Resp/C responded to the different plot management pointing on the higher substrate utilisation at all “old” windthrow plots.

The community structure based on the utilisation of different carbon source showed differences especially between the REX and FIR plot. At the FIR plot higher utilisation of L-arginine, L-asparagine, N-acetyl-D-glucosamine, glycyl-L-glutamic acid and putrescine, and lower utilisation of D-glucosaminic acid was observed in comparison to the REX plot.

The present study showed that the absence of significant differences in microbial characteristics and a similarity of microbial community composition between the extracted and not-extracted plots persist ten years after the windthrow despite the fact that a significant difference between plots in C/N ratio appeared as a consequence of the input of the organic matter of different quality. A beneficial effect of the reduction of thick surface organic layers after wildfire and consequent nutrients release persists over a decade and is reflected in still higher microbial activity at the burnt plot in comparison to the other windthrow plots. Microbial characteristics at the “new” windthrow plot did not differ from the plots with different management except of the burnt plot. The results indicate that at a high elevation the regional mesoclimate seems to have more distinct effect on soil microorganisms than a different post-disturbance management (removing or leaving fallen trees) in contrast to a wildfire, whose distinct effect exceeds a decade.

*Keywords: forest soil, soil microorganisms, windthrow, fire, postdisturbance management*

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8-P

## **Changes of the water repellency and physical properties with decomposition rate of deadwood of different species in temperate climate**

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### **ABSTRACT**

The aim of the study was to demonstrate the changes in physical properties and water repellency of deadwood depending on the species and decomposition stage. An innovative element in the study is the use of the methods of measurements of droplets contact angle to the wood surface, which are used in the areas of sciences in the field of material engineering. In addition, this study implements water repellency assessment methods from soil research for deadwood research. Five species of trees in three decomposition stages were selected for the present study. The deadwood silver fir (*Abies alba*), common hornbeam (*Carpinus betulus*), common ash (*Fraxinus excelsior*), common alder (*Alnus glutinosa*), common aspen (*Populus tremula*) were selected. The basic physical properties of the wood were determined. Water repellency (WR) was determined based on water drop penetration time. Surface Free Energy (SFE) was determined based on the measured angles. Owens-Wendt and Van Oss-Chaudhury-Good models were used to determine SFE. The advancement of deadwood decomposition is accompanied by its physical properties change. Marked changes in density, moisture, porosity and weight loss in subsequent stages of degradation were found in the wood of all the tested species. Changes in the physical properties of wood result in changes in water repellency and wettability. As the degree of decomposition increases, wood of different species with natural moisture content absorbs water more quickly, and its surface becomes more hydrophilic. The water repellency evaluation method implemented from soil research has proved to be successful in the case of wood analysis. The obtained results concerning surface free energy (SFE) suggest the possibility of using this method in deadwood analysis.

*Keywords: Contact angle, Decay classes, Surface free energy, Water content*

*Acknowledgments: The project was financed by the National Science Centre, Poland: decision no. DEC-2016/21/D/NZ9/01333).*



9-P

## **Climate and litter quality effect on three tree species leaf litter decomposition**

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### **ABSTRACT**

Litter decomposition represents one of the largest fluxes in the global terrestrial carbon cycle. Environmental conditions, litter quality and the microbial community are the main factors affecting decomposition, and Climate Change might change these fluxes as litter decomposition depends on temperature and water availability. In this context, the aim of this study is to improve our understanding of the effect that climate has in the decomposition of leaf litter of tree species that differ in litter quality and to test if there is home field advantage (HFA) through a reciprocal litter transplant assay. We hypothesized that decomposition rate will be lower in colder and drier climate and that decomposition will be different between tree species. Moreover, we expect that the microbial community might have been adapted to a specific composition of litter. Basque Country (in the north of Spain) is located in a transitional zone between Atlantic and Mediterranean climates. We established 2 study sites: (i) Artikutza (Atlantic climate) presents an annual mean temperature of 16.5°C, 2527mm of annual precipitation and 31 frost days and (ii) Montoria (sub-Mediterranean climate) shows an annual mean temperature of 10.5°C, 653 mm of annual precipitation and 58 frost days. Three different stands were selected at each study site dominated by oak (*Quercus robur* L.), beech (*Fagus sylvatica* L.) and Scots pine (*Pinus sylvestris* L.). In those locations, two types of standard litter (green tea and rooibos tea) and natural litter were buried. Litter samples were sequentially sampled at 7 different dates along a year. To quantify HFA, litter exchange was done between 2 study sites and HFA index was calculated. The results showed that higher decomposition rates were found in warmer and more humid climate in the case of Scots pine and the two standard litters. However, no significant differences in decomposition rates between sites for oak and beech litter were found, suggesting that climate may have a different effect on the decomposition of litter of different characteristics. HFA was not found to be the case showing that litter quality between sites may have similar characteristics.



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10-P

## **Carbon stocks in coarse woody debris in natural temperate forest to moisture gradient of sites**

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### **ABSTRACT**

Deadwood is an important element of properly functioning forest ecosystem and play a very important role in the maintenance of biodiversity soil fertility and carbon sequestration. The main aim of the study was to present effect of site conditions, including moisture content on the amount of deadwood and its rates of decomposition. Our intention was to fill the gaps in the knowledge about carbon stock in coarse woody debris in different site conditions. The investigation was carried out in Czarna Różga Reserve in central Poland. 40 circular plots of 0.1ha were established. The plots were located in a regular grid of points (100x300m). The study included the sequence of site humidity: fresh, moist and wet sites. All the live and dead trees were measured on each test plot. The stocks of deadwood in the studied moisture gradient are strongly differentiated and followed the order: fresh > wet > moist sites. The site moisture affects the quantity and quality of deadwood and through carbon stock. The highest resources of deadwood were related to sites without the permanent impact of groundwater. On the site in wet variant the increase in stock volume can be explained by the slowdown of decay processes during periods of excessive humidity, causing periodic anaerobic conditions. Coarse woody debris plays an important part in carbon pools in forest ecosystems. The carbon stock in deadwood depends on wood species, rate of decomposition and site conditions. In addition to the effect of moisture on the decay rate of deadwood, site conditions affect the quality of deadwood by influencing the species composition and structure of the stand.

*Keywords: Carbon stock, Deadwood, Forest ecosystem, Moisture gradient, Site conditions*

**Acknowledgments:** The project was financed by the National Science Centre, Poland: decision no. DEC-2016/21/D/NZ9/01333.



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11-P

## **Spontaneous mycorrhization of seedlings in Serbian forest nurseries – first report**

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### **ABSTRACT**

During a three-year survey in 14 forest nurseries in Serbia we observed seedlings of 19 species and 96 stocktypes of both conifers and broadleaves. Seedlings taken from the seedbeds and containers (autumn season) were put into plastic bag and transport to the laboratory where their roots were carefully washed under the running tap water. Roots were immediately examined under the 100x-500x microscope for the presence of ectomycorrhiza. Mycorrhization by artificial inoculation is not an operational practice in Serbian forest nurseries. However, the results of a three-year survey show the presence of ectomycorrhizal fungi in all nurseries, seedbeds and containers, and for all species. The rate and diversity of mycorrhizae naturally established in the nursery depends on environmental – nursery conditions, cultural practices and host species (Croghan 1984; Molina and Trappe 1984). It is known that high fertilization rates (Cram and Dumroese 2012), especially with nitrogen and phosphorus (Flykt et al. 2008) can suppress mycorrhizae development, and low level of fertilization rates in Serbian nurseries can be one of the reasons for colonization success. The presence of mycorrhiza in all samples is not unusual. In six forest nurseries in South Tyrol, on conifer seedlings, the rate of mycorrhization was 100% in autumn and about 97% in spring in all nurseries and on all plant species (Bacher et al. 2010). Pietras et al. (2013) founded that artificial inoculation of beech seedlings in the nurseries is not necessary due to abundant natural colonization. The fact that the mycorrhization in the container is not erratic indicates the abundance of fungus spores landing and washing in the growing media (Castellano and Molina 1989). Additional research should to provide type of mycorrhizal fungi and level of mycorrhization.

*Keywords: mycorrhiza, seedlings, forest nursery*

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12-P

## **Long-term effect of experimental drought and nitrogen addition manipulations on ectomycorrhizal fungi**

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### **ABSTRACT**

We performed the study on ectomycorrhizal (ECM) fungi in the former sites of artificial drought (D) and nitrogen experiments (N) in a 75-year-old *Pinus sylvestris* stand in central Lithuania. The experiments were performed in 2003–2006, in nine randomly distributed 15 x 20 m plots, three plots for every treatment: D, N and control (C). Fungi were studied in 2013–2014. No statistically significant differences in soil chemistry (organic and mineral layers) were found between the studied plots (MRPP test;  $p < 0.05$ ), contrary to the results obtained immediately after the experiment (Ozolinčius et al. 2009), meanwhile deviations in vascular plant and bryophyte cover were still apparent. Previous manipulations still had effect on ECM fungi, too. Numbers of ECM root tips in the organic soil layer were almost twice higher in C plots compared to D ( $A=0.238$ ;  $p=0.052$ ) and N ( $A=0.269$ ;  $p=0.040$ ). In the mineral layer some difference was found only between C and D plots ( $A=0.218$ ;  $p=0.049$ ). Number of fruiting ECM species was highest in D plots and lowest in N plots, but there was no statistical difference between the species diversity ( $A=0.052$ ;  $p=0.324$ ). In the organic soil layer, EMC communities differed between C and N plots ( $A=0.310$ ;  $p=0.026$ ) and between D and N plots ( $A=0.310$ ;  $p=0.025$ ). In the mineral layer, ECM communities showed weak differences between C and N plots ( $A=0.167$ ;  $p=0.041$ ), but no statistical differences were found between C and D or D and N plots. It can be concluded that even spatially limited disturbances (artificial drought and nitrogen experiments in our case) have a lasting effect on ECM fungi although its full range of impact is not yet clear.

**Keywords:** *Pinus sylvestris*, *ecm root tips*, *organic layer*, *mineral layer*

**Acknowledgments:** The study was financed by a PostDoc grant No 004/109 from the Lithuanian Research Council.

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13-P

**Effect of salinity on the microbiome of *Alnus glutinosa* roots**

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**ABSTRACT**

Black alder (*Alnus glutinosa* Gaertn.) forms symbiotic association with ectomycorrhizal (EM) and arbuscular (AM) fungi, as well as represents actinorrhizal plants that associate with nitrogen-fixing actinomycete *Frankia* sp. (Thiem et al., 2017). We hypothesized that the unique ternary structure of symbionts can affect community of other plant associated microorganisms (bacteria and fungi), particularly under seasonally changing salinity in *A. glutinosa* roots.

**MATERIAL AND METHODS**

In our study we analyzed bacterial and fungal microbiome of black alder roots at two forest test sites (saline and non-saline) in two different seasons (spring and fall). The physicochemical soil parameters were combined with new generation metagenomic analysis performed using MiSeq Illumina sequencing.

**RESULTS/DISCUSSION**

The dominant type of root microsymbionts of alder were ectomycorrhizal fungi, whose distribution depended on the salinity level. Fungi belonging to *Tomentella*, *Lactarius* and *Phialocephala* were more abundant at the saline site while *Mortierella* and *Naucoria* displayed the opposite tendency. Arbuscular mycorrhizal fungi belonged to Glomeromycota (orders Paraglomales and Glomales) and represented less than 1% of all identified fungi. Bacterial community structure depended on test site (salinity). *Rhodanobacter*, *Granulicella* and *Sphingomonas* dominated at the saline sites, while *Bradyrhizobium* and *Rhizobium* were more abundant at the non-saline site. Moreover, genus *Frankia* was observed only at the saline site. In conclusion, bacterial and fungal community structure of alder root microsymbionts depends on soil chemical parameters: salinity, phosphorus, pH, SP and total organic carbon but not seasonality.

*Keywords: salinity, ectomycorrhiza, arbuscular mycorrhiza, Frankia sp., metagenomics*

**Acknowledgments:** This investigation was conducted under the framework of COST Action (European Cooperation in Science and Technology) FP1305 Linking belowground biodiversity and ecosystem function in European forests, and financially supported by the National Science Centre (Poland) PRELUDIUM 2016/23/N/NZ8/00294.





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14-P

### **Comparison of forest soil fungi between four differently age stands of Norway spruce (*Picea abies* (L.) Karst.)**

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#### **ABSTRACT**

Monitoring of belowground fungal communities and environmental parameters in forest soils will allow a more comprehensive assessment of forestry management regimes. Fungi have important role in forest ecosystems as symbionts, decomposers, etc. therefore fungi can be used as an indicator for environmental processes. Biodiversity of fungal communities can be linked to above-ground plant diversity and other processes, however more research is required. We assessed fungal biodiversity and common soil parameters in four sampling sites (*Picea abies* stands) – three year old clearcut, naturally regenerated site after wind damage in 1969, 50 and 85 year old stands growing on fertile mineral soils in Oxalidosa forest type in northwest of Latvia. Each sampling site consisted of four plots in a square 50 m apart. Each sample (from one plot) was made combining seven random samples collected from a circle with radius of 2 m, using 0-10 cm topsoil. For each of sequenced sample (n=16), soil physicochemical analysis were done, quantifying soil pH (in water), total phosphorus, nitrogen, organic carbon, and organic soil matter. For metabarcoding we used fungal internal transcribed spacer (ITS2) and high throughput sequencing (Ion Torrent). Sequencing data were analysed using SEED2, CraftPipe, and DADA2 workflows. Diversity was estimated using Shannon-Wiener index and the Gini-Simpson index. The difference in fungal compositions between sites were visualised with non-metric multidimensional scaling (NMDS) ordination using Bray-Curtis dissimilarity matrices. Calculations were done in Project R using Vegan and DADA2 packages.

Preliminary results showed high operational taxonomic unit (OUT) richness in the samples, but community composition in general was significantly different between plots in each of the sites. Soil variables did not explain differences in fungal communities. Bray-Curtis ordination showed that only the clearcut samples formed a distinct OTU cluster. Overall preliminary results indicate that spatial variation in fungal communities could be a significant challenge for the assessment of community composition in large sampling sites and it also is a challenge for long term forest soil monitoring systems.

*Keywords: ITS2, Metabarcoding, biodiversity, Forest, Soil, Microbiome*

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15-P

### **Effects of DDE on a soil natural microbial community in presence/absence of the *Solanum lycopersicum* plant**

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#### **ABSTRACT**

Although the use of DDTs has been banned in most Countries over 20 years ago, its residues (DTTr) still persist in the environment. DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene) is the most toxic DDT metabolite and it is reported to be more persistent than its parental compound (Ortiz and Velasco, 2013). Plant-assisted bioremediation can be a promising and cost-effective clean-up technology to contaminated soil remediation. It relies on synergistic action between plant rhizosphere where microorganisms support toxic substance removal. *Solanum lycopersicum* is known to be able to accumulate DDTs in roots, owing to exudate production, which make these contaminants more bioavailable for soil microorganisms. Moreover, several natural bacterial strains have been shown to degrade organochlorine pesticide through their enzymes able to break-down complex molecules (Verma et al., 2014; Qu et al., 2015). In this context, a greenhouse experiment was performed to study the effects of tomato plant presence on DDE persistence in an agricultural soil. The experimental set up consisted of pots filled with contaminated soil (1ppm of DDE) in presence/absence of tomato plants and control soils (with/without plant and/or DDE). The plots were sampled 40 days from DDE exposure. The effects of DDE and/or plant presence on the natural microbial community were evaluated in terms of microbial abundance, viability, structure, dehydrogenase activity and DDE residual concentration. The overall results showed that although the plant presence stimulated the soil microbial community activity, it did not increase DDE degradation. On the other hand DDE presence not only did not negatively affect the natural microbial community, but the latter was able to degrade DDE independently from the plant presence, showing a preacquired capability presumably due to a soil previous contact with this pesticide.

*Keywords: plant-assisted bioremediation, pesticide, microbial community structure, greenhouse experiment, soil microbial activity*

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16-P

**Root colonization ability of indigenous bacteria from the olive rhizosphere displaying effective biological control against *Verticillium dahliae***

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**ABSTRACT**

The soil-borne fungal pathogen *Verticillium dahliae* Kleb., the causal agent of Verticillium wilt of olive (VWO), is one of the most important biotic constraints affecting this woody crop. VWO is very difficult to control and the implementation of an integrated disease management strategy is the most effective measure. A promising control measure within this holistic framework is the use of beneficial microorganisms (López-Escudero and Mercado-Blanco, 2011). Biological control agents (BCAs) can be used either as preventive (before-planting) or palliative (post-planting) tools within the target agro-ecosystem. Important prerequisites for an effective BCA are good colonization ability and endurance in the target niche where it will be deployed. A collection of bacterial isolates was previously obtained from healthy nursery-produced olive plants (Gómez-Lama Cabanás *et al.*, 2018; Ruano-Rosa *et al.*, 2017). The efficiency of five strains, namely PIC25 (*Pseudomonas indica*), PIC28 (*Bacillus* sp.), PIC66 (*Rhizobium* sp.), PIC105 (*Pseudomonas* sp.), and PICF141 (*Pseudomonas* sp.) to control the defoliating (D) pathotype of *V. dahliae* has been previously demonstrated (Gómez-Lama Cabanás *et al.*, 2018; unpublished). In order to evaluate the olive root/rhizosphere colonization ability and the capacity to enter the root interior, spontaneous rifampicin-resistant (Rif<sup>R</sup>) mutant derivatives of each of these strains were generated. Box-PCR and Rep-PCR assays were performed to confirm that preselected spontaneous mutants (5 per strain displaying similar colony morphology) showed identical electrophoretic patterns than those observed for their respective wild-type parents. *In vitro* antagonist tests against a representative *V. dahliae* D isolate showed that the Rif<sup>R</sup> mutants displayed similar *in vitro* pathogen's growth inhibition ability than their wild-type parents. Then, an experiment was set up to assess the performance of one selected Rif<sup>R</sup> mutant per each of the olive rhizobacteria under study in the olive rhizosphere at different times (7, 14 and 28 days after inoculation; DAI). Twelve nursery-produced plants (cv. Picual) were inoculated for each strain, and three plants were sampled as each time-point. Initial inocula densities ranged from  $6.5 \cdot 10^8$  ufc/mL (PIC25Rf) to  $9.6 \cdot 10^4$  ufc/mL (PIC28Rf). Results showed that all Rif<sup>R</sup> mutants were found in the olive rhizosphere at 7 DAI, although a sharp decrease in their population sizes (cfu/mL) was evident (i.e. PIC25Rf:  $1.23 \cdot 10^4$ ; PIC28Rf:  $3.47 \cdot 10^3$ ; PIC66Rf:  $9.28 \cdot 10^3$ ; PIC105Rf:  $3.23 \cdot 10^3$ ; and PIC141Rf:  $5.50 \cdot 10^2$ ). However, only Rif<sup>R</sup> mutant derivatives of strains PIC25 ( $5.33 \cdot 10^2$ ), PIC66 ( $3.98 \cdot 10^3$ ) and PICF141 ( $8.33 \cdot 10^1$ ) were detected in the root interior. At 14 DAI, mutant PIC141Rf could not be detected in the rhizosphere, in contrast to PIC25Rf ( $7.72 \cdot 10^3$ ), PIC28Rf ( $7.82 \cdot 10^3$ ), PIC66Rf ( $2.05 \cdot 10^4$ ) and PIC105Rf ( $1.75 \cdot 10^4$ ). At this time-point, only PIC25Rf ( $4.20 \cdot 10^2$ ), PIC28Rf ( $1.17 \cdot 10^1$ ) and PIC66Rf ( $3 \cdot 10^1$ ) were found colonizing the olive root endosphere. Finally, at 28 DAI mutants PIC28Rf ( $9.05 \cdot 10^3$ ) and PICF141Rf ( $1.63 \cdot 10^3$ ) remained detectable in the olive rhizosphere, and PIC28Rf ( $5 \cdot 10^1$ ) and PIC66Rf ( $6.67 \cdot 10^1$ ) in the root interior. The Box-PCR patterns of three randomly-selected Rif<sup>R</sup> colonies of each rhizobacteria were

analyzed at the end of the experiment (28 DAI) to ensure the goodness of the re-isolation and counting procedure. In conclusion, all bacterial strains under study were able to colonize olive roots although showing different performances. Fluctuations in the population sizes were also observed along the experiment, as well as an overall and rapid decrease over time. At the end of the experiment only three bacterial strains remained detectable in the olive rhizosphere, strain PIC28 showing better consistency in colonization and endurance abilities, even endophytically. These results provide valuable information on the colonization ability and persistence of these BCAs upon their release in the olive rhizosphere and under non-gnotobiotic conditions. This knowledge will be useful for the future design of effective bioformulations to control VWO.

**Keywords:** *Biocontrol; Olea europaea; Rhizobacteria; Root colonization; Verticillium dahliae*

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**17-P**

**An ecological explanation for *Tuber* spp-host symbiosis.**

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**ABSTRACT**

Some authors indicate that increase in size and tissue nutrient concentration improves seedling survival in Mediterranean forest plantations. In Mediterranean areas, soils have a low nutrient pool. Mediterranean climates are not dry the entire year but usually have two seasons of contrasting water availability. Mycorrhizal is a good way to obtain water and nutrients for soil. We propose a symbiotic model for seedling survival in Mediterranean-climate plantations for improve competitive options of forest seedling. The model considers the association truffles-host to explain seedling survival, growth and nutrient absorption. The basis of the model is the formation of active carbonate by root eating that maintains high levels of  $\text{Ca}^{2+}$  in the soil solution, regulates soil pH and modifies the exchangeable cations complex. This process is produced by *Tuber* spp. mycelium and could help to host in front of another competitors, like herbs. The model considers that survival of forests seedlings in Mediterranean climates is linked to high growth capacity during the wet season. High growth is achieved when seedlings can invert large amount of resources to support new root and shoot growth. This high productivity is important for *Tuber* spp, and it could permit a bigger growth of mycelium and a better nutrients absorption. We discuss its implications and the factors that may explain this process.



18-P

**Maximizing soil ecosystem services for ecological restoration: plant root traits influence runoff and erosion processes**

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**ABSTRACT**

The ecological restoration of degraded and constructed environments has usually aimed at reducing onsite (e.g. decline of soil quality) and off-site effects (e.g. contamination of water downstream) and to avoid irreversible situations where ecological succession is arrested. However, with the recent rise of ecosystem service theory and the attempts to quantify these services, there has been a change in the paradigm of restoration ecology in recent years. Restoration ecology can now benefit from the ecosystem services framework and aim to incorporate the maximization of these ecosystem services into projects.

Here, we aimed to study the ecosystem services related to soil processes provided by 12 different species regularly used for restoration in the south of France. We studied the soil characteristics and root traits of these 12 species. Each species was grown from seed in a monoculture in 72 inclined steel boxes (0.7 x 0.7 x 0.3 m) and every monoculture was replicated six times with additional six control (bare soil) boxes. Three replicate boxes were used for soil and root sampling and three were equipped with rhizotrons (PVC windows to observe root growth). Every 4 months, root physical properties were assessed and aggregate stability tests were performed. Additionally, rainfall simulations were performed at two vegetation stages (seedling and mature) and before and after cutting the aerial biomass (to disentangle the effects of above- and belowground biomass and to relate results to root traits). Runoff and sediments were also collected after each natural rainfall event.

Results showed that species from the *Fabaceae* family were more efficient at controlling erosion and increasing infiltration rates compared to members of the *Poaceae* family. However, no significant effect of species or family was found regarding soil aggregate stability during the study period. Our results suggest that erosion and infiltration processes are influenced by plant biomass and traits in the short term, whilst the development of soil aggregate stability is a longer-term process. Therefore, we suggest that herbaceous species of the *Fabaceae* family have a major potential for rapidly improving soil structure on road embankments compared to *Poaceae* family. A longer-term experiment is now being performed to study the effects of vegetation on soil aggregate stability.

*Key words: Infiltration, erosion, sediment yield, root properties, soil aggregates.*

**Acknowledgments:** This research was possible thanks to the TALVEG2 project financed by the FEDER (European Fund for Economic and Regional Development) and the Région Occitanie.



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19-P

**Assessment of methodologies for the quantification of Glomalin Related Soil Proteins (GRSP): Identification of interferences and methodological improvements**

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**ABSTRACT**

The formation and stabilization of soil aggregates is a key factor in the functioning of soils (Bronick et al., 2005). High intensity precipitation events on dry forest soils (which are predicted to increase with the climate change) can cause aggregate breakdown due to entrapped air compression, and as a consequence, an increased runoff, waterlogging and soil erosion (Le Bissonnais et al., 1997). Therefore, new forest soil management strategies are needed to avoid soil erosion, and the subsequent loss of ecosystem services provided by forest soils, as well as to improve degraded soils by increasing aggregate stability. Arbuscular mycorrhizal fungi (AMF) have been considered important biotic agents of the rhizosphere affecting aggregate stability (Jastrow et al., 1998). Physical stability has been found to be strongly correlated with a fraction of organic matter reputed to be a thermostable glycoprotein released by AMF, called glomalin. However, in the last few years, the role of this compound has become controversial due to two main issues: the soil-extraction and quantification methodologies, and the exact role of these proteins on the stability of aggregates (Lehmann et al., 2015). However, Glomalin Related Soil Proteins (GRSP) as an operationally defined fraction of soil organic matter is still a good biological indicator (Jorge-Araujo et al., 2015). The measurement of GRSP could be used as a proxy to study the biotic effects on aggregate formation and stabilization and to compare different soils and management strategies. The aim of this study was to investigate the source of interferences in the Bradford quantification of GRSP and propose several methodological improvements based on identified interferences. The easily extractable and total GRSP in five contrasted top-soils were compared using different methodologies (e.g. standard addition, controlled dilution, color correction). Results showed that: 1) the extent of interference varied between soils, 2) the direct assay underestimated GRSP content, while the standard addition method overestimated, 3) controlled sample dilution with color correction may give the best estimate of soil protein. Therefore, estimation of protein content from the dilution curve of the soil extract appeared to be the most suitable method.

*Keywords: Soil structure, GRSP, soil protein quantification, humic substances.*

**Acknowledgments:** This work was funded with a Short-Term Scientific Missions (STSMs) from the COST ACTION Biolink (FP1305). Main results were communicated in the SOM symposium 2017 and submitted in an article entitled "Towards meaningful quantification of Glomalin Related Soil Protein (GRSP)" in the EJSS. Soil samples were obtained from two European research projects: CHARFOR (RTA2012-00048-00-00) and FIXSOIL (Project ID: 626666 Funded under FP7-PEOPLE). Special thanks to Nahia Gartzia-Bengoetxea, Ander Arias-González and Rob. Griffiths for initial discussions and access to sites and to Josiane Abadie for her help in the laboratory.

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20-P

## **High-throughput sequencing shows higher spatial variation in soil fungal diversity and community structure of planted forest stands as compared to natural forests**

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### **ABSTRACT**

The rapid advances in molecular techniques provide unprecedented opportunities for understanding soil biodiversity. During the last decades studies on belowground microbial communities and their diversity increased substantially and recent data revealed their important functional role. The combination of DNA-based identification methods and high-throughput sequencing has proven to be a powerful tool to study and monitor biodiversity in environmental samples. Different amplicon targets were used for sequencing-based studies of fungal communities revealing high cryptic diversity in environmental samples (Lindahl et al. 2013).

In the present study soil fungal communities of two forest ecosystems – planted Douglas fir stand and European beech forest were compared using ITS1 metabarcoding. Soil samples were collected from four permanent sampling plots in two mountain regions (the Balkan and Rhodopi Mountains) in Bulgaria. Fungal diversity and community structure are analysed at different taxonomic levels. Basidiomycota and Ascomycota were the prevalent phyla accounted for 57 (43-71)% and 22 (9-37)% of all OTUs respectively, followed by Mortierellomycota 12 (4-20)%. Fungal communities of both regions differ considerably with complexes of the Douglas fir stands having greater genetic and taxa composition variability as compared to the natural beech forests. The genetic richness (OTUs numbers) and diversity (Shannon Indices) were high (232-373 and 3.3-4.5) with *Russula*, *Mortierella*, *Solicoccozyma*, *Inocybe*, *Xerocomellus* being the most common and abundant genera. This study is the first attempt to characterise soil fungal microbiome diversity of forest ecosystems using high-throughput DNA sequencing in the country.

**Keywords:** *biodiversity, Fagus sylvatica, metabarcoding, Pseudotsuga menziesii*

**Acknowledgments:** The authors are grateful to BioLink COST FP1305 project for supporting this presentation.

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21-P

***In-situ* belowground decomposition dynamics**

Dynamics of decomposing fine roots of European beech  
(*Fagus sylvatica*) and Norway spruce (*Picea abies*)

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**ABSTRACT**

Despite known contributions of decomposing absorptive roots to soil carbon pools and nutrient cycles, a paucity of data addresses *in situ* root decomposition dynamics. The paradigm of belowground decomposition dynamics has been established with techniques which artificially alter the decomposition media and soil environment. Current findings indicate that higher-order, older, fine root sections with secondary development decompose at a faster rate than lower-order, younger absorptive fine roots. These fine roots represent a stage of transition from primary to secondary development and commonly have mycorrhizal associations which may alter their anatomy. The traditional approaches to quantify root decomposition, e.g. litter bags or intact soil cores, can significantly influence early mass loss, nutrient, and carbon mobilization rates, which can range between 20-40% within the first six months of decay.

We are currently investigating the relative rate of mass loss, changes in root anatomy, root nutrient dynamics, as well as the *in situ* decomposer community of dying fine root tips (1 cm long, < 0.5 mm in diameter) of decapitated, intact whole root systems from three-year-old tree seedlings of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*).

We present preliminary mass loss of dying, absorptive fine root tips, as well as initial changes in root anatomy based on cross sectioning during the first 12 months of the project. In contrast to the previous decomposition paradigm, dying fine root tips, harvested from intact, decapitated root systems did not show any significant decrease in mass loss during the first 12 months for European beech.

Fine root tips of Norway spruce lost 40% of their average initial mass after 12 months, which contradicts the previously reported 6 month decay period. Our preliminary findings reveal that decomposition rates are delayed in undisturbed, *in-situ* dying and decomposing fine roots, which differs from the previously reported trends in carbon and nutrient loss using traditional decomposition approaches. Therefore, we need to rethink future research on belowground dynamics.

**Keywords:** *in-situ* root decomposition, initial mass loss, *Picea abies*, *Fagus sylvatica*, root anatomy

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22-P

## **Influence of site and season on spruce ectomycorrhizal fungal diversity**

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### **ABSTRACT**

Norway spruce (*Picea abies* L.) is a regionally important timber species for wood industry but is also a significant host for various ectomycorrhizal fungi including edible species. This species reaches its southernmost limit in the mountainous regions of south Serbia. The study focused on the diversity of ectomycorrhizal fungi on Norway spruce natural sites and the potential effects of two contrasting seasons (spring and autumn) and three different sites on spruce ectomycorrhizal communities. Three national parks or parks of nature, namely Nature Park Stara Planina, Kopaonik National Park and National Park Tara were selected where spruce reaches its southern-most distribution areal. Using standardised sampling in combination with anatomical and molecular identification of ectomycorrhiza we recorded 29 different types of ectomycorrhiza. The number of unique types of ectomycorrhiza was high, 6 ectomycorrhizal types were characteristic for Stara planina, 9 for Tara and 10 for Kopaonik sites. *Cenococcum* sp. was found on all three studied sites, while *Tomentella* sp. 2 was common for sites Stara planina and Kopaonik, *Amphinema byssoides* was found on Kopaonik and Tara and *Clavulina cristata* was observed on Stara planina and Tara sites. At studied sites Stara planina, Kopaonik and Tara only 5, 4 or 2 types of ectomycorrhizae were recorded in both analysed seasons, respectively. Number of ectomycorrhizal types and value of the Species richness index differed between Stara planina and Tara in autumn. However, influence of site and season on studied diversity indices (number of ectomycorrhizal types, Species richness index, Shannon Wiever index, Evenness, Equitability and Berger-Parker evenness index) was insignificant.

**Keywords:** *ectomycorrhiza, Picea abies* L., *community structure, Stara planina, Kopaonik, Tara*

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23-P

**The influence of soil on anatomical features of Bald cypress  
(*Taxodium distichum* (L.) Rich.) at different localities in Serbia**

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**ABSTRACT**

The aim of this research was to determine differences in secondary anatomical structure based on anatomical features of Bald cypress (*Taxodium distichum* (L.) Rich.) xylem and to determine the influence of soil on it. The Bald cypress trees were selected in the protected natural area Veliko ratno ostrvo (The Great War Island) and near Bačka Palanka. Veliko ratno ostrvo is the wetland located in the city of Belgrade that has been created as an alluvial accumulative and sedimentary formation, due to stopping of sandy sediments at the confluence of the Danube and Sava rivers. Bačka Palanka is a locality where the only seed stand of Bald cypress in Serbia is situated. In order to determine the texture composition and chemical properties of the soil, the samples from four pedological profiles were obtained (3 pedological profiles from Veliko ratno ostrvo and 1 from Bačka Palanka). Based on the study of the granulometric composition and chemical properties of the soil, it was found that at both sites the soil belongs to a group of hydromorphic soils - type fluvisol. There was determined that growth rings were on average wider by trunks from Veliko ratno ostrvo than by the others from Bačka Palanka. It could be related to content of available water in the soil. As for proportion of late wood zone inside the growth rings, it was more expressed by narrower (Bačka Palanka) than by wider growth rings (Veliko ratno ostrvo). The width of the growth rings, determined by detailed macroscopic and microscopic analysis of stems from both localities, indicates a faster radial increment in the stems from Veliko ratno ostrvo, whose growth rings are wider, and the transition between early- and latewood zone is gradual, unlike the stems from Bačka Palanka, with narrower growth rings and sharper transition zone. More dynamic growth of stems from Veliko ratno ostrvo is mainly related to the physical and chemical soil features. Soil textural classes that appear at this locality are sandy loam and loam and are characterized by their high retention capacity, and thus high content of water affordable to the plants. On the other side, in Bačka Palanka, the textural soil classes are loamy sand and sand, which contain significantly less water available for the plants, which is reflected in the growth rate. It should be emphasized that the soil at the locality Veliko ratno ostrvo is significantly higher in fertility compared to Bačka Palanka, as can be seen from the values of the components that determine this feature (content of dust, clay, humus, nitrogen, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O).

*Keywords: Bald cypress, soil, anatomical features, growth rings, radial increment*

**Acknowledgments:** The research was financed by the project: „Establishment of forest plantations to increase the afforested areas in Serbia” (TR 31041), Ministry of education and science of the Republic of Serbia.

24-P

## **Assessment of the microbial community in poplar rhizosphere of a heavy metal contaminated soil**

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### **ABSTRACT**

A plant-assisted bioremediation strategy have been applied in a heavy metal contaminated soil using a specific poplar clone (Monviso).

In this work, the results obtained at 900 days from the poplar-assisted bioremediation of a historically heavy metal (HM) contaminated area in Southern Italy are reported. The Monviso clone was selected for its tested capability to promote organic contaminant rhizodegradation and heavy metal phytoremediation in previous field (Gamalero et al., 2012; Ancona et al., 2017b) and laboratory studies

Chemical (heavy metals and organic carbon) and microbiological (microbial abundance, viability, structure and dehydrogenase activity) analyses were performed on soil samples collected at 900 days both in the rhizosphere and in soil samples collected at 1 m distance from the trunk and at 20-40 cm depth. A significant decrease in most metals was observed where the poplar trees were present. Results obtained revealed that:

- an overall increase in microbial abundance, cell viability and dehydrogenase activity and differences in microbial structure, particularly evident between rhizosphere and at 1 m distant from the trunk, were observed.
- soil concentration of HMs (V, Cr, Sn, Pb), strongly decreased and in most cases was below the Italian national law limit (D.Lgs 152/06). The calculation of HM bioaccumulation factor (BAF, considering roots and leaves) and translocation factor (TF) suggests that poplars were able to phytostabilise HMs.
- organic carbon increased compared to the previous sampling in all planted plots; the highest values were observed in rhizosphere soils of each target trees.

Overall results show that the poplar based phytoremediation strategy was able to promote the phytostabilization of the heavy metals and to improve the soil quality.

Further investigations will be carried to analyse the microbial composition in more detail in order to identify the bacterial populations involved in different ways in HM bioremediation.

*Keywords: phytoremediation, plant-microbial interactions, microbial community structure, heavy metals phytostabilization, soil quality*

**Acknowledgments:** Authors thank Claudia Campanale and Barbara Casale for their help in performing soil chemical analyses.

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25-P

## **Does above- and belowground diversity reflect forest management history in mountain spruce forests?**

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### **ABSTRACT**

The high diversity is supposed to improve ecosystem functioning and resilience and to be negatively influenced by management practices. In order to test the hypothesis under local conditions we established permanent plots (circle 500 m<sup>2</sup>) in three types of mountain spruce (*Picea abies*) forests in the Giant Mts. (Czech Republic): (i) high elevated unmanaged forest, (ii) high elevated managed forest and (iii) low elevated managed forest on sites originally occupied by beech, in six replicates per each. We measured soil properties, tree characters, vegetation, natural regeneration, root density, ectomycorrhizal (ECM) mycelium using mesh bags and fungal diversity by three approaches: Illumina sequencing of soil fungi, ectomycorrhizae morphotyping and fruit body monitoring.

Surprisingly a management history was most pronouncely written in soil. Forest types differed in thickness of horizons, pH and C:N. On the other hand we found no difference in most quantitative parameters (amount of ECM mycelium, number of ECM species, number of subordinate ECM species) among forest types.

ECM fruit bodies, saprotrophic fungi in soil and tree regeneration were mainly influenced by altitude, which indicates that suitable abiotic factors (temperature, precipitation) were in this case more important than disturbance caused by management practices. However, the highest numbers of indicator fungal species of natural habitats, assessed by field mycologists, were present in unmanaged forests. Concerning herbaceous species dominants, *Calamagrostis villosa* was more abundant than *Vaccinium myrtillus* in high elevated unmanaged forests while in high elevated managed forests it was vice-versa.

Spruce crown characters, ECM communities on spruce roots and in soil were shaped mainly by position of plots in west-east direction, which corresponds to actual forest damages due to acidification.

*Keywords: mountain spruce forests, ectomycorrhizal fungi, saprothrophs, bellowground diversity, forest management*

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26-P

**Characterization of *Pseudomonas fluorescens* PICF7 mutants and assessment of their altered phenotypes on endophytism, rhizosphere survival and biocontrol of Verticillium wilt of olive**

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**ABSTRACT**

Cultivated olive (*Olea europaea* L. subsp. *europaea* var. *europaea*) is widely distributed in the Mediterranean Basin. It constitutes one of the main economic engines for the agricultural sector of the region. Moreover, the crop is expanding to other Mediterranean-type climatic areas worldwide. One of the major biotic constraints for olive cultivation is Verticillium wilt (VWO), caused by the soil-borne fungus *Verticillium dahliae* Kleb. a disease very difficult to control. Therefore, an integrated management strategy is recommended (López-Escudero and Mercado-Blanco et al., 2011). Within this framework, the use of beneficial microorganisms (i.e. biocontrol approaches) represent an excellent option either to be used alone or in combination with other disease control tools. *Pseudomonas fluorescens* PICF7 is a natural colonizer of the olive rhizosphere, able to endophytically colonize root tissues, and an effective biocontrol agent against VWO (Maldonado-González et al., 2015). However, most of the genetic mechanisms concerning these processes remain to be elucidated (Gómez-Lama et al., 2017). Thus, the identification and characterization of genes involved in phenotypes such as rhizosphere/soil persistence (e.g. copper resistance, 1-aminocyclopropane-1-carboxylate [ACC; the immediate precursor of ethylene] deaminase activity), root colonization (e.g. biofilm formation), and plant growth promotion (e.g. phytase activity) are aims pursued in this study. The sequencing, gene annotation and *in silico* analysis of strain PICF7's genome previously allowed the identification of a putative ACC deaminase-coding gene (Martínez-García et al., 2015). However, ACC deaminase activity has not been confirmed in our study. Instead, the presence of a closely-related gene, coding for a putative D-cysteine desulfhydrase, was found. ACC deaminase activity can thus be discarded as involved in the phenotypes here under investigation. Some 5500 tetracycline-resistant colonies from an available Tn5 random insertion mutant bank (Maldonado-González et al., 2015) were screened to seek for mutants affected in one of the traits mentioned above. A collection of 78 mutants were eventually selected, including 34 showing either no (or reduced) growth or colony color change in medium supplemented with Cu<sup>2+</sup>, 6 impaired in biofilm formation, 18 unable to grow (or with altered morphology) in YEM (Yeast Extract Mannitol) medium, and 20 displaying reduced or absence of phytase activity. The identification of the disrupted genes (i.e. the insertion site of Tn5) was performed for 42 of these mutants using a nested-PCR approach and DNA sequence analysis. Results so far showed that: i) disruption of putative genes *CopZ* and *CusR* reduced or impaired growth of PICF7, respectively, in media supplemented with Cu<sup>2+</sup>; ii) insertions affecting genes coding for a ppGpp synthetase and flagellar biosynthesis protein were defective in biofilm formation; and iii) the disruption of a gene coding for a histidinol-phosphate aminotransferase abolished phytase activity. These mutants will be used in *in planta* experiments to evaluate their olive rhizosphere/roots colonization ability and VWO biocontrol effectiveness.





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**Keywords:** *Biocontrol agent, Mutant analysis, Olea europaea, Root colonization, Verticillium dahliae*

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27-P

### **Analysis of rhizobial endosymbionts of legumes species used to maintain mountain firewalls in Sierra Nevada National Park (South Spain)**

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#### **ABSTRACT**

Forest fires are the cause of the disappearance of numerous natural ecosystems. It is especially important when they happen in areas of special protection or affect endemic or threatened species. This is the case of wildfires in the Sierra Nevada National Park (Southeast Spain). Firewall areas were created to try to limit the devastating effects of wildfires in forests. The maintenance of these firewalls areas can be carried out mechanically or by using traditional extensive livestock by grazing sheep and goats without mechanical intervention [1, 2].

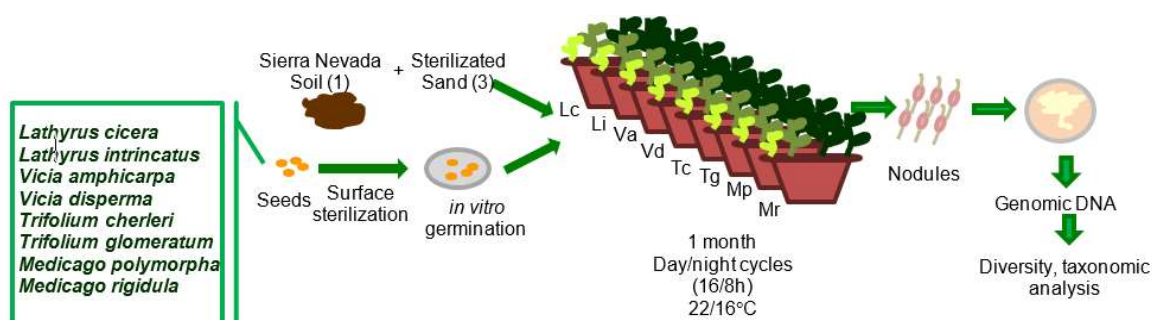
In order to make these firewalls areas rich in attractive pastures for livestock, we have isolated and studied various rhizobia obtained from nodules with several leguminous plants, which have a high protein content and low dependence on N fertilizers [3], from the genera *Vicia*, *Lathyrus*, *Trifolium* and *Medicago* in a firewall zone established in Lanjarón (Granada) close to the Sierra Nevada National Park (Spain).

The results showed strains isolated from nodules of *Vicia*, *Lathyrus* and *Trifolium* with a high genetic diversity that had 3, 16, 14 and 13 different types of *rrs*, *recA*, *atpD* and *glnII* genes, respectively, were phylogenetically close to the species from the *Rhizobium leguminosarum* group, although they were not identified as any of them. The isolated strains belonged to the symbiovars *viciae* and *trifolii* but high phylogenetic diversity was found within both symbiovars, since there were 16 and 14 *nodC* gene types, respectively. Some of these strains formed *atpD*, *recA*, *glnII* and *nodC* clusters and lineages only found to date in Sierra Nevada National Park [4].

However, strains isolated from *Medicago* nodules showed less genetic diversity with only 2 *rrs* genes slightly different in sequence and 3 *recA* genes. The phylogenetic diversity was also lower, with only 8 types of *nodC* genes, 3 of them exclusive from *Medicago polymorpha* isolates and 1 from *Medicago rigidula*. In any case all the isolated strains from *Medicago* plants were phylogenetically close to *Sinorhizobium meliloti* group.

#### **MATERIAL AND METHODS**

Isolation of nodulating strains of 8 species of legumes from Sierra Nevada firewalls [5]



The *rrs*, *atpD*, *recA*, *glnII* and *nodD* genes were amplified and sequenced according to [7, 8, 9, 10]. The bands corresponding to the different genes were purified and sequenced. The sequences obtained were compared to those held in GenBank, aligned and inferred phylogenetic trees with the neighbour-joining method [11] and MEGA5 software [12]. Confidence values for nodes in the trees were generated by bootstrap analysis using 1000 permutations of the data sets [4].

## RESULTS AND DISCUSSION

A total of 43 strains were isolated from *V. amphicarpa*, 38 from *V. disperma*, 36 from *L. intricatus*, 40 from *L. cicera*, 42 from *T. cherleri*, 6 from *T. glomeratum*, 43 from *M. rigidula* and 40 strains from *M. polymorpha*. They were all able to renodulate the plant from which they were isolated and formed effective nodules. The genetic diversity of the isolated strains was analysed by RAPD fingerprinting. The results of the RAPD analysis showed a high diversity of the isolated strains and the existence of groups or branches with a similarity lower than 70%. A representative strain of each different branch and hosts plant was selected for core and symbiotic gene analyses.

The *rrs* (16S rRNA) gene is the current basis of rhizobial classification within the family *Rhizobiaceae*, new genera have recently been described and several others have been recovered following the analysis of this gene. All strains isolated in this study from *Vicia*, *Lathyrus* and *Trifolium* had closely related *rrs* genes and belonged to the phylogenetic group of *R. leguminosarum*. Most strains were *rrs* type 1, the *rrs* gene of the strains *rrs* type 2 differed by 1 nucleotide to those with *rrs* type 1, and the strains with *rrs* type 3 differed by 3 nucleotides with respect to strains that had types 1 and 2. Several recently described species within the genus *Rhizobium* have identical *rrs* genes, the strains showing *rrs* types 2 and 3 could belong to undescribed species within this genus. The high similarity of this gene in the species of the *R. leguminosarum* phylogenetic group makes the analysis of other genes necessary for the differentiation of these species. The analysis of *recA*, *atpD* and *glnII* genes showed that they presented 16, 14 and 13 different types of these genes. These analyses confirmed that all these strains were related to the species of the *R. leguminosarum* phylogenetic group but they could not be assigned to any of these species because the distances of the *recA*, *atpD* and *glnII* genes were similar or higher than those found between different species, which had similarity values for these genes lower than 97%.

All strains isolated from *Medicago* had closely related *rrs* genes and belonged to the phylogenetic group of *Sinorhizobium meliloti*, only 2 strains from *M. polymorpha* differed by 1 nucleotide of *rrs* gene from all the others *Medicago* strains. These same strains differed in 7 nucleotides also with *recA* gen of the majority of strains isolated from *Medicago*, but here appeared other *recA* sequence in 1 strain which is intermedia between both groups, differed only 4 nucleotides from the major group and 3 from the small group of 2 strains. All the strains isolated from *Medicago* plants had the same *atpD* sequence. Regarding the sequences of the *nodD* gene, two very different sequences clearly appear and then there are some small variations within these majority sequences.

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**Keywords:** *Firewall, Rhizobium, Sinorhizobium, Lathyrus, Vicia, Trifolium, Medicago, Identification, Phylogeny*

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28-P

**Fine root production in hemi-boreal coniferous forests: comparison of ingrowth core and net method**

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**ABSTRACT**

Fine roots (diameter  $\leq 2$  mm) play a key role in the global carbon cycle because the majority of carbon accumulation in the soil is inextricably linked to fine root production and turnover. In present study fine root production has been measured by two methods- ingrowth core and ingrowth nets in contrasting forest stands in Estonia. We aimed to estimate the effect of research method on the assessment of fine root production and turnover.

The ingrowth core and nets have been installed in 2015 in 8 Scots pine (*Cladonia*, *Vaccinium vitis-idaea*, *Myrtillus*, *Fragaria*) and Norway spruce (*Polytrichum*, *Myrtillus*, *Oxalis*, *Calamagrostis alvar*) stands with varying soil moisture and fertility conditions. Ingrowth cores and nets were extracted annually, in 2016 and 2017. Fine roots from samples taken by both methods were washed free of soil and separated

into living and dead roots. Roots were divided into three categories: fine living root of trees, roots of ground vegetation, dead roots. Root samples were dried at 65 °C for 48 hours and weighed for biomass assessment.

The effect of root research method on annual estimate of fine root production for trees as well for understory plants will be discussed.

*Keywords: ingrowth core, ingrowth net, fine root production, fine root turnover, hemi-boreal forest*



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## **Changes of specific physico-chemical and biological properties of soil due to different tree species composition**

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### **ABSTRACT**

The main objective of our research was to evaluate the effect of different tree species on some physico-chemical and biological properties of soil at the locality Vrchdobroč (Central Slovakia). The study was focused on two coniferous trees (*Picea abies* and *Pseudotsuga menziesii*) and two deciduous species (*Acer pseudoplatanus* and *Fagus sylvatica*). Soil samples were taken in three pure stands of each tree species from the O- and A-horizons in five replications. In soil samples, basic physico-chemical (soil moisture, soil acidity, C and N content, base cation contents) as well as microbial (microbial biomass, basal respiration, substrate-induced respiration, N-mineralisation, catalase activity, functional groups of soil microorganisms) characteristics were determined. We observed a significant effect of different tree species on the most soil properties. Interestingly, while *Pseudotsuga m.* showed the most distinct effect in the O-horizon, the soil properties in the A-horizon were affected mainly by *Acer p.* In stands with *Pseudotsuga m.* we also observed the most distinct fall of microbial characteristics from the O to A soil horizon in comparison with other tree species. In conclusion we can claim that according to our results the effect of tree species cannot be divided simply on the effect of broadleaves and conifer trees because within each group the tree species influence soil properties in a different way.

*Keywords: physico-chemical properties, biological properties, soil, tree species*

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***Carya ovata* on old plantations outside of natural range are a rich reservoir of ectomycorrhizal fungi**

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**ABSTRACT**

Information about the ectomycorrhizal (ECM) fungal assemblages of hickory trees (*Carya* spp.) is very limited, and restricted to the pecan hickory (*C. illinoensis*) (Bonito et al. 2011), which is economically important to the USA pecan nut industry. How the mycorrhizal associations of other hickories are structured has not been determined, either from North America, or from outside their natural distribution ranges. Recently (Rudawska et al. 2018) we found that 40 ectomycorrhizal fungal taxa are accompanying roots of exotic *C. laciniosa* and *C. cordiformis* successfully acclimated to local habitat condition of historic Kórnik Arboretum in Poland.

As a next step of research we like to recognize how ECM communities are structured when the exotic *Caryas* are grown as monoculture plantation surrounded by native forests. For this purpose, an old *Carya* plantation established in the end the nineteenth century by German and Austrian foresters Schwappach, Wiedemann, and Ciešlar (Bellon et al. 1977) and existing to the present time has been analyzed. The studies were performed at three mature *C. ovata* stands situated in western Poland and located within the temperate deciduous forests dominated by hornbeam, oak, beech and pine. Ectomycorrhizal fungal community of the surrounded forests was also determined in order to evaluate the ECM fungal linkages between exotic

*C. ovata* and native trees. Here we present how are structured above- (epigeous sporocarps) and below-ground (ectomycorrhizal root tips) ECM fungal communities of *C. ovata* and neighboring native trees from one of our three stands located in the Smolarz Forest District. Ectomycorrhizal fungi were identified by combination of morphotyping and direct sequencing of the PCR-amplified internal-transcribed spacer of rDNA. By identifying sporocarps and ectomycorrhizas, we detected a total of 88 taxa of ECM fungi: 67 taxa at the *C. ovata* stand and 53 in the surrounding forest. From 57 fungal taxa identified from ectomycorrhizal root tips 22 taxa were found on *Carya ovata* and 15 taxa on the native trees around *Carya* stands. Twenty taxa were shared in common on tested hickories and on trees from native forest. The molecular analysis of sporocarps revealed 31 further ectomycorrhizal fungal taxa undetected for belowground fungal root communities and 20 taxa previously found as ectomycorrhizal root tips. Under the canopy of *C. ovata*, sporocarps of 24 ECM fungal taxa were exclusively found. Under the neighboring trees 11 taxa were exclusively noted. The 14 taxa were shared and commonly observed in analyzed communities regardless tree species.

The most abundant species in below-ground ECM fungal community of *C. ovata* were *Cenococcum geophilum*, *Craterellus cornucopioides* and *Elaphomyces* sp., in the community of neighboring forest they were *C. geophilum*, *Genea hispidula* and *Lactarius decipiens*.

Thus can be concluded the ectomycorrhizal community of exotic *C. ovata* has particular high species richness above the level of surrounding communities and therefore can provide the reservoir for ectomycorrhizal fungal taxa.

*Keywords: Hickory; ectomycorrhizal fungi; exotic trees; Juglandaceae*



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31-P

**Increased plant growth and photosynthetic pigment production of *Arabidopsis thaliana* after treatments with *Trichoderma reesei* peptaibols**

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**ABSTRACT**

Peptaibols are toxic compounds due to their ability of opening ion channels in bilayer membranes. They are short peptides usually consisting of 18-20 residues, which include unique amino acids like  $\alpha$ -aminoisobutyric acid (Aib), isovaline (Iva), and the C-terminal 1,2-amino alcohol. Peptaibols are produced by most members of the genus *Trichoderma*. Studies on their bioactivities against various fungal plant pathogens revealed that peptaibols have the potential to be included in the arsenal to control plant pathogenic fungi affecting agricultural crops and forest trees. Positive effects of *Trichoderma* strains on plants are also commonly known from the literature, and the role of peptaibols in plant growth promotion and induction of systemic resistance has been suggested.

In the present study, an *Arabidopsis thaliana* plant-based biotest was used to examine the direct effects of *Trichoderma* peptaibols on plant growth. *Trichoderma reesei* - a species commonly used in biotechnology due to its ability to produce hydrolytic enzymes degrading cellulose or hemicellulose - was selected for the experiments on plants. A certain group of peptaibols, paracelsins are produced by *T. reesei*. Known paracelsin sequences are available in the Online Peptaibiotics Database (<https://peptaibiotics-database.boku.ac.at/django/>). After the HPLC-ESI-MS investigation of the crude extracts deriving from *T. reesei* SZMC 22616 (Rut-C30) cultures, 21 paracelsin-like peptaibol compounds could be identified along with 6 previously described (paracelsin B, D and H; saturnisporin SA IV and *Trichoderma citrinoviride* sequence 4 and 5) and 3 entirely new compounds. Many of the known peptides are positional isomers of each other therefore the chances to identify new peptaibol compounds after the identification of the isomeric types of the amino-acid residues were high. The extracts were purified by preparative HPLC and plants were treated with a dilution series from 10 mg ml<sup>-1</sup> to 0.005 mg ml<sup>-1</sup> of the purified peptaibol extract. The treatment with high peptaibol concentrations proved to be deterrent for the growth of *A. thaliana* seedlings, though after a certain level of dilution, more intense growth could be observed. The quantification of photosynthetic pigments extracted from *Arabidopsis* plants treated with *T. reesei* also proved the positive effects of peptaibols on plants. Folding dynamics of paracelsin B and H examined by molecular dynamics simulations revealed a linear helical structure for both peptaibols. *Arabidopsis thaliana* was used as a model plant organism and thus further studies will be carried out to evaluate the applicability of peptaibol compounds for growth promotion of seedlings in forest nurseries.

**Keywords:** *Trichoderma*, *peptaibol*, *HPLC-ESI-MS*, *Arabidopsis*, *bio-test*



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32-P

**Ozone, phosphorus and nitrogen effects on root biomass, fine root morphology, ectomycorrhiza, C, N and P fine root concentrations in an ozone-sensitive poplar clone**

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**ABSTRACT**

A widespread increase in nitrogen deposition increases N:P ratio of organisms, decreases growth rates and induces changes in community structure, species diversity and ecosystem functions in terrestrial ecosystems (Peñuelas et al. 2013). In combination with other environmental stressors, these effects might be even more profound. In our study, effects of nutritional imbalances were investigated in conjunction with ozone stress. The study was performed on poplar, which is an ecologically and economically important deciduous broadleaf species, and above all, a model tree species. Its roots establish symbiotic relationships with ectomycorrhizal and arbuscular mycorrhizal fungi.

Cuttings of ozone sensitive poplar clone *Populus maximoviczii* Henry × *berolinensis* Dippel (Oxford poplar clone) were propagated in December 2015 and potted into small pots in February 2016. In April 2016, they were replanted into 10 L pots using the peat:sand:local soil mixture in 1:1:1 ratio. Potting substrate was amended with three levels of P (0, 40 and 80 kg ha<sup>-1</sup>) in combination with two levels of N (0 and 80 kg ha<sup>-1</sup>) distributed in several applications over the growing season. Potted cuttings were exposed to three levels of ozone (ambient, 1.5 x ambient and 2.0 x ambient) at a Free-Air O<sub>3</sub> exposure facility in Sesto Fiorentino, Italy, from May 2016 till October 2016. After harvest, root systems were analysed for ectomycorrhizal fungi by combination of morpho-anatomical (Agerer 1991, Agerer & Rambold 2004-2017) and molecular (Grebenc & Kraigher 2007) methods. Fine roots ( $\varnothing < 2$  mm) were scanned and analysed with WinRhizo to obtain fine root morphological parameters. Dry weight of fine and coarse roots was recorded. C and N concentrations in fine roots were measured by dry combustion in CN analyser and P concentrations were determined by ICP-OES after microwave pressure digestion.

Negative effects of elevated ozone were observed on fine and coarse root biomass, fine root N:P ratio, percentage of fine root system in 0.1-0.2 mm diameter class and ectomycorrhizal species richness, but positive effects occurred on phosphorus concentration in fine roots and percentage of root system in 0.0-0.1 mm diameter class.

Increasing phosphorus amendment had a negative effect on carbon concentration in fine roots, N:P ratio in fine roots and percentage of ectomycorrhizal root tips, and positive effects on phosphorus concentration in fine roots.

Nitrogen addition had negative effects on phosphorus concentration in fine roots and percentage of fine root system in 0.0-0.1 mm diameter class, while positive effects on N:P ratio in fine roots, root surface area per soil volume and branching density were observed.

Interactive effect of ozone and nitrogen was observed on fine and coarse root biomass: biomass increments due to nitrogen amendment were lower at elevated ozone. Elevated

ozone in combination with nitrogen resulted in lower phosphorus content in fine roots compared to control ozone levels. At ambient ozone levels, nitrogen addition resulted in significantly higher root tip density and root length density, while at elevated ozone, nitrogen addition had no effects on these two parameters. Phosphorus addition at ambient ozone level resulted in higher increase in fine root phosphorus concentrations compared to elevated ozone. Altogether, 6 ectomycorrhizal taxa were found on roots of poplar cuttings: *Tomentella ellisii*, *Geopora* sp., *Sphaerosporella brunnea*, *Inocybe* sp., *Peziza ostracoderma* and *Trichophaea woolhopeia*. *Sphaerosporella brunnea* had higher abundance at elevated ozone levels, while for nutrients no consistent pattern was observed.

Our study has shown important belowground impacts of single investigated factors on many poplar root characteristics, while interactive effects of investigated factors were less frequent and less prominent.

*Keywords: nitrogen-phosphorus imbalance, interactive stress effects, Populus sp., N:P ratio*

**Acknowledgments:** The authors acknowledge the financial support from Slovenian Research Agency (research core funding No. P4-0107), LIFE GENMON project (LIFE ENV/SI/000148), MOTTLES project (LIFE15 ENV/IT/000183), TROZGRODIV project (CNR-RA bilateral agreement 2017-2019) and DESFOR PN 1633/01-02 project.

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33-P

**Influence of the land history on oak mycorrhiza in a temperate forest**

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It is well known that majority of terrestrial plant species interact with mycorrhizal symbionts. The selection of specific fungal symbionts by the roots depends on the properties of the surrounding soil. The main aim of this experiment was to study the effect of the land history (soil from the stands of different tree species) on the fungal communities in (1) roots and (2) soil compartments of oak seedlings. Soil was collected from stands of oak, beech and spruce in a temperate forest in the Czech Republic. The pot experiment was set by planting oak seeds in pots containing each type of soil. Pots without trees were used as controls. Once the oak seedlings have established (4 months), roots, rhizosphere soil and bulk soil were collected. Following DNA extraction, ITS2 region of the fungal rRNA gene was amplified and sequenced on Illumina MiSeq. Ecology was assigned to fungal taxa using FUNGuild ([www.funguild.org](http://www.funguild.org)) to one of the four categories/guilds: ectomycorrhiza, saprotrophs, pathogens and endophytes. The results have shown that there is no clear separation of fungal communities in the soil compartments (rhizosphere and bulk soil) and controls. However, the fungal communities in roots differ from the soil compartments in all cases. Moreover, as revealed by statistical analysis (PERMANOVA), there seems to be significant influence of land history on symbiotic fungi in roots, but not on saprotrophs and endophytes. These results show a complex feedback between the plant and the surrounding soil, where symbiotic ectomycorrhizal fungi are clearly an important component.

*Keywords: oak seedlings, fungal communities, ectomycorrhiza*

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34-P

## **Tree species alters soil faunal community effects on leaf litter decomposition**

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### **ABSTRACT**

Leaf litter decomposition is one of the key processes of the recycling of C and nutrients in terrestrial ecosystems (Fujii et al. 2018). Although climatic, litter quality (Hättenschwiler et al. 2005), and microbial organism controls are reasonably well understood, soil fauna effects on leaf litter decomposition process remains elusive. Here, we assessed how tree species alter soil fauna community and thereby influence leaf litter decomposition, and whether shredders (isopods and diplopodas) control the main decomposition process in a 44-year-old common garden experiment in Denmark.

We studied the soil faunal community apart from earthworms in forest floor under five common European tree species in November 2017. Earthworms were previously studied (Schelfhout et al. 2017), and we hypothesized similar pronounced tree species effects on other functional soil faunal groups. Two study sites were former agricultural land while the remaining sites were old forest sites. Each site was planted with 0.25 ha adjacent unreplicated monoculture stands of common ash (*Fraxinus excelsior* L.), European beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur*), small-leaved lime (*Tilia cordata* Mill.), sycamore maple (*Acer pseudoplatanus* L.) (Vesterdal et al. 2008), except for Kragelund, where the ash stand was missing. In each stand, we used a wooden frame with dimensions of 25 cm × 25 cm randomly selected five representative plots (the total number of plots were 70) with a minimum distance of 10 m from the stand border. The results showed that tree species affected the soil fauna community, and that the effect differed among the sites. Data analysis is currently ongoing, and the poster will present the very first results on different functional soil faunal groups.

*Keywords: isopoda, diplopoda, common garden design, forest floor, European tree species*

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## **Investigating the population structure and diversity of *Amanita caesarea* (Scop.)**

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### **ABSTRACT**

The Mediterranean basin is one of the European hot spots for biodiversity. In particular, Mediterranean forests contain numerous endemic tree species which support one of the highest concentration of ectomycorrhizal (ECM) species worldwide (Tedersoo et al. 2012).

In the current context of climate change and strong anthropic pressure on forests, it is of crucial relevance to understand the ecology and the biology of the organisms which realize essential functions for trees. The study of emblematic model species, like *Amanita caesarea* (Scop.) commonly known as the Caesar's mushrooms, provides promising insights into this direction, by allowing the integration of ECM belowground fungi as a component of the biotic niche of trees (Taudière et al. 2015).

The principal objective of the study is to understand the reproductive regime and explore the longevity and growth of *A. caesarea*, in order to better understand the sustainability of the links between the fungus and his host.

To reach this goal, it is studied the population structure (genealogy among individuals) and the dynamics (temporal persistence of individuals in places) of *A. caesarea* from sporocarps accurately mapped, and collected in 14 different provenances in France during eleven years, representing an exceptionally large sampling (Richard et al. 2010). This collection and the investigation of the genetic basis of the monitored populations is part of the OSU-OREME observatory of Mediterranean Biodiversity devoted to the understanding of the response of fungi to environmental changes (OREME).

Insight the large dataset of individuals, I have studied the population structure (genealogy among individuals) and the dynamics (temporal persistence of individuals in places) of *A. caesarea* from the sporocarps collected in 3 different provenances in France (in each site, sporocarps were collected from several sub-sites):

- 7 different sub-sites in the Haute-Corrèze area (Limousin region).
- 40 different sub-sites in the Basse-Corrèze area (Limousin region).
- 4 different sub-sites at the Lot department.

During four consequently years (from 2013 to 2016) a total 341 individuals were collected and analyzed in order to better understand the sustainability of the links between the ectomycorrhizal fungus and its host.

### **MATERIAL AND METHODS**

Simple sequence repeat genotyping (SSR) was performed using pairs corresponding to 13 polymorphic SSR markers (V. Salomon 2011).

The resulting DNA fragments were separated in an ABI PRISM R 3100 Genetic Analyzer using 500 LIZ (Life Technology, Carlsbad, USA) as size marker.

Multilocus genotype identification was performed with the microsatellite data using the software analysis GeneMapperR Software (Applied Biosystems, Foster City, CA USA). Allele patterns were described according with the previous studies that have been done by the same group in *A. caesarea* (from V. Salomon 2011) and by the new observations that have been done during the exchange period.

## RESULTS

From the 341 samples of sporocarps of *A. caesarea*, the percentage of success was really high: 93 % of the total number of samples with more than 50 % of the locus identified (six or more loci from 11), 67 % with all the locus identified and just 1.76 % of the samples to discard because the amplification failed for all loci.

*Keywords:* *A. caesarea*, *SSR markers*, *population structure*, *population dynamics*, *ECM*

*Acknowledgments:* This research was supported by an STSM grant from the COST Action FP1305: Linking belowground Biodiversity and ecosystem function in European forests (BioLink)

## References

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36-P

**Can enzymatic stoichiometry be used to determine growth-limiting nutrients for microorganisms? – a critical assessment in two subtropical soils**

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**ABSTRACT**

The measurement of potential enzymatic activities has been proposed as an efficient and informative method to infer nutrient limitations for heterotrophic soil microorganisms in environmental samples, and is currently one of the dominant strategies used. To validate the power and precision of using enzymatic assessments to determine limiting factors for microbial growth in soil, confirmation with direct methods of microbial growth response to resource addition are required. To address this, we experimentally manipulated soils from the afro-montane subtropics with additions of C, N and P in a multifactorial design for one month in order to shift the microbial community towards C-, N- and P-limitation. We then measured the most commonly measured indicator enzymes used to infer growth limiting nutrients, i.e.  $\beta$ -1,4-glucosidase,  $\beta$ -1,4-N-acetylglucosaminidase, leucine aminopeptidase and acid phosphatase. In addition, we determined the responses in bacterial and fungal growth rates to nutrient supplements by measuring the incorporation of leucine and acetate into bacteria and fungi, respectively, and also verified the microbial growth responses with biomass responses to factorial nutrient amendments. Enzymatic C:N, C:P and N:P ratios indicated that the grassland was primarily P-limited, and secondarily C- and N-limited, while the forest soils were co-limited by C and P, with N playing a minor role as limiting nutrient. However, both short-term and long-term responses to nutrient additions in the soils strongly pointed towards C-limitation of bacterial growth, fungal growth and respiration. Thus we conclude that enzymatic ratios do not capture the growth-limiting factors for bacterial growth, fungal growth, or respiration in either the grassland and forest soil. Furthermore, long-term addition of C shifted the fungal community into N limitation, while bacteria were limited by CN, revealing that both bacteria and fungi can be limited by different nutrients.

*Keywords: nutrient limitation, enzymatic ratios, fungal growth, bacterial growth*



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## **General Information on FPS COST Action FP1305, Linking belowground biodiversity and ecosystem function in European forests (BioLink)**

European forests are of immense importance to both society and the environment, providing a range of products and ecosystem services many of which are threatened by climate change. Our understanding of forest diversity, especially belowground, is currently limited and spread over distinct trophic levels. Little is known about the redundancy and functional diversity in forest soils. Numerous factors threaten existing belowground biodiversity, the simplification of forest ecosystems in pursuit of higher productivity being one of the prominent ones. There is an urgent need to link up existing scientific expertise at different levels to fully explain the connection between diversity, stability and function. Concurrently, forest modelling lacks coordinated activity aimed at bringing biodiversity into the fold - current emphasis is on yield and forest gap models. Elsewhere, food web models and ecosystem network models are well developed, but their application to forests is limited. This COST Action will provide a forum where current understanding of functional belowground biodiversity in European forests will be synthesised and its role communicated in a form suitable for modellers, policy makers and end-users.





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**COST Action FP1305 BioLink - Linking belowground biodiversity and ecosystem function in European forests**

Management Committee

Mc Chair : **Dr Martin Lukac** (UK); Mc Vice Chair: **Dr Ivika Ostonen-Märtn** (EE);  
Science Officer: **Dr Federica Ortell**; Administrative Officer: **Ms Cassia Azevedo**

**Action details**

MoU	063/13
CSO Approval date	15/11/2013
Start of Action	14/05/2014
End of Action	13/05/2018

Participations

Country	Date	Status
<b>Austria</b>	28/11/2013	Confirmed
<b>Belgium</b>	29/01/2014	Confirmed
<b>Bosnia and Herzegovina</b>	20/11/2013	Confirmed
<b>Bulgaria</b>	05/02/2014	Confirmed
<b>Croatia</b>	17/12/2013	Confirmed
<b>Czech Republic</b>	14/01/2014	Confirmed
<b>Denmark</b>	27/11/2013	Confirmed
<b>Estonia</b>	29/11/2013	Confirmed
<b>Finland</b>	07/04/2014	Confirmed
<b>France</b>	13/12/2013	Confirmed
<b>Germany</b>	07/01/2014	Confirmed
<b>Greece</b>	11/12/2013	Confirmed
<b>Hungary</b>	14/03/2016	Confirmed
<b>Iceland</b>	25/11/2013	Confirmed
<b>Ireland</b>	21/01/2014	Confirmed
<b>Israel</b>	20/07/2015	Confirmed
<b>Italy</b>	02/04/2014	Confirmed



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<b>Latvia</b>	13/02/2014	Confirmed
<b>Lithuania</b>	03/02/2014	Confirmed
<b>Montenegro</b>	14/03/2016	Confirmed
<b>Netherlands</b>	19/12/2013	Confirmed
<b>Norway</b>	05/03/2014	Confirmed
<b>Poland</b>	02/12/2013	Confirmed
<b>Portugal</b>	21/01/2014	Confirmed
<b>Romania</b>	05/12/2013	Confirmed
<b>Serbia</b>	26/03/2014	Confirmed
<b>Slovakia</b>	12/01/2014	Confirmed
<b>Slovenia</b>	20/03/2014	Confirmed
<b>Spain</b>	28/11/2013	Confirmed
<b>Sweden</b>	25/02/2014	Confirmed
<b>Switzerland</b>	17/01/2014	Confirmed
<b>Turkey</b>	14/01/2014	Confirmed
<b>United Kingdom</b>	21/11/2013	Confirmed

Total 33

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Lithuania	Dr Audrius Skridaila	Switzerland	Dr Beat Frey
Lithuania	Dr Reda Irsenaite	Switzerland	Dr Martin Hartmann
Netherlands	Prof Godefridus M.J. (Frits) Mohren	United Kingdom	Dr Andy Smith
Netherlands	Prof Karsten Kalbitz		

**COST Participants subject to MC permission and MoU acceptance**

<b>Country</b>	<b>MC Member</b>	<b>Country</b>	<b>MC Substitute</b>
Hungary	Dr Gergely Boros Dr Laszlo Kredics	Montenegro	Dr Jelena Lazarevic

## **Biolink Previous meetings:**

### **Kick-off meeting**

Brussels, Belgium, 14th May 2014

Four Points by Sheraton Brussels (Rue Paul Spaak 15, Brussel)

#### **AGENDA:**

- Welcome
- Adoption of the agenda
- Presentation of the delegates
- Introduction to COST
- Grant System and the Grant Holder
- Status of the COST Action
- Agreement on the internal rules of procedure for the Management Committee of the COST Action
- Election of the Chair and Vice-Chair
- Working plan for the implementation of the MoU
  - Presentation of the Action
  - Objectives and working programme
  - Working method/organisation and management
  - Distribution of tasks and election of WG leaders
  - Time-table
  - Budget plan for the first period
- Approval of IPC participation, if any.
- Place and date of next meeting
- AOB





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**The 1<sup>st</sup> annual meeting of Cost Action FP1305 Biolink: *What are we linking?***  
University of Reading, UK 4-6 November 2014

**Meeting programme**

**Tuesday 4<sup>th</sup> November 2014**

16:00 – 20:00 Registration, refreshments

**Wednesday 5<sup>th</sup> November 2014**

8:30 – 9:15 Teas & Coffees

9:15 – 9:30 Welcome

9:30 – 12:30 Brainstorming, led by Idenk

12:30 – 13:20 Lunch

*Session chaired by Ivika Ostonen*

13:20 – 14:00 **Mary Edwards** (University of Southampton)

New DNA approaches to understanding Late-Quaternary and recent biodiversity changes – potential and problems.

14:00 – 14:40 **Inger Greve Alsos** (The Arctic University of Norway)

The use of lake sedDNA as proxy for reconstructing vegetation change: comparisons with known afforestation history and macrofossils.

14:40 – 15:20 **Annamaria Bevivino** (ENEA Roma)

Soil bacterial diversity: from culture-based to metagenomic approaches.

15:20 – 15:40 Teas & Coffees

15:40 – 17:00 Networking + posters

17:00 – 18:00 MC meeting

**Thursday 6<sup>th</sup> November 2014**

*Session chaired by Elena Vanguelova*

9:00 – 9:40 **Thomas Bell** (Imperial College London)

Soil biodiversity and ecosystem functioning: linking laboratory experiments to natural systems

9:40 – 10:20 **Stefano Mocali** (ENTECRA Italy)

Exploring soil microbial diversity in forest ecosystems: new perspectives

10:20 – 10:50 Teas & Coffees

10:50 – 11:30 **Paul Henning Krogh** (Aarhus University)

The role of soil invertebrates in forest ecosystems

11:30 – 12:10 **Robert Griffiths** (CEH Wallingford)

Large scale drivers of soil bacterial biodiversity

12:10 – 12:25 **Lars Vesterdal** (University of Copenhagen)

Do tree species identity and diversity influence soil carbon stocks in temperate and boreal forests?

12:25 – 12:40 **Petr Baldrian** (Charles University Prague)

Fungal communities in forest soils: diversity, dynamics and functioning.

12:40 – 12:55 **Isabel Abrantes** (University of Coimbra)

The ecology of pinewood nematodes.

12:55 – 13:45 Lunch

13:45 – 15:30 WG sessions

15:30 – 16:00 Teas & Coffees

16:00 – 16:30 Closing remarks

19:00 – 22:00 Conference dinner

**Friday 7<sup>th</sup> November 2014: Departure**



**University of  
Reading**



## **2<sup>nd</sup> Action meeting: *Belowground biodiversity in changing environment***

Krakow, Poland, 17<sup>th</sup>-19<sup>th</sup> March 2015

Buildings of Collegium Novum, Golebia 24 street and Collegium Maius, Jagiellonska 15 street of Jagiellonian University, Krakow.

### **Meeting programme**

#### **Day 1, 17th March (Tuesday)**

- 12:15 Start of the field excursion (at the front of Coll. Novum). Approximate duration of excursion: 6 hours.  
17:00-21:00 Registration, poster session (with wine; in Coll. Novum)

#### **Day 2, 18th March (Wednesday)**

- 09:00-09:30 Welcome  
09:30-11:00 Plenary session, part 1 (in Coll. Novum); *chaired by Ivano Brunner*  
09:30-09:55 **Petr Baldrian** (Czech Republic)  
Forest trees determine the composition of soil fungal and bacterial communities and drive their activity  
09:55-10:20 **Monique Carnol** (Belgium)  
Microbial biomass increases with tree species diversity in European forest soils  
10:20-10:40 **Erika Gomoryova** (Slovakia)  
Soil microbial community changes at the windthrow plots in Tatra Mts. (Slovakia)  
10:40-11:00 **Marcin Pietras** (Poland)  
Ectomycorrhizal communities in managed oak forests in central Poland: effect of chronosequence and host decline  
11:00-11:30 Coffee break (including poster session)  
11:30-11:50 **Darta Klavina** (Latvia)  
ECM community in conifer stands on peat soils 12 years after wood ash treatment  
11:50-12:15 **Oskar Franklin** (Austria)  
Modeling ectomycorrhizal forest: A plant-microbe system perspective  
12:15-12:35 **Martina Vašutová** (Czech Republic)  
Fungal community structure across treeline ecotone  
12:35-13:00 **Edda S. Oddsdottir** (Iceland)  
The effects of afforestation on Collembola density and species number  
13:00-14:00 Lunch  
14:00-17:00 WGs meeting, part 1 (with coffee break; in Coll. Novum and Coll. Maius)  
17:00-18:00 MC meeting  
19:30-22:00 Conference dinner (in Dom Polonii)

#### **Day 3, 19th March (Thursday)**

- 09:00-13:00 Plenary session, part 2 (in Coll. Novum), *chaired by Katarzyna Hryniewicz*  
09:00-09:20 **Paola Grenni** (Italy)  
Soil microbial community and their role in ecosystem functioning  
09:20-09:40 **Mathias Mayer** (Austria)  
Exploring the drivers of post-disturbance soil CO<sub>2</sub> efflux in the Austrian Alps  
09:40-10:05 **Nadejda Soudzilovskaia** (Netherlands)

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	Quantifying differential impacts of arbuscular and ectomycorrhizae on ecosystem carbon budget
10:05-10:30	<b>Lixia Wang</b> (Austria)
	Ectomycorrhizal and soil enzyme activity profiles at the tree line
10:30-10:50	<b>Mark R. Bakker</b> (France)
	Mixed forest stands and biodiversity belowground: mycorrhizal infection and enzymatic activities
10:50-11:30	Coffee break (with poster session)
11:30-11:50	<b>Manuel Fernández-López</b> (Spain)
	<i>Arthrobacter</i> survival in the holm-oak rhizosphere after a wildfire
11:50-12:10	<b>Jesús Mercado-Blanco</b> (Spain)
	The tripartite interaction olive- <i>Pseudomonas fluorescens</i> PICF7- <i>Verticillium dahliae</i> as study system to unravel biocontrol performance of an endophytic bacterium and plant genetic responses to root colonization
12:10- 12:35	<b>Diogo Neves Proença</b> (Portugal)
	The role of endophytic microbial community in Pine Wilt Disease: diversity and function of microbes
13:00-15:00	Lunch (sightseeing of Collegium Maius after lunch - optional)
15:00-17:00	WGs meeting, part 2 (in Coll. Novum and Coll. Maius)
16:45-17:15	Coffee break (in Coll. Novum)
17:15-18:00	Closing remarks and meeting end





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**3<sup>rd</sup> Action meeting: Soil Biological Communities and Aboveground Resilience**  
Rome, Italian Geographical Society and Consiglio Nazionale delle Ricerche, 17-19 November 2015



**Programme**

17<sup>th</sup> November, *Italian Geographical Society, Villa Celimontana, via della Navicella 12, Rome*

16:00-18:30 Registration

17:00-17:15 Opening Ceremony and Meeting Introduction by Anna Benedetti, Head of Research Centre for the Soil-Plant System, CREA-RPS, Italy  
*Soil Global partnership*

17:15-17:30 Welcome speech by Pierfrancesco Sacco, Italian Ambassador to FAO, leader of Italy's permanent Delegation at the Organizations of the United Nations

17:30-18:15 Main Lecture by Stefano Grego, WAHF, World Agricultural Heritage Foundation, Research Council of Italy, CREA-RPS, Rome  
*Transdisciplinary Approaches for Building a New Sustainable and Resilient Agroecosystem*

18.15-18:30 Discussion

18:30-20:00 Refreshments

18<sup>th</sup> November CNR, Piazzale Aldo Moro 7, Aula Convegni

09:00-09:30 Welcome

**Mauro Gamboni**, Head of the Sustainable Agriculture Project, Biology, Agriculture and Food Sciences Dept., CNR, IT, Biolink WG3 Coordinator

**Francesco Loreto**, Head of Biology, Agriculture and Food Sciences Dept., CNR, IT

**Massimo Iannetta**, Head of Biotechnologies and Agro-Industry Division, ENEA, IT

**Stefano Bisoffi**, Scientific Director of the Council for Agricultural Research and Analysis of Agricultural Economics, CREA, IT

09:30-11:00 Plenary Session, Part 1 Chairs: *Douglas Godbold*, Institute of Forest Ecology, Forest and Soil Sciences Dept., Natural Resources and Life Sciences Univ.-BOKU, Vienna, AT, Biolink WG1 Coordinator; *Aurelio Ciancio*, CNR, Institute of Sustainable Plant Protection (CNR-IPSP), Bari, IT

09:30-09:55 **Brian S. Griffiths**, SRUC, Crop and Soil Systems Research Group, Edinburgh EH9 3JG, UK. *An overview of the resilience of soil microbial processes and the interactions with above-ground processes*

09:55-10:20 **Erica Lumini**, CNR-Institute for Sustainable Plant Protection (CNR-IPSP), Turin, IT  
*Unravelling Soil Fungal Communities from Different Land-Use Backgrounds*

10:20-10:40 **Beat Frey**, Swiss Federal Research Institute WSL, Birmensdorf, CH  
*Resistance and resilience of the forest soil microbiome to logging-associated compaction*

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- 10:40-11:00 **Luis G. García-Montero**, Forest Engineering Dept., E.T.S.I. Montes, Technical University of Madrid (UPM), Madrid, ES  
*Could mycorrhizae perform “ecosystem engineering” on the host plants? Basis on rhizoculture of agroforestry systems to improve their resilience*
- 11:00-11:30 Coffee break
- 11:30-13:00 Plenary Session, Part 2. Chairs: *Johannes Rousk*, Lund Univ., Biology Dept., MEMEG, LUND, SE, Biolink WG2 Coordinator, *Anna Barra Caracciolo*, CNR, Water Research Institute (CNR-IRSA) Rome IT
- 11:30-11:55 **Jose Julio Ortega-Calvo**, Instituto de Recursos Naturales y Agrobiología de Sevilla, CSIC, Sevilla, ES. *Microbial and plant influences on bioavailability of hydrophobic organic pollutants in soil*
- 11:55-12:20 Andrey Yurkov, Leibniz-Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, DE. *Diversity, distribution and functions of yeasts in soils*
- 12:20-12:40 **Petr Baldrian**, Laboratory of Environmental Microbiology, Institute of Microbiology of the ASCR, Prague, CZ. *Tracing the activity of individual microbial taxa in forest soils*
- 12:40-13:00 **Luis Merino-Martín**, INRA, UMR AMAP, Montpellier, France and Centre for Ecology & Hydrology, Crowmarsh Gifford, Wallingford, Oxfordshire, UK. *Understanding the factors driving soil aggregate stability: the role of plant-soil-root-microbe interactions*
- 13:00-14:00 Lunch
- 17:00-18:00 MC Meeting: Aula Convegna
- 19:30-22:00 Conference dinner, *Royal Art Café Restaurant*, Piazza del Colosseo, 1.
- 19<sup>th</sup> November- CNR, Piazzale Aldo Moro 7, Rome, Aula Convegna
- 08:30-09:30 Guided tour of selected Posters. Chair: *Alessandra Lagomarsino*, CREA-ABP, Agrobiology and Pedology Research Centre, Florence, IT
- 09:30-11:00 Plenary Session, Part 3. Chairs: *Gabrielle Deckmyn*, Antwerpen Univ., Antwerpen, BE, Biolink WG2 Coordinator; *Annamaria Bevivino*, ENEA Casaccia Research Center, Rome, IT
- 09:30-09:55 **Mark Tibbett**, School of Agriculture, Policy and Development, Reading Univ., Reading, UK. *Microbial functional diversity in restored biodiverse forest soil*
- 09:55-10:20 Jorge Curiel Yuste, Museo Nacional de Ciencias Naturales, CSIC, Madrid, ES. *Forest fragmentation and drought as engines of change of the plant-soil-microbial ecological interactions in the Mediterranean basin*
- 10:20-10:40 **Mona N. Högborg**, Forest Ecology and Management Dept., Swedish Agricultural Sciences, Univ. Umeå, SE. *How does soil microbial community composition and nitrogen supply change with ecosystem age in primary boreal forests?*
- 10:40-11:00 **Relena R. Ribbons**, 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



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- Resource Management Dept., Copenhagen Univ, DK. *Microbial communities, functional genes and nitrogen cycling processes in forest floors under four tree species*
- 11:00-11:30 Coffee break (including poster session)
- 11:30-13:00 Plenary Session, Part 4. Chairs: *Olaf Schmidt*, College Dublin Univ., Agriculture and Food Science Centre, Dublin, IE; *Paola Grenni*, CNR, Water Research Institute (CNR-IRSA) Rome IT
- 11:30-11:55 **Hans Göransson**, Forest ecology, Natural resources and life sciences Univ., Vienna, AT. *Effects of geometrid moth attacks on belowground processes in sub-arctic birch forests*
- 11:55-12:20 **Franco Nigro**, Università degli studi Aldo Moro, Dipartimento di Scienze del suolo, della pianta e degli alimenti (Di.S.S.P.A.) Bari, IT. *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.*
- 12:20-12:40 **Jérôme Cortet**, CEFE UMR 5175, CNRS, Université Paul-Valéry Montpellier, EPHE, Bat Fabre, Montpellier Cedex, FR. *The use of traits for soil invertebrates: state of the art and perspectives*
- 12:40-13:00 **Oskar Franklin**, IIASA, International Institute for Applied Systems Analysis, Laxenburg, AT. *Modeling plant – soil interactions in an ectomycorrhizal forest*
- 13:00-14:00 Lunch
- 14:00-15:00 Poster Session
- 16:45-17:15 Coffee break with all the WG participants and brief relations by WG leaders (Aula Convegni)
- 17:15-18:00 Closing Remarks and meeting end (Aula Convegni)





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**4<sup>th</sup> BioLink meeting:**  
**Soil Biodiversity and Ecosystem Services**  
Sofia, Bulgaria, 12<sup>th</sup>-14<sup>th</sup> April 2016. Park  
Hotel Moskva



## MEETING PROGRAMME

**April 12<sup>th</sup>** Europe Hall (ground floor)

- |             |   |
|-------------|---|
| 08:30-09:30 | Registration  |
| 09:30-10:00 | Welcome Dr Martin LUKAC–BioLink Project Coordinator Dr Miglena ZHIYANSKI–Forest Research Institute, Bulgarian Academy of Sciences Assoc. Prof. Dr Anna GANEVA–Director of Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences Prof. DSc Hristo TSAKOV–Director of FRI-BAS; Prof. Dr Svetla ROUSSEVA–Director of Institute of Soil Science “Nikola Poushkarov” |
| 10.00-11.00 | Plenary Session Part 1 Session chaired by Elena Vangelova and Douglas Godbold   |
| 10.00-10.30 | <b>Kevin R. BUTT</b> University of Central Lancashire, Preston, UK. <i>Earthworms and ecosystem services</i>  |
| 10.30-11.00 | <b>Lyubomir PENEV</b> Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria. <i>One Ecosystem of journals: Data and narrative-integrated publishing on Pensoft’s ARPHA Publishing Platform</i>  |
| 11:00-11:30 | Coffee break  |
| 11:30-13:00 | Plenary Session Part 2 Session chaired by Boyko Georgiev and Jérôme Cortet  |
| 11.30-11.50 | <b>Gergely BOROS</b> Centre for Ecological Research, Institute of Ecology and Botany, Hungary. <i>The effects of forestry treatments on enchytraeid worms (Annelida, Oligochaeta) in a Hungarian sessile oak-hornbeam forest</i>  |
| 11.50-12.10 | <b>Francis ASHWOOD</b> Forest Research, Alice Holt Lodge, Farnham, Surrey, UK. <i>Woodland restoration on landfill sites: Earthworm activity and ecosystem service provision</i>  |
| 12.10-12.30 | <b>Vlada PENEVA</b> Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria. <i>Soil nematode diversity -ecosystem functions, services and disservices</i>  |
| 12.30-12.50 | <b>Diogo Neves PROENÇA</b> CEMUC, University of Coimbra, Coimbra, Portugal. <i>Microbiomes of potato-cyst-nematodes isolates kept in laboratory conditions and from environment</i>   |
| 12.50-13.00 | <b>Isabel ABRANTES</b> (short communication)  |
| 13:00-14:00 | Lunch   |
| 14:00-15:30 | Plenary Session Part 3 Session chaired by Annamaria Bevivino and Ivano Brunner  |



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- 14.00-14.30 **Alessio MENGONI** (Invited speaker) Department of Biology, University of Florence, Sesto Fiorentino, Italy. *The pan-bacteriome approach. A way to decipher the puzzling diversity and dynamics of bacterial community in soil*
- 14.30-14.50 **Manuel FERNÁNDEZ-LÓPEZ** Soil Microbiology Dept., Estacion Experimental del Zaidin, Granada. *Analysis of the rhizospheric microbial communities of Quercus pyrenaica along an altitudinal gradient*
- 14.50-15.10 **Taina PENNANEN** Natural Resources Institute Finland (Luke), Vantaa, Finland. *Digging the mechanisms behind the growth of spruce –role of diversity of ectomycorrhizal fungi*
- 15.10-15.30 **Petr BALDRIAN** Laboratory of Environmental Microbiology, Institute of Microbiology of the CAS, Praha, Czech Republic. *Forest microbiome –habitat diversity, dynamics, and ecosystem functioning*
- 15.30-16.00 Coffee break
- 16.00-17.40 Plenary Session Part 4 Session chaired by Gabrielle Deckmyn and Mauro Gamboni
- 16.00-16.20 **Diogo PINHO** Biocant Park, Cantanhede, Portugal. *Bacterial and fungal communities in rhizosphere of corkoak (Quercus suber L.): Linking microbiome and forest health*
- 16.20-16.40 **Lars VESTERDAL** Department of Geosciences and Natural Resource Management, University of Copenhagen, Frederiksberg, Denmark. *Is tree species diversity or tree species identity the most important driver of European forest soil carbon stocks?*
- 16.40-17.00 **Brynhildur BJARNADÓTTIR** University of Akureyri, Iceland. *The effect of deforestation on stream water ecosystems in Iceland*
- 17.00-17.20 **Dessie ASSEFA** Institute of Forest Ecology, BOKU, Vienna, Austria. *Variability in fine root morphology, biochemistry, and litter quality indices of ten tropical tree species*
- 17.20-17.40 **Marc GOEBEL** Department of Natural Resources, Cornell University, Ithaca, USA. *Fine root production and phenology among temperate, mature tree species in a common garden in Poland*
- 18.00-20.00 Wine reception (Restaurant “Panorama”, Park Hotel Moskva) 5
- April 13th**
- 09.30-10.30 Networking and poster session (Groundfloor)
- 10.30-11.00 Coffee break
- 11.00-13.00 WGs sessions, Part 1
- 13.00-14.00 Lunch
- 14.00-16.00 WGs sessions, Part 2
- 16.00-16.30 Coffee break
- 16.30-17.15 MC Meeting
- 19.00-22.00 Conference dinner (Restaurant “Edno vreme”, Borisova gradina PARK)
- April 14th**
- 09.30-11.00 WGs sessions, Part 3
- 11.00-11.30 Coffee break

11:30-12:00 Closing remarks  
WP4 Modelling Workshop (Thursday afternoon and Friday, 15th)



**5<sup>th</sup> meeting of COST Action**  
***Belowground biodiversity and global change***  
Prague, Czech Republic, 24<sup>th</sup>-26<sup>th</sup> October 2016  
Congress and Educational Centre 'Floret',  
Pruhonice, Prague



## Programme

### Monday 24th October 2016

16:00 18:00 Registration  
18:00 20:00 Poster session, refreshments

### Tuesday 25th October 2016

#### Session 1

9:00 9:20 **Welcome**

9:20 9:50 **Matty Berg** (invited speaker) Department of Ecological Science / Section Animal Ecology, Vrije Universiteit Amsterdam. *Reactions of communities and ecosystems to environmental change: a trait approach.*

9:50 10:10 **Christoph Rosinger** Institute of Forest Ecology, Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences, BOKU Wien, AT. *The significance of rare EM species for biodiversity and ecosystem functioning in European forests.*

10:10 10:30 **Elena Vanguelova** Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK *Does tree species have an effect on soil microbial community?*

**10:30 11:00 Coffee**

#### Session 2

11:00 11:30 **Paolo Nannipieri** (invited speaker) Department of Agrifood Production and Environmental Sciences, University of Firenze, Italy. *Soil as a biological system and the role of omics in analyzing it.*

11:30 11:50 **Johannes Rousk** Microbial Ecology – Dept Biology, Lund University, 22362 Lund, Sweden *Fungal-to-bacterial dominance of soil food-webs in a changing world: Consequences for the priming effect and resource-use efficiency.*

11:50 12:10 **Petr Baldrian** Laboratory of Environmental Microbiology, Institute of Microbiology of the CAS, Praha, Czech Republic *Fungal communities and enzymes involved in decomposition respond to root dieback after clearcut*



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12:10 12:30 **Tereza Mašínová** Laboratory of Environmental Microbiology, Institute of Microbiology of the CAS, Praha, Czech Republic *Combining metagenomics and cultivation approach to understand soil yeast communities of temperate forest topsoil*

**12:30 13:30 Lunch**

**Session 3**

13:30 14:00 **Flavia Pinzari** (invited speaker) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Rome, Italy; Department of Life Sciences, Natural History Museum, London, UK. *Recycling and mobilisation of mineral nutrients in forests: the role of fungal networks and biodiversity*

14:00 14:20 **Ina C. Meier** Plant Ecology, University of Göttingen, Göttingen, Germany *Influence of the type of mycorrhization on root functions of temperate trees*

14:20 14:40 **Taina Pennanen** Natural Resources Institute Finland, Vantaa, Finland *Early root growth and architecture of fast- and slow-growing Norway spruce families*

14:40 15:00 **Evsey Kosman** Institute for Cereal Crops Improvement, Tel Aviv University, Tel Aviv, Israel. *Dispersion, Diversity and Decomposing Functional Variability in Communities and Populations*

**15:00 15:30 Coffee**

15:30 17:00 WG sessions

17:00 17:30 MC meeting

**Wednesday 26th October 2016**

**Session 4**

9:00 9:30 **Marie Spohn** (invited speaker) Department of Soil Biogeochemistry, Bayreuth Center of Ecology and Environmental Research (BAYCEER), University of Bayreuth, Germany. *Phosphorus cycling in Fagus sylvatica forests*

9:30 9:50 **Katarzyna Hryniewicz** Department of Microbiology, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland. *Characterization of siderophore producing bacterial strains (SPB) associated with birch and alder at heavy metal contaminated areas and their potential in phytoremediation*

9:50 10:10 **Iftekhar U. Ahmed** Institute of Forest Ecology, Universität für Bodenkultur (BOKU), Vienna, Austria. *Soil microbial biomass and enzyme activities in changing environment of Ethiopian Highland*

10:10 10:30 **Edda Sigurdís Oddsdóttir** 'The effects of elevated soil temperatures on ectomycorrhizal community and litter decomposition in a Sitka spruce forest in southern Iceland.'

**10:30 11:00 Coffee**

**Session 5**

11:00 11:30 **Brian Pickles** (Invited speaker) 'Impacts of mycorrhizal biodiversity on plant responses to global change'

11:30 11:50 **Irena Maček** Icelandic Forest Research, Mogilsa, Reykjavik, Iceland. *Arbuscular mycorrhizal fungal communities pushed over the edge – lessons from extreme ecosystems*

11:50 12:10 **Mathias Mayer** Institute of Forest Ecology, Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences – BOKU, Vienna, Austria. *The last survivors – pre-disturbance tree recruitment retards post-disturbance soil decomposition*

12:10 12:30 **Martina Vašutová** Global Change Research Institute, Czech Academy of Sciences, České Budějovice, Czech Republic; Department of Botany, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic. *Management influence on ectomycorrhizal species community – ten years after windthrow*

12:50 12:50 **Tobias Guldberg Frøslev** Department of Biology, Terrestrial Ecology Section, University of Copenhagen, Copenhagen, Denmark. *BIOWID'*

12:50 13:30 Lunch

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13:30 15:00 WG Sessions  
15:00 15:30 Coffee  
15:30 16:00 Closing remarks  
17:00 23:00 Evening tour of Prague, including conference dinner  
**Thursday 27th October 2016:** Departure



(Photos from: <http://www.vlastiveda.cz/unesco/pruhonicky-park>)





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**6<sup>th</sup> meeting of COST Action jointly with 7<sup>th</sup> International Symposium on Physiological Processes in Roots of Woody Plants**  
University of Tartu, micum building (Riia 23B, Tartu), Estonia,  
June 26<sup>th</sup> - 29<sup>th</sup>, 2017



Meeting Programme



**Sunday 26<sup>th</sup> June**

17.00-19.00 Welcome Reception

Drinks & snacks, Omicum building (Riia 23B, Tartu)

Monday 26<sup>th</sup> June

08.00: Arrival/registration

09.00-9.15 Welcome: Ivika Ostonen, Chair of Woody Root 7 symposium

9.15-12.00 Session: *Water acquisition and nutrient uptake*; Chair: Ivano Brunner

9.15-10.00 Keynote and COST invited speaker: **Arthur Gessler**, The role of nutrients in drought-induced tree mortality and recovery

10.00-10.15 **Annie DesRochers**, Physiological integration of connected *Populus balsamifera* ramets

10.15-10.30 **Tapani Repo**, Biophysical changes in roots of Scots pine seedlings by cold acclimation and freezing damage

10.00-10.30 Coffee

11.00-12.00 Chair: Jhonathan Ephrath

11.00-11.15 **Taina Pennanen**, Unearthing mechanisms behind variable growth rate in Norway spruce

11.15-11.30 **Leena Hamberg**, Early root growth and architecture of fast- and slow-growing Norway spruce (*Picea abies*) families differ – potential for functional adaptation

11.30-11.45 **Sonia Meller**, The potential of beech seedlings to adapt to low P availability in soil – linking plant P and biomass allocation with rhizosphere processes

11.45-12.00 **Mana Mukai**, Effects of tree-root exudates on the solubilization of phosphorus adsorbed to allophane in the rhizosphere on Andisols, Yakushima Island, Japan

12.00-13.00 Lunch

13.00-16.00 Session: *Belowground assimilate allocation*, Chair: Krista Lõhmus

13.00-13.45 Keynote speaker: **David Eissenstat**, Plant Functional Traits Associated with Mycorrhizal Root Foraging in Arbuscular Mycorrhizal and Ectomycorrhizal Trees

13.45-14.00 **Jaana Leppälammi-Kujansuu**, Fine root dynamics in the boreal soil fertility gradient

14.00-14.15 **Rabbil Bhuiyan**, Estimating fine-root production by tree species and understory functional groups in peatland forests

14.15-14.30 **Yasuhiro Hirano**, Contrasting root systems of *Pinus thunbergii* in soils with different groundwater levels in a coastal forest

14.30-15.00 Coffee

15.00-16.00 Chair: Yasuhiro Hirano

15.00-15.15 **Ina Christin Meier**, Root exudation depends on soil depth, root morphology, and environment

15.15-15.30 **Bartosz Adamczyk**, How root chemistry affect sequestration of carbon from fungal biomass

15.30-15.45 **Heljä-Sisko Helmisaari**, Fine root carbon compounds of Norway spruce and Scots pine on a latitude gradient

15.45-16.00 **Pille Mänd**, Acclimation of Silver birch (*Betula pendula*) roots in artificial gradient of soil moisture

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16.00-16.15 **Dominika Thiem**, Tripartite mutualism of black alder (*Alnus glutinosa* L.) at saline area  
16.15-18.15 WG activities, MC meeting  
17.00-18.00 Hands-on PlutoF course "Create and manage soil biodiversity database" (Vanemuisse 46)

**Tuesday 27<sup>th</sup> June**

8.30-16.15 Session: *Linking root functioning and belowground biodiversity*; Chair: Martin Lukac  
8.30-9.15 Keynote and COST invited speaker: **Björn Lindahl**, Influence of ectomycorrhiza on decomposition across biomes  
9.15-9.30 **Petr Baldrian**, Feed in summer, rest in winter: Microbial carbon utilization in coniferous forest soil reflects root activity  
9.30-9.45 **Ivano Brunner**, The root of the matter - decomposition of Scots pine roots and its microbial communities  
9.45-10.00 **Sannakajsa Velmala**, Root architecture of Norway spruce families with contrasting growth phenotypes in response to nutrient-rich patches within a nutrient-poor environment  
10.00-10.30 Coffee  
10.30-12.00 Chair: Mari Moora  
10.30-10.45 **Oskar Franklin**, The role of mycorrhiza for carbon storage in forests – an ecosystem perspective  
10.45-11.00 **Rodica Pena**, Partitioning of soil fungal biomass between saprotrophic and ectomycorrhizal guilds in deciduous and coniferous temperate forests  
11.00-11.15 **Nadia Soudzilovskaia**, Environmental predictors of plant species richness within distinct mycorrhizal types  
11.15-11.30 **Tanja Mrak**, Belowground response to combined effects of ozone and drought in seedlings of three oak species (*Quercus ilex*, *Q. pubescens* and *Q. robur*)  
11.30-11.45 **Marc Goebel**, Decomposition dynamics of undisturbed, dead fine roots in temperate tree species  
11.45-12.00 **Saori Fujii**, Soil microarthropod communities involved in decomposition of leaf and root litter  
12.00-13.00 Lunch  
13.00-14.30 Chair: Leho Tedersoo  
13.00-13.45 Keynote speaker: **Wim van der Putten**, Feedback interactions between plant roots and soil biodiversity during range shifts and ecosystem development  
13.45-14.00 **Diogo Pinho**, The rhizosphere microbiome in the oak decline  
14.00-14.15 **Rasmus Kjeller**, Use of in-growth mesh bags to assess the physiological status of ectomycorrhizal fungi  
14.15-14.30 **Siim-Kaarel Sepp**, Effects of moderate land use on arbuscular fungal communities  
14.30-15.00 Coffee  
15.00-16.15 Chair: Maarja Öpik  
15.00-15.45 Keynote speaker: **Martin Zobel**, Macroecology of arbuscular mycorrhiza (AM)  
15.45-16.00 **Irena Maček**, Locally Extreme Environments as Natural Long-Term Experiments in Community Ecology of Arbuscular Mycorrhizal Fungi  
16.15-19.00 Poster session with drinks and snacks



### **Wednesday 28<sup>th</sup> June**

9.30-12.15 Session: *Roots, global change and ecosystem services* Chair: Mark R Bakker  
9.30-9.15 Keynote and COST invited speaker: **Phil Ineson**, Assessing the Functional Activity of Roots in the Field: Novel Techniques and Old Questions  
9.15-9.30 **Lars Vesterdal**, Are tree species diversity and tree species identity effects on soil carbon stocks across Europe linked with fine root biomass?  
9.30-9.45 **Leena Finér**, Relationships between fine root biomass and tree species diversity and functional group in European forest types  
9.45-10.00 **Joanna Mucha**, Response of ectomycorrhizal fungal community of boreal and temperate host species near their range limits to experimental warming  
10.30-11.00 Coffee  
10.30-12.00 Chair: Tarja Lehto  
10.30-10.45 **Tuula Larmola**, Impact of simulated atmospheric nitrogen deposition on nutrient cycling and carbon sink via mycorrhizal fungi in two nutrient-poor peatlands  
10.45-11.00 **Inga Dirks**, P uptake and use efficiencies of Palestine oak respond to N availability and drought  
11.00-11.15 **Timo Domisch**, Forest tree seedlings may suffer from predicted future winters with less snow  
11.15-11.30 **Krista Lõhmus**, Elevated atmospheric humidity shapes fine root foraging strategies and the carbon cycle of a silver birch forest ecosystem: a FAHM study  
11.30-11.45 **Arvo Tullus**, Regeneration of hybrid aspen from root suckers and stump sprouts under elevated air humidity in the FAHM experiment  
11.45-12.00 **Seung Hyun Han**, Effect of soil moisture on fine root production under experimental warming and precipitation manipulation  
12.00-12.15 **Kaining Zhou**, The Effects of Photoselective Nets on Shoot and Root Development of Young Grafted Orange Trees in the Field  
12.15-13.20 Lunch  
13.20-13.30 Intro: ISRR 10 in Israel. **Jhonathan Ephrath**  
13.30-16.45 Session: *Root traits, models, concepts* Chair: David Eissenstat  
13.30-14.15 Keynote speaker: **Daniel C Laughlin**, Integrating fine root traits into predictive models of community assembly  
14.15-14.30 **Martin Lukac**, How do plant roots drive soil biodiversity?  
14.30-14.45 **Frida Andreasson**, Fine root growth, production and morphology of *Pinus pinaster* and its understory species  
14.45-15.00 **Hidetoshi Ikeno**, Reconstruction of root systems of *Cryptomeria japonica* using coordinate points and root diameters  
15.00-15.30 Coffee  
15.30-16.30 Chair: Heljä-Sisko Helmisaari  
15.30-16.00 COST invited speaker: **Ari Laurén**, Using laboratory manipulation experiment to improve ROMUL-decomposition model  
16.00-16.15 **Gaby Deckmyn**, Keylink: An integrative soil representation for inclusion in ecosystem scale models  
16.15-16.30 **Mirco Rodeghiero**, Investigation of spatial and temporal oxygen evolution during root-pathogen interaction using planar optodes  
16.30-17.00 Final discussions and symposium wrap up  
19.30-01.00 Symposium dinner at Ahhaa Science Centre, Sadama

### **Thursday 29<sup>th</sup> June**

Excursions. Buses leave in front of Vanemuise 46, Tartu at 9:00 a.m.  
Trip 1: Free Air Humidity (FAHM) experimental site – SMEAR station – Järvelja Nature Reserve – Uniküla;

*Soil biodiversity and European woody agroecosystem  
FP1305 Biolink Cost Action Annual Meeting  
Granada, 14-16 March 2018*

Trip 2: Soontaga forest station – Meenikunno hiking trail – Giants of Ootsipalu  
**Friday 30<sup>th</sup> June** 9.00 - 16.00 Post-Symposium CAR-ES Workshop, Vanemuise 46, room 327



Photos from: <http://woodyroot7.ut.ee/node/11221>



[www.bio-link.eu](http://www.bio-link.eu)

*Soil biodiversity and European woody agroecosystem  
FP1305 Biolink Cost Action Annual Meeting  
Granada, 14-16 March 2018*

## **Working Groups**

### **WG1: Linking belowground biodiversity to ecosystem function**

Coordinator: Prof Douglas GODBOLD, Institute of Forest Ecology BOKU, Austria

[douglas.godbold@boku.ac.at](mailto:douglas.godbold@boku.ac.at)

Vice leader: Nadia Soudzilovskaia (NL)

### **WG2: Microbial and faunal functional biodiversity in belowground food-webs**

Coordinator: Dr Johannes ROUSK, Lund University Department of Biology, Sweden

[johannes.rousk@biol.lu.se](mailto:johannes.rousk@biol.lu.se)

Vice leader: Rasmus Kjøller (DE)

### **WG3: Belowground biodiversity in plantations and tree crops**

Coordinator: Dr Mauro GAMBONI, Consiglio Nazionale delle Ricerche, Italy

[mauro.gamboni@cnr.it](mailto:mauro.gamboni@cnr.it)

Vice leader: Christos Athanassiou (GR)

### **WG4: Functional diversity in forest models**

Coordinator: Dr Gabrielle DECKMYN, Plant and Vegetation Ecology Sciences University of Antwerpen, Belgium

[gaby.deckmyn@uantwerpen.be](mailto:gaby.deckmyn@uantwerpen.be)

Vice leader: Jorge Curiel-Yuste (ES)

### **Short-term scientific missions (STSM)**

Coordinator: Dr Miglena ZHIYANSKI, Forest Research Institute – Bulgarian Academy of Sciences, Bulgaria

[zhiyanski@abv.bg](mailto:zhiyanski@abv.bg)

## STSMs

BioLink has supported 14 STSMs in its first grant period, 6 in the second grant period, with one call for STSM currently open to offer support for missions at the beginning of 2016. Many of the STSMs have resulted in The list of STSMs carried out so far is as follows:

### Grant Period 1:

Name	Gender	From	To
Martina Di Lenola	F	Italy	Portugal
Carla Cruz Paredes	F	Denmark	Sweden
Isabella Pentimone	F	Italy	Spain
Tereza Polacikova	F	Czech Rep	Portugal
Jovana Devetakovic	F	Serbia	Poland
Diogo Proenca	M	Portugal	Denmark
Lieven Michelsen	M	Belgium	Germany
Jose Francisco Cobo Diaz	M	Spain	France
Zhun Mao	M	France	UK
Marina Katanic	F	Serbia	Slovenia
Maria Sakka	F	Greece	Italy
Yasmine Pinuela	F	Slovenia	Spain
Nadia Soudzilovskaia	F	Netherlands	Sweden
Edith Hammer	F	Sweden	Netherlands

### Grant Period 2

Name	Gender	From	To
Dominika Thiem	F	Poland	Slovenia
Romina Lorenzetti	F	Italy	Greece
Natasa Sibanc	F	Slovenia	UK
Antonio Fernandez Gonzales	M	Spain	Czech Rep
Nadia Soudzilovskaia	F	Netherlands	Sweden
Gergely Boros	M	Hungary	Switzerland
Sandrine Malchair	F	Belgium	Czech Rep



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Granada, 14-16 March 2018*

**Grant Period 3:**

<b>Name</b>	<b>Gender</b>	<b>From</b>	<b>To</b>
Jonathan Bonfanti	M	France	Denmark
Christoph Rosinger	M	Austria	Sweden
Emira Hukic	F	Bosnia	Slovenia
Jelena Lazarevic	F	Montenegro	Sweden
Isabella Pentimone	F	Italy	Spain
Mauro Lanfranchi	M	UK	Spain
Diogo Proenca	M	Portugal	Germany
Natasha Sibanc	F	Slovenia	UK
Yasmine Pinuela	F	Slovenia	France
Diogo Pinho	M	Portugal	UK
Diana Navratilova	F	Czech Rep	Sweden

## **WG1: summary of the activities**

The main activity of WG1 has been the work leading to a review publication as described below.

### **Belowground biodiversity is positively related to ecosystem service supply in European forests**

*Bakker MR<sup>1</sup>, Brunner I<sup>2</sup>, Ashwood F<sup>3</sup>, Bjarnadottir B<sup>4</sup>, Børja I<sup>5</sup>, Carnol M<sup>6</sup>, Cudlin P<sup>7</sup>, Dalsgaard L<sup>5</sup>, Erktan A<sup>8,9</sup>, Godbold DL<sup>7,10</sup>, Kraigher H<sup>11</sup>, Lehto T<sup>12</sup>, Suz LM<sup>13</sup>, Meier IC<sup>9</sup>, Merino Martin L<sup>8</sup>, Motiejunaite J<sup>14</sup>, Mrak T<sup>11</sup>, Oddsdóttir E<sup>15</sup>, Ostonen I<sup>16</sup>, Pennanen T<sup>17</sup>, Püttsepp Ü<sup>18</sup>, Vangelova E<sup>3</sup>, Vesterdal L<sup>19</sup>, Soudzilovskaia NA<sup>20</sup>*

*<sup>1</sup>Bordeaux Sciences Agro France, <sup>2</sup>WSL Switzerland, <sup>3</sup>Forest Research UK, <sup>4</sup>Uni Akureyri Iceland, <sup>5</sup>NIBIO Norway, <sup>6</sup>Uni Liège Belgium, <sup>7</sup>GCRI-CAS Czech Republic, <sup>8</sup>AMAP INRA France, <sup>9</sup>Uni Göttingen Germany, <sup>10</sup>BOKU Austria, <sup>11</sup>Slovenian Forestry Institute Slovenia, <sup>12</sup>Uni Eastern Finland Finland, <sup>13</sup>RBGK UK, <sup>14</sup>Nature Research Centre Lithuania, <sup>15</sup>Icelandic Forest Research Iceland, <sup>16</sup>Uni Tartu Estonia, <sup>17</sup>LUKE Finland, <sup>18</sup>Uni Life Sciences Estonia, <sup>19</sup>Uni Copenhagen Denmark, <sup>20</sup>Uni Leiden Netherlands.*

[douglas.godbold@boku.ac.at](mailto:douglas.godbold@boku.ac.at)

## **Background**

The concept of Ecosystem Services can be described as the many different benefits that ecosystems provide to people (Millennium Ecosystem Assessment 2005). The Millennium Ecosystem Assessment divided the ecosystem services into four categories: i. Provisioning Services, ii. Regulating Services, iii. Cultural Services and iv. Supporting services. Provisioning Services relate to products obtained from ecosystems, and examples include food, fuel, genetic resources, nutrients and fresh water. Regulating Services refer to the benefits obtained from the regulation of ecosystem processes and the services of this group contain for instance air quality-, climate-, water- or erosion regulation and pollination. Cultural Services are the non-material benefits people obtain from ecosystems through spiritual enrichment, cognitive development, reflection, recreation, and aesthetic experiences. Supporting Services are those services that are needed to enable all other ecosystem services, as well as their impacts on people. Supporting Services are often indirect, or occur over a very long time, whereas changes in the other groups have relatively direct and short-term impacts on people.

The COST FP1305 consortium consists of experts in different fields of belowground organisms originating from 32 European countries. According to the assumed relevance of different biotic groups of soils in providing ecosystem services (expert opinions expressed in workgroup sessions) and the expertise in the consortium, a selection of soil biotic groups (and subgroups) was made for our analyses. These biotic groups do not strictly cover all possible organismal groups of forest belowground diversity, but the most important groups based on our expert opinion. Expertise in our group was stronger for plants, fungi and soil fauna, but prokaryotes and protists were also included in our approach.

## **Key word searches**

We performed the main queries based on standardized sets of three to four key-words for each of the combinations of ecosystem services and biotic groupings under the Web of Science (December 2015 to Spring 2016). In total these queries represented 37 ecosystem services per 13 biotic subgroupings. A first screening was applied to the list of total hits based on general



exclusion / inclusion guidelines, using titles and abstracts of the references encountered for each combination of key words. We used the following guidelines for inclusion of potentially useful references for the next steps of evaluation: the source should 1) refer to a European ecosystem; 2) relate to forested ecosystems; 3) include or focus solely on belowground functions, processes and selected biotic groupings; and 4) address the relationship between the diversity of a specific grouping and an ecosystem service. The *a priori* exclusion of references from the list of hits was based on the reference not fulfilling any of the above conditions or being clearly out of scope.

### **Abstract evaluation and in-depth examination of the obtained references**

Biotic-group experts went through the list of publications obtained and the selected potentially useful hits to check the relevance of the references found. Then, based on the abstract, the potentially useful references were accessed for inspection of the full paper content and the effects of belowground biodiversity on a given service for a given biotic grouping were recorded (positive, negative, neutral or could not be assessed based on the source). The article screening was carried out per biotic group. This permitted also to refine the selection (discarding irrelevant sources from the list of potentially useful hits). Based on expert opinions expressed during the process of abstract screening and on full paper evaluation, some additional queries (using alternative key words) were considered necessary and these allowed to add a few relevant references to the standardized initial search. In addition, other sources known by these experts, and not occurring in the queries, or in queries run for other ecosystem services, were added. Finally, an additional set of key words was deployed for the largest biotic groupings of plants, fungi and soil fauna using Google Scholar regarding the cultural services specifically, and combined with this, a snowball approach was used cultural ecosystem services having no or few hits in the main queries.

### **Step-by-step synthesis of the query results**

The search findings of the biotic-group experts (number of potentially useful hits, and final number of relevant hits used) were presented and incremented during the COST meetings (i.e. in Sofia (April 2016), Prague (October 2016) and Vienna (March 2017) and discussed at the ecosystem services grouping level (i.e. Provisioning, Regulating, Cultural and Supporting services). Then, we accounted for the total number of used sources and the number of sources showing a positive, negative or neutral relationship between biodiversity of a biotic grouping and a given ecosystem service. Summary data sets were subjected to further synthesis by panels of experts for each ecosystem services group.

## **Results**

### **Main queries per ecosystem service and biotic groups**

The main queries resulted in 10557 hits with an average 25 hits (SE=53 hits) for each of the 481 queries (37 ecosystem services, 13 biotic subgroupings) and varied from zero (no hits) to 503 hits. The four main ecosystem services had comparable numbers of total hits and these ranged from the lowest value of 2357 hits for Cultural services to the highest value of 2788 hits for Provisioning Services. The relative frequency of the total hits for the biotic sub-grouping differed between ecosystem service, with stronger variation between ecosystem service groups for pathogenic fungi, bacteria and nematodes relative to their share of the total hits. Most total hits corresponded to roots, bacteria, nematodes and to a lesser extent pathogenic fungi and protozoa. Overall, hits relating to roots made up 16–19% of the total hits, mycorrhizal fungi 7–12%, saprotrophic fungi 3–4%, pathogenic fungi 3–16%, bacteria 3–28%, archaea 4–8%, earthworms 2–5%, enchytraeidae 0–3%, collembola 1–3%, acari 2–5%, nematodes 2–27%, protozoa 2–10% and algae 4–7%. The distribution of total hits per main ecosystem services group showed relatively similar patterns for Provisioning and Supporting services

relative to the five main biotic groupings, whereas Cultural services showed less hits for soil fauna and protists and Regulating services higher numbers for soil fauna.

### **Abstract evaluation and expert inputs**

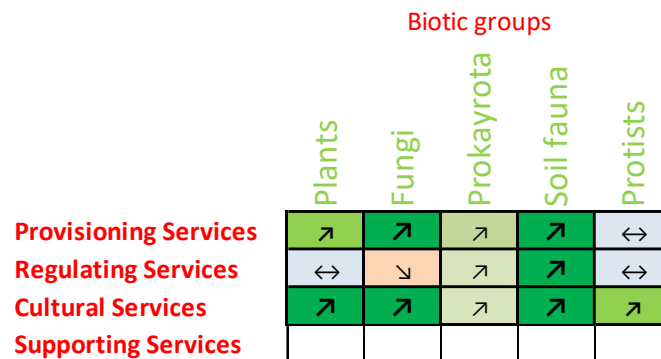
Based on the inspection of the 10557 abstracts, we explored whether the belowground diversity within each biotic grouping could be unidirectionally linked to given individual ecosystem services in forested ecosystems within Europe. After abstract inspection, only 602 out of the 10577 initial hits were potentially useful studies with 161 for Provisioning, 140 for Regulating, 243 for Supporting Services, and only 58 for Cultural Services. The relative frequency of the potentially useful studies also changed relative to the initial results obtained in the key word queries: the proportion of some biotic subgroupings (such as roots) decreased whereas the proportion for others (such as mycorrhizal fungi) increased or in the case of bacteria remained similar. Overall, fungi contributed with comparable numbers (but representing 24–74% of the total number of potentially useful hits depending on the ecosystem service grouping) to the number of potentially useful references. Plant roots contributed less than expected from total number of hits (only 3–6%). The biotic groupings prokaryotes, soil fauna and protists contributed with variable numbers (representing 14–23%, 3–34% and 0–17%, respectively). Cultural services were the least represented, with the exception of those obtained for fungi. Due to the difficulty in finding references related to Cultural Services, specific queries were run for this group of services. Expert evaluations of the full references and the work on the gathered data, along with the implementation of the references encountered during other queries, the input from expert advice as well as additional queries for cultural services, led to a final number of >458 references.

### **Soil biodiversity and ecosystem services**

The relationships between belowground biodiversity and ecosystem services were first evaluated by accounting for all the >458 studies obtained from the 481 combinations of ecosystem services and biotic sub-groupings. The large majority (>375 studies) showed a positive relationship between diversity and an ecosystem service, and a lower number indicated neutral and negative relationships. In a second step, the dominant trends for each of the 481 potential relationships were listed.

In the case of Provisioning Services, 24% of the investigated relationships between belowground biodiversity of a given biotic sub-grouping and an associated ecosystem service showed a positive relationship. These relationships, with one exception, were based only on one to three sources each. Three negative relationships were recorded (3%), and one relationship was evaluated as neutral (1%), but the large majority of possible combinations yielded no sources (no data available). For Regulating Services ten positive relationships were found (7% of all), two negative (1%), six neutral (4%) and 125 of the combinations had no data to evaluate whether there was a relationship or not (88%). Again, the relationships were all based on few sources only (1–4 sources). Comparatively, Cultural Services featured many more relationships: 54 (38%) were positive, two (1%) negative and three (2%) were neutral. As a result, only in the case of 84 combinations (54%) there were no data to evaluate the type of relationship.

The overall picture of the dominant relationships between the four main ecosystem service groups and five main biotic groups shows a majority of positive relationships (Figure 1).



**Figure 1.** Overall trends of the relationships between belowground biodiversity of different biotic groups and ecosystem services. Green, red and blue colours indicate positive, negative and neutral relationships, respectively. The intensity of the colour (and size of the arrows) indicate the number of studies underlying the dominant relationship with three levels: 1-3, 4-7 and 8 or more sources.

#### References

Millennium Ecosystem Assessment (2005) *Ecosystems and Human Well-being: Synthesis*. Island Press, Washington, DC. 155 pp.

## **WG2: summary of the activities**

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### **Introduction**

The subtitle describing WG2 is “*Microbial and faunal functional biodiversity in belowground food-webs*”. The ambition of our group since its inception has been to consider the interlinkages between ecosystem functions, the biotic community structure and its traits, and the environment (Fig. 1). Naturally, this has been an ambitious undertaking with a considerable width of scope. Over the course of the duration of the COST action, we have spent the WG-sessions of each BioLink meeting, along with a specific WG2-workshop, developing a range of focused activities where specific topics have been distilled into deliverable units that can be communicated and published. Some of these activities are still in development and are unlikely to be completed within the duration of the COST-action, but will instead provide the seeds to ongoing activities after its completion. Examples include (1) a review paper that explores the topic “On the dispersal and establishment of microbial species in soil”, (2) a database for methods in soil ecology linked to groups within our COST action that can host the use of such methods, and (3) a perspective paper focused on “Plant-soil-links: Achieving a holistic system analysis with trait-based approaches”. These seeds suggest that significant potential still remains within the community connected through the COST Action that can yet be tapped and translated into productive collaborative research endeavours. In addition to this range of topics that remain to be developed, the members of the WG2 of the COST action have also successfully pursued and developed research deliverables that are, or are very nearly, completed. These activities, along with contributor lists, are summarised in greater detail in the three subsections here following.

### **1. Towards a top-down framework to make microbial ecology predictive**

Type of activity: A perspective paper. Stage of completion: to be re-submitted for publication.

Contributors and co-authors: Johannes Rousk<sup>\*1</sup>, Lettice C. Hicks<sup>1</sup>, Kristin Rath<sup>1</sup>, Beat Frey<sup>2</sup>, Rasmus Kj  ller<sup>3</sup>, Martin Lukac<sup>4</sup>, Mari Moora<sup>5</sup>, and James T. Weedon<sup>6</sup>

<sup>1</sup>Section of Microbial Ecology, Department of Biology, Lund University, Ecology Building, 22362 Lund, Sweden; <sup>2</sup>Forest Soils and Biogeochemistry, Swiss Federal Research Institute WSL, Birmensdorf, Switzerland; <sup>3</sup>Department of Biology, Terrestrial Ecology Section, University of Copenhagen, Universitetsparken 15, 2100, Copenhagen, Denmark; <sup>4</sup>School of Agriculture, Policy and Development, University of Reading, RG6 6AR, UK; <sup>5</sup>Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu, Lai 40, 51005 Tartu, Estonia; <sup>6</sup>Systems Ecology, Department of Ecological Science, Vrije Universiteit Amsterdam, 1081 HV Amsterdam, The Netherlands.

**Summary:** We propose a framework to determine how microbial communities influence ecosystem processes. Rather than the established bottom-up approach where information on individual taxa is assembled to construct the function puzzle, we propose a top-down approach in three steps. 1. Develop tools to determine the dependence of microbial community processes on environmental factors (e.g., intrinsic temperature dependence of bacterial community growth rates) as measures of community response trait distributions. 2. Determine the whole-community contribution to ecosystem functions, by parametrising response trait distributions (from 1.) with current environmental conditions. 3. Synthesize 1. and 2. with taxonomic community composition by comparative analysis of characterisations of environmental gradients combined with experimental findings. This could identify “biomarkers” that can capture microbial communities’ regulation of ecosystem function and predict the susceptibility of

community structure and functions to environmental change. This framework can offer an alternative “top-down” complement to the “bottom-up” approaches currently dominating microbial ecology.

## **2. Seasons belowground**

Type of activity: A review paper. Stage of completion: Manuscript near completion.

Tentative list of contributors and co-authors: James Weedon, Marc Goebel, Tijana Martinovic, Jacob Heilmann-Claussen, Alvaro Lopex Garcia, Irena Macek, Saori Fujii, Johannes Rousk, Ina Meier, Mari Moora, Ivan Janssens, Hans De Boeck, Andrea Jesssen, Lettice Hicks

**Summary:** Seasonal variation in physiological processes, populations and communities of organisms are fundamental ecological phenomena that contribute to ecosystem patterns at every spatial scale from single plants to the global atmospheric CO<sub>2</sub> concentration. The annual cycle in terrestrial ecosystems of spring budburst, canopy expansion, senescence and litter fall are so familiar as to almost seem trivial – yet the timing and duration of these phenological events set important constraints on the temporal dynamics of ecosystems. Although the phenology of above-ground processes in terrestrial ecosystems is a well-studied field, comparatively little is known about the existence and nature of analogous patterns in below-ground organisms and processes. Such knowledge is important for a complete understanding of the functioning of soil ecosystems, and for predicting the possible consequences of ongoing and projected alterations in climate and hydrology in many systems. This project aims to perform a thorough literature search and produce quantitative syntheses of data and qualitative review of concepts related to seasonal patterns of soil populations and processes, at every level of organization from nutrient cycling (e.g. N mineralization / immobilization), to soil enzyme activities, to populations of micro- and macro-organisms (e.g. root turnover, microbial biomass, soil invertebrate populations). Our focus is on the following three questions: 1) How does seasonality in climate and hydrology translate to annual patterns in soil populations and processes? 2) Can seasonality of soil processes be predicted by the response of environmental processes to variation in seasonally changing conditions (e.g. temperature, moisture, substrate input)? 3) What are the key knowledge gaps related to understanding intra-annual dynamics in soil ecosystems? Our review will identify recurring patterns, links to climatic and vegetation seasonality, and highlight important directions for future research.

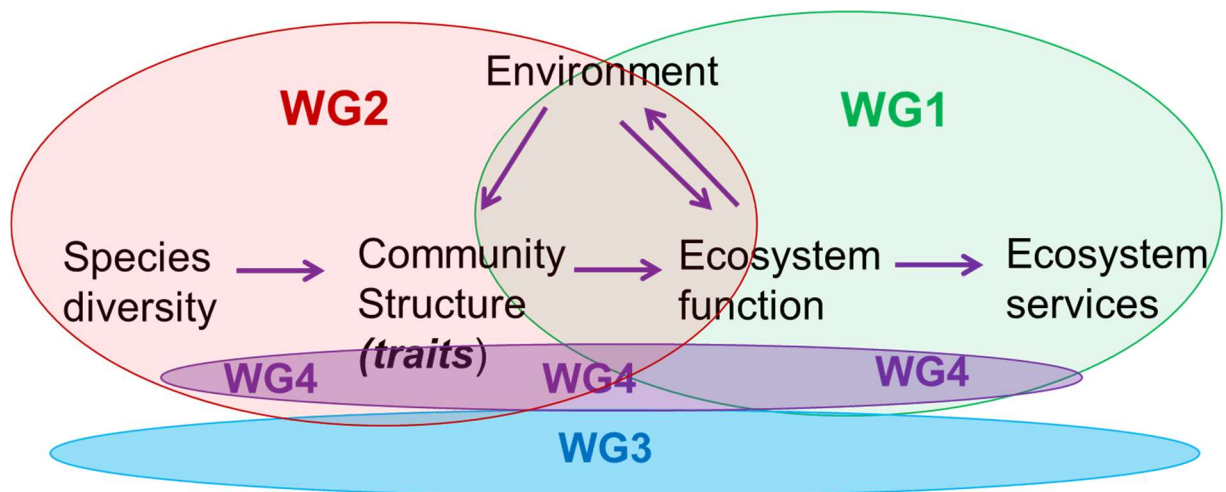
## **3. The analysis of the CENTURY – investigating how the microbial adaptation to environmental temperature affect the predictions of a global cycling model**

Type of activity: A research paper. Stage of completion: Data-analyses completed, MS in draft form.

Tentative list of contributors and co-authors: Lettice Hicks, Eva Berglund, David Wårlind, Adrian Gustafsson, Stefan Olin, Albert C. Brangarí, Johannes Rousk

**Summary:** Soils are a globally important store of terrestrial carbon (C), vulnerable to loss due to climate change. Empirical studies have shown that soil microorganisms – which regulate soil C stocks – can become temperature adapted, which will affect their response to future climatic warming. However, this is not represented in current Earth System models, which are currently parameterised by a single fixed rate-modifier for temperature for all ecosystems of the globe, and with no differentiation between the temperature responses of microbial growth and respiration. We investigated how microbial temperature adaptation influenced global simulations of soil C cycling, by integrating a flexible temperature rate modifier in an Ecosystem model (LPJ-GUESS). The temperature responses of microbial growth and respiration were allowed to vary as a function of local climate (mean temperature of three warmest months), based upon empirical results from a survey of 56 European soils spanning a wide climatic gradient (–4 – 18 °C MAT, 300 – 2000 mm year<sup>–1</sup> MAP), and observations from a long-term soil-warming experiment in a temperate forest (“Harvard Forest LTER”, +5 °C for 9 years). Global simulations,

including the new flexible temperature rate modifiers for growth and respiration, resulted in differences in soil C pool sizes and net mineralisation rates, compared to simulations where a single fixed temperature function was used. In particular, use of the flexible modifier resulted in increased soil C in Northern boreal systems, and a seasonal change in net mineralisation with feedbacks to plant nutrition and consequently for the plant functional group composition. Use of separate flexible modifiers for growth and respiration also lead to differences in microbial carbon use efficiency, with implications for C release from soil to the atmosphere and simulated soil C pools.





### **WG3: summary of the activities**

Mauro Gamboni

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<b>WG3 LEADER:</b>	MAURO GAMBONI
<b>LEADER ASSISTANT</b>	PAOLA GRENNI
<b>VICELEADER</b>	CHRISTOS ATHANASSIOU
<b>VICELEADER</b>	MARTINA DI LENOLA (ECI)

#### **INTRODUCTION**

In the framework of the BioLink Cost Action, the Working Group No 3 (WG3) is involved in a peculiar task. While the general topic of belowground biodiversity dealt with in the Action concerns forests, WG3 pays attention to plantations and tree crops. Its objective is “to collate and exchange recent findings from experimental and observational studies of belowground biodiversity in perennial tree crops and simplified ecosystems”, as well as “to compare experimental and model results from natural forests and tree crops concerning effects on soil biodiversity”. This leads to a direct link with the agricultural practices. The participants are therefore called to share their scientific knowledge and experiences and assess the importance of belowground diversity with a precise focus on making more sustainable the intensively managed agro-ecosystems. With this aim, WG3 finalized its effort on the role of soil biological communities in fostering resilience and productivity in tree crops. Aware that belowground biodiversity is a key resource for maintaining the functioning of tree crop ecosystems, in the first WG3 meeting held in Reading (UK), a brainstorming among all participants led to identify possible topics mainly to consider and tasks to be done (annex 1) and a Working Paper has been prepared, giving some ideas on how to lead the WG3 activities (annex 2). Following the suggestions emerged by this preliminary meeting and after further considerations, a number of themes and actions were selected and discussed in the subsequent WG3 meetings, allowing to reach some concrete outcomes. They include elaborating scientific reports and publications, proposing STSM, organizing training school as well as establishing partnerships for submitting research project proposals.

#### **ACTIVITY**

More than 20 researchers, coming from different Countries, participated to the different WG3 meetings (tab. 1). Their large expertise, scientific competencies and skills guaranteed an interdisciplinary and holistic approach in addressing the study on belowground biota. On the basis of this, WG3 members have been divided into four sub-groups concerning belowground biodiversity in tree crops and plantations:

- SOIL BIODIVERSITY
- INTERACTION BETWEEN SOIL ORGANISMS AND ABOVEGROUND ECOSYSTEMS
- INTEGRATED CULTIVATION SYSTEM, SUSTAINABLE MANAGEMENT AND INTEGRATED PEST MANAGEMENT
- SOIL CONSERVATION

Apart from the scientific community, WG3 considered as a target audience different stakeholders including farmers and tree crop growers. Data and information collected, discussed and deepened have been useful for defining activity and reaching expected results

#### *Sub-group “Soil Biodiversity”*

Led by Andy Smith from UK. Its specific objective is to exchange knowledge and to deepen studies on the main factors affecting belowground biodiversity in tree crop systems, such as intensive

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management; previous land use; plant species composition. Different topics to investigate emerged from this, including the role of different species and functional diversity in driving ecosystem processes; how belowground communities adapt to rapid environmental change; what are the mechanisms that shape biological communities at different spatial and temporal scales and the role of belowground biodiversity in promoting resilience to stress.

*Sub-group “Interaction between soil organisms and aboveground ecosystems”*

Led by Jesús Mercado-Blanco from Spain. The work addresses some crucial aspects in the sustainable management of tree crops. Although the close relationship between soil organisms and plants is known there is still many aspects to be investigated. A number of possible areas of investigation has been proposed and include: how the plants respond to the interaction with soil microbes; how microbiome promote the plant growth; how the soil microbiome composition and interconnection with plant can change passing from natural forest to tree crops.

*Sub-group “Integrated Cultivation System, Sustainable Management and Integrated Pest Management”*

Led by Christos from Greece and specifically devoted to binding the “BioLink” concept to sustainable agro-practices in tree crops. The aim of this sub-group is to merge this knowledge with those coming from the other sub-groups. This includes understanding belowground biodiversity in “self-defense” mechanisms, improving productivity and reducing use of resources;

*Sub-group “Soil Conservation”*

Led by Miglena Zhiyanski from BG and addresses to promote alternative regimes combining conservation and sustainable use of soil in tree crops. In this framework some objectives have been proposed: look at intensive plantations and tree-crops in relation to historical management; provide direction for future and more sustainable use of soil; deliver new knowledge on key indicators of changes in soil functions and its biodiversity in intensive plantations and tree-crops.

## **MAIN FINDINGS**

1. A 350-page book has been published in 2017. Different WG3 members provided significant contributions and three of them are editors. A final chapter is devoted to tree crops.  
Springer – Series “Sustainability in Plant and Crop Protection”

### **Soil Biological Communities and Ecosystem Resilience**

**Martin Lukac<sup>1</sup>, Paola Grenni<sup>2</sup>, Mauro Gamboni<sup>3</sup> (Editors)**

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<sup>2</sup>Water Research Institute, National Research Council of Italy (CNR-IRSA), Italy

<sup>3</sup>Department of Biology, Agriculture and Food Sciences, National Research Council of Italy (CNR-DiSBA), Italy,

#### *Summary*

This volume provides an overview of some of the most recent developments in understanding the contribution of soil biodiversity to ecosystem function. The findings and methodologies presented in individual chapters present a window into the largely invisible world of soil-dwelling organisms. Their biodiversity and the range of functions they provide will be affected by environmental changes in the near future – likely agents including climatic change, nitrogen deposition or spread of invasive species. This volume aims to showcase to current knowledge of belowground diversity and thus contribute to our effort to preserve biodiversity in all of its forms. The last chapter provides a review concerning the perspective on the potential of studies in the management of tree crops, by identifying main drivers and services underpinning the implementation of sustainable strategies.

2. A review paper concerning the relationship between soil biota and aboveground ecosystems has been submitted to the Journal Frontiers in Microbiology in the Special Section “Plant Microbe Interaction”.

### **Belowground Biotic Interactions for Healthy Tree Crops**

**Jesús Mercado-Blanco<sup>1</sup>, Isabel Abrantes<sup>2</sup>, Anna Barra Caracciolo<sup>3</sup>, Annamaria Bevivino<sup>4\*</sup>, Aurelio Ciancio<sup>5</sup>, Paola Grenni<sup>3</sup>, Katarzyna Hryniewicz<sup>6</sup>, László Kredics<sup>7</sup>, Diogo N. Proença<sup>8</sup>**

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<sup>8</sup>CEMMPRE and Department of Life Sciences, University of Coimbra, Portugal

#### **Summary**

The review discusses below-ground biotic interactions influencing the growth of tree crops. The study of tree-(micro)organism interactions taking place at the belowground level is crucial to understand how they contribute to processes like carbon sequestration, regulation of ecosystem functioning, and nutrient cycling. A comprehensive understanding of the relationship between roots and their associate microbiome can also facilitate the design of novel sustainable approaches for the benefit of these relevant agro-ecosystems. This review summarizes the methodological approaches to unravel the composition and function of belowground microbiomes, the factors influencing belowground microbiomes associated with tree crops, their benefits, and harm, with a focus on representative examples of Biological Control Agents (BCA) used against relevant biotic constraints of tree crops. Finally, we add some concluding remarks and suggest future perspectives concerning the microbiome assisted management strategies to sustain tree crops.

In September 2017, WG3 promoted the organization of one week training school. 25 students (master and PhD) attended the school following practical and theoretical lessons. The location was the international institution CIHEAM-IAMB with a long experience in high training activities.

### **Training School on “Soil Biodiversity in Tree Crops”**

**Trainers and organizers from WG3: Isabel Abrantes, Aurelio Ciancio, Paola Grenni, Jesús Mercado-Blanco<sup>1</sup>, Isabel Abrantes, Annamaria Bevivino, Diogo Poenca, Mauro Gamboni**

#### **Summary**

The main aim of the training school is to provide updated concepts and methodologies on soil biodiversity in tree crops. The school addressed a multidisciplinary approach that include deepening of knowledge in different topics concerning soil, such as: biodiversity composition; physical, chemical and biological properties; food webs and ecosystem services; methods for assessing diversity; molecular markers; metagenomic analysis; relationship between below-ground and up-ground biodiversity.

3. WG3 members carried out a survey on the perception of importance of belowground biodiversity by the farmers. This activity should be followed by the preparation of guidelines. Results of the survey will be presented at the BioLink Granada meeting and a publication is under consideration.

### **Quantifying the power of awareness of belowground biota: perceptions differ among European countries.**

**Maria K. Sakka<sup>1</sup>, Isabel Abrantes<sup>2</sup>, Marina Katanic<sup>3</sup>, László Kredics<sup>4</sup>, Arianna Latini<sup>5</sup>, Tamás Marik<sup>4</sup>, Silvana Moscatelli<sup>6</sup>, Mauro Gamboni<sup>6</sup>, Martin Lukac<sup>7</sup>, George Viontzos<sup>1</sup>, Christos G. Athanassiou<sup>1</sup>**

<sup>1</sup>University of Thessaly, Department of Agriculture, Crop Production and Rural Development, Greece.

<sup>2</sup>University of Coimbra, Coimbra Department of Life Sciences/Centre for Functional Ecology (CFE), Portugal.

<sup>3</sup>University of Novi Sad, Institute of Lowland Forestry and Environment, Serbia.

<sup>4</sup>University of Szeged, Department of Microbiology, Hungary.

<sup>5</sup>ENEA Casaccia Research Center, Energy Efficiency Department, Italy.

<sup>6</sup>National Research Council of Italy, Department of Biology, Agriculture and Food Sciences, Italy.

<sup>7</sup>University of Reading, School of Agriculture, Policy and Development, UK.

#### **Summary**

The awareness level of farmers on the importance of soil biodiversity in tree crop ecosystems has been tested. More than 380 farmers from Greece, Hungary, Italy, Portugal and Serbia joined the survey. Basically, a general farmers' attention on the relevance in adopting practices to promote belowground



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biodiversity has been registered. These preliminary results call for elaborating useful guidelines destined to farmers in order to enlarge the knowledge and encourage the use of agricultural practices towards soil biodiversity maintenance and improvement.

4. In June 2017, an ERA-NET project proposal titled “Effect of climate conditions on belowground carbon turnover and soil biodiversity in European forests - backbone” has been done by a partnership emerging from discussion inside BioLink. The proposal has not passed but the experience was very instructive for any subsequent proposals to be prepared on the basis of evaluation obtained. A new project proposal have been submitted and is now under assessment.

## **ANNEXES**

1. **Findings on the first WG3 meeting**
2. **Position paper**
3. **List of BioLink WG3 participants**

**NOTE ON THE FIRST OF WG3 MEETING  
Reading, 6 November 2014**

**LIST OF MAIN TOPICS EMERGED DURING BRAINSTORMING  
Reading, 5 November 2014**

**Issues:**

- Effect of tree crops intensive management on soil biodiversity
- Exploring same tree crops with different soil management and cultivation systems
- Plant species with complementary functions and soil biodiversity
- How does land-use history affect soil biodiversity?
- Integrated Pest Management (IPM) and soil biodiversity
- Soil conservation potential in:
  - increasing yield
  - mitigating of negative impacts
- Relationship between tree crops resilience capacity and belowground biodiversity
- Relationship between soil biodiversity and plant nutrition

**LIST OF MAIN TOPICS EMERGED DURING WG3 MEETING  
Reading, 6 November 2014**

**General issue: more detailed information on functions linked aspects**

**Issues or key words:**

- Carbon storage
- Ecological services
- Alien invasive species
- Bio-corridors effects
- Who is linked to who?
- Flux of microbiota from soil to rhizosphere
- Influence of different plant root species identity on belowground biota
- Deforestation and substitution with tree crops and reforestation
- Inter and intra management after disturbance
- Bio-control agents/phytoremediation on overall biodiversity

**TASKS**

1. consider what initiatives, in the framework of the tasks assigned to WG, can be carried out (conference and workshop and related abstract books, training schools, scientific articles, state-of-art reports, technical handbook, STSM and ESR involvement, etc.)
2. better define topics on which the WG should concentrate, mainly considering the Task 4 of the MoU;
3. start to finalize next WG meeting in March/April (Bulgaria, Poland):
  - a. propose few large topics (maximum 4/5 topics) that can be better detailed in sub-topics and usefully investigated, thanks to the expertise present in the WG;
  - b. this work could be concluded at the beginning of next year (January/February);
4. define a large subject around which to base the next annual Cost Action meeting in November in Rome:
  - a. Preliminarily, we thought to the great concept of Resilience that could contain all the considered topics.;
  - b. We should find a title of the annual meeting understandable, largely comprehensive and appealing;



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- c. Title, rationale and possible preliminary program of the next annual Cost Action Meeting could be launched during the WG3 meeting in March/April

**Some earliest suggestions for the title of the next Cost Action meeting:**

- Belowground and aboveground biodiversity: how is the linking?
- Belowground and aboveground interrelation: more biodiversity & more resilience
- Belowground interactions: soil biodiversity, function, ecosystem processes





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November 2014

*prepared by Mauro Gamboni - WG3 Coordinator*

*Contributions from Anna Barra Caracciolo, Paola Grenni, Martina Di Lenola*

**COST ACTION FP1305**

**“Biolink: Below-ground biodiversity and ecosystem function in European Forests”**

**WG3**

**“Belowground biodiversity in plantations and tree crops”**

**Working Paper n. 1**

Rome, 29 November 2014

**PRELIMINARY REMARKS**

One of the main objectives of *Biolink Cost Action* is to create a forum where current understanding of functional belowground biodiversity can guide the development of prescriptions for sustainable forest and tree crops management.

This overall objective has to be pursued through the activities of four Working Groups (WGs) whose tasks are described in the MoU. This includes a collaborative program of knowledge exchange and training, taking into account the specific objectives and expertise of each WG.

**THE WORK IN PROGRESS**

Following this approach, the WG3 “Belowground biodiversity in plantations and tree crops” has firstly analyzed its specific objective and identified the task that better fits with it. In this regard, the expertise present in the WG3 was collected, through delivered profiles from each member. The findings of the *Reading brainstorming* and *WG3 meetings* have been also analyzed and organized, allowing the definition of preliminary major issues and related topics to be considered in order to reach the specific WG objective and fulfill the relevant task. This has led to recognize the main area of investigation and to consider the opportunity to carry out the work of WG3 in sub-groups devoted to deal with specific themes. Finally, preliminary considerations on the kind of activities that could be carried out have been done. They should be shared and further evaluated for their concrete realization. At the same time, the WG3 members have identified the need for further expansion to additional areas of expertise (i.e. systematic and molecular experts etc.).

**THE WG3 OBJECTIVE**

The objective of WG3 regards the assessment of the importance of belowground diversity in intensively managed agro-ecosystems, taking into account that belowground biodiversity is a key resource for maintaining the functioning of ecosystems. To reach this objective, the WG3 members will furnish research results from Institutions/Country to which they belong, specifically in the investigation sphere concerning plantations and tree crops. These data and information will be useful for preparing scientific reports, publications, case study analyses and any other initiatives, including workshops, conferences, as well as networking for preparing eventual research project proposals.

#### SCIENTIFIC TASK TO BE PERFORMED

The task 4, included in the MoU, is what that better fits with WG3 objective. It concerns the promotion of remote knowledge on the role of soil biological communities in fostering resilience in tree crops. These crops could be considered as a succession to previous forest ecosystems and/or as agro-ecosystems integrated with contiguous forests. Their resilience and productivity are affected by functional diversity of soil biota and its stability, which in turn depends on the genetic diversity of the concerned species. A knowledge exchange should be also provided on the principle of “*biodiversity insurance hypothesis*” for verify its applicability to these ecosystems. The WG3 scientific task findings could include recommendations destined to the practitioners, managers and policy makers, as to the feasibility of harnessing the belowground biodiversity to support production sustainably.

#### COLLECTION OF THE WG MEMBERS EXPERTISE

Around 20 researchers have joined the WG3. Their expertise includes many scientific competences, allowing to perform the work in a very interdisciplinary way. In addition, the WG3 members come from different Countries and are active in different types of eco-zones. This will allow covering the major part of Europe and represents an important issue because of the possibility to have data and information coming from a large geographical area. The profiles provided by each WG3 member will be in depth analyzed and organized to create an internal database of skills useful in carrying out the activities in a very effective way.

#### REPORT ON THE READING FINDINGS

In annex 1, the presentation performed during the last WG3 meeting is reported while, in annex 2, the findings of Reading meetings are summarized. They show a wide range of issues, topics and key words to be considered in the ongoing work. However, a clear recommendation seems to generally emerge from the WG3 meeting debate. It concerns to focus on functional linked aspects.

#### THE MAIN AREA OF INVESTIGATION AND ITS DEVELOPMENT

The focal elements, around which the WG3 activities will be developed, are therefore mainly related to the “ecological functions” of soil biodiversity and its linkage with the aboveground tree crops (some tree crops can be considered such as walnut, chestnut, olives, oak, vineyard or other perennials). This consideration derives from the main outcomes registered during the WG3 meeting and represents the large investigation area on which WG3 should try their hand. This is coherent with the WG3 objective and related scientific task. Following this approach, it could start from the theoretical models related to the distribution of functional groups in soil ecosystem and then to continue in the recognition of practical experiences on different agro-ecosystems along a gradient ranging from natural systems through to more or highly/intensively managed ecosystems. Based on that, it intends to create a connection between three basic concepts: BIODIVERSITY, FEATURES and SERVICES, considering FUNCTION as topic core of the correlation among these concepts. In particular, the WG3 aims at defining how soil biodiversity is related to soil functions and how ecosystem services, in turn, depend on soil biodiversity.

The starting point concerns the different role that organisms have in the belowground. According to the EC Final Report (2010) on *Soil biodiversity: functions, threats and tools for policy makers*, this role can be subdivided into three large functional groups:

- Soil ecosystem engineers
- Biological regulators
- Chemical engineers/microbial decomposers.

This could be the basis of approaching the work in this first phase. Each WG3 member will be asked to distribute along the three functional groups, maintaining of course and anyway a global vision due to the systematic interaction among the groups.

#### HOW TO EARLY GO FORWARD

Each WG3 member, based on his/her experience, can make a contribution to knowledge in the different functional groups. The WG3 members are therefore invited to check the annexed matrix (annex 3) and fill it in. In the first column, each WG3 member should indicate issues or topics connected to the functional group to which he/she would like to join (highlight the functional group) and that deserve to be investigated further, under the basis of a holistic approach in belowground biota. For choosing issues and topics, what is reported in annex 2 could be taken into account (the list is not exhaustive). This leads to have a distribution based on the different expertise, allowing to carry out the ongoing work finalized to perform studies on research results, to elaborate scientific reports, to organize workshops as well as to prepare the next WG3 meeting to be held on March/April 2015.

#### IDENTIFICATION OF TARGET AUDIENCES

In the framework of WG3, apart from the scientific community, a target audience regards tree crop growers. For this purpose, within WG activities, state-of-the-art reports, final reports and case study reports in technical journals, as well as review papers have to be developed.

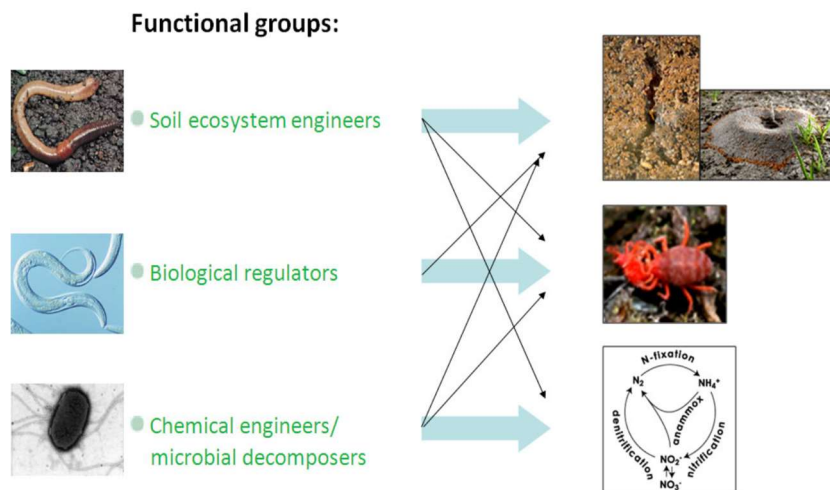
#### FINAL REMARKS

In order to go on in an effective way, the WG3 members are asked to:

- make comments and provide suggestions on the above illustrated approach
- deliver the matrix (annex 3) filled
- remember the COST ACTION general objective, scope and tasks of WG3 (annex 1)
- consider the conclusion of Reading meeting included in the note (annex 2)
- think about the activities to be carried out (point 3 of annex 2).

#### ACKNOWLEDGEMENT

The conceptual basis of this preliminary paper was discussed in a workshop in Rome with the following invited experts: Anna Barra Caracciolo, Aurelio Ciancio, Martina Dilenola, Paola Greni and Isa Pentimone (*via skype*). These issues have been also directly discussed with Christos Athanassiou and Annamaria Bevivino. All their valuable comments are hereby gratefully acknowledged.

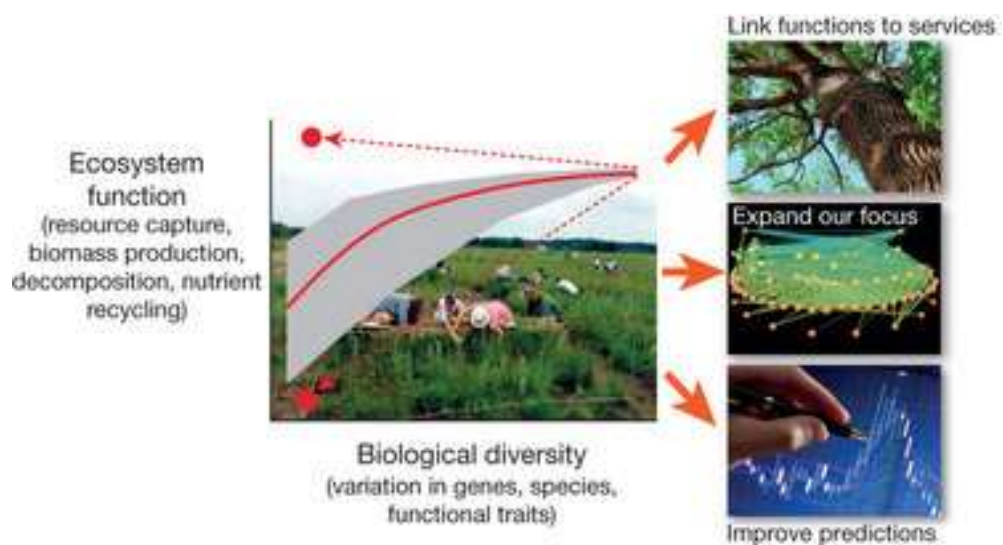


*European Commission, 2010. Soil biodiversity: functions, threats and tools for policy makers.  
Technical Report - 2010 – 049.  
[http://ec.europa.eu/environment/archives/soil/pdf/biodiversity\\_report.pdf](http://ec.europa.eu/environment/archives/soil/pdf/biodiversity_report.pdf)*

### ***THE FORM OF A TYPICAL DIVERSITY–FUNCTION RELATIONSHIP***

*This Figure below reported conceptual diagram summarizes what we know about the shape of the biodiversity–ecosystem functioning (BEF) relationship based on summaries of several hundred experiments.*

*The red line shows the average change across all combinations of genes, species, and/or traits. The grey polygon represents the 95% confidence interval, whereas red dots give maximum and minimum values of the most or least productive species grown alone in monoculture (see main text about uncertainties associated with the upper bound). To improve our predictions of how diversity loss influences the goods and services of ecosystems, we must now take this experimental relationship and (1) link the ecosystem functions measured in experiments to the provisioning and regulating services of ecosystems; (2) expand the focus of research to better mimic realistic extinction scenarios and trophic structures of natural ecosystems; and (3) develop mathematical models that can scale experimental results to whole landscapes.*



*Images from D.T., N. Martinez and Shutterstock.com; used with permission.*

*(B.J. Cardinale, J. E. Duffy, et al. 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67).*

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List of BioLink WG3 participants						
	MAIN GENERAL TOPICS					
	SOIL BIODIVERSITY	INTERACTION BETWEEN SOIL ORGANISMS AND ABOVEGROUND ECOSYSTEMS	INTEGRATED CULTIVATION SYSTEM, SUSTAINABLE MANAGEMENT and INTEGRATED PEST MANAGEMENT	SOIL CONSERVATION	e-mail	Affiliation
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Nonic Marina (Serbia)	X		X		<a href="mailto:marina.nonic@sfbg.ac.rs">marina.nonic@sfbg.ac.rs</a>	University of Belgrade · Department of Forestry
Pentimone Isabella (Italy)			X	X	<a href="mailto:isapentimone@yahoo.it">isapentimone@yahoo.it</a>	IPSP-CNR
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Proença Diogo		X	X		<a href="mailto:diogo.proenca@gmail.com">diogo.proenca@gmail.com</a> <a href="mailto:diogo.proenca@uc.pt">diogo.proenca@uc.pt</a>	Universidade de Coimbra, Faculdade de Ciências e Tecnologia, IMAR-CMA Marine and Environmental Research Centre, Portugal
Sijacic-Nikolic Mirjana (Serbia)		X	X		<a href="mailto:svacic68@open.telekom.rs">svacic68@open.telekom.rs</a>	University of Belgrade, Faculty for Forestry, Serbia
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## **WG4: summary of the activities**

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### **Modelling soil biodiversity: key goals**

At the start of this COST action, very little was defined. And the first meetings were necessary to pin down exactly what the feasible goals were within a COST action framework. They were decided as:

- Review the existing knowledge on different scales and types of diversity (functional diversity, number of species,...) and how they affect soil functioning (C, nutrients and water). To be published as review, submission spring 2018
- Create a 'plug-in' soil model at an ecosystem scale which does not require too many parameters but does include major effects of soil diversity. To be submitted spring 2018.

### **Core conceptual model**

The core of the developed BIOLINK model is combining the foodweb, traditional SOM concepts and soil structure as key determinants of soil functioning, developed within BIOLINK and KEYSOM COSTactions.

- Litter and SOM decomposition is an active process by microbes and soil fauna
- The distinction between the fungal and bacterial pathway is necessary
- Mycorrhizal fungi need to be represented in the model concerning their interaction with the plant (important input of C to the soil), decay of SOM and effect on aggregation
- Soil fauna play a crucial role in soil in SOM transformation and stabilization as well as in engineering (bio pore formation and bioturbation)
- Besides soil engineers the absolute minimal number of food web levels is 3 steps (microbes-microbivores & non-engineer detritivores-predators). The diversity and number of levels changes with soil types/ecosystems.
- SOM decomposition is controlled both by accessibility (pore size) and by quality (recalcitrance and CN ratio)

- Pore size distribution determines the accessibility to all soil biota, but also the hydrology and the availability of O<sub>2</sub>
- Pore size distribution is a dynamic function of biota and initial soil structure

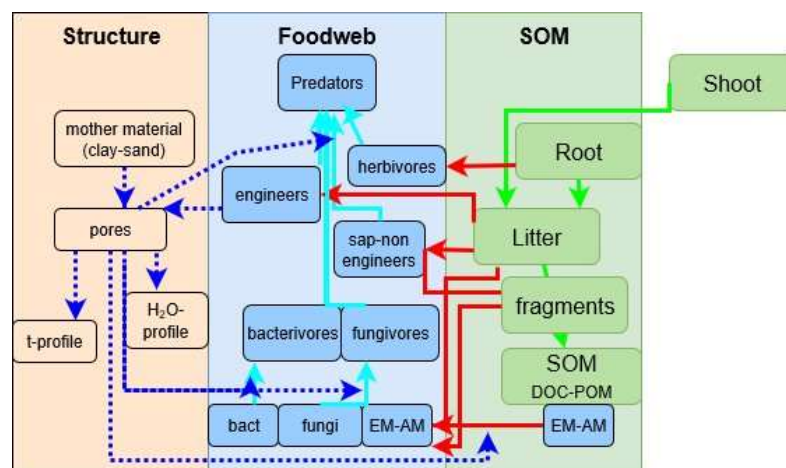
**Inaccessible pores** (<0.1 µm): inaccessible (within the micro-aggregate, organo-clay interaction), water is held there but not available to plants (measured from wilting point), linked to clay content & type. This is the residual soil water content

**Bacterial pores** (0.1 µm-2 µm): accessible only to bacteria, within macro-aggregates, pores in loam. Water in these pores is accessible to the plants.

**Micropores** (2-30 µm): these pores are not accessible to macro-and meso-fauna and most predators, but accessible to bacterivores, fungivores, fungi, mycorrhizae and bacteria. Water is held at field capacity but available to plants. Pores in sandy soil are in this range, as are the pores within macro-aggregates (>25 µm).

**Mesopores**: (30µm-1.5mm) pores where most soil mesofauna can penetrate (not macrofauna), also formed by fine roots and between large macro-aggregates. Mesopore volume can be determined from field capacity (but this includes macro pores). These pores are aerated also at field capacity, but dry out below field capacity.

**Macropores** (>1.5mm): cracks or biopores formed by soil engineers. They are of vital importance to calculate hydrology as preferential flow through these pores has a major impact on infiltration rate. The first pores to have O<sub>2</sub> when water level is above field capacity, but dry out very fast below field capacity. Macropore volume cannot be measured from soil water retention only, but can be visually assessed.



**Fig. 1.** Model flowchart. Red and blue full lines: C flows, dotted lines: influence.



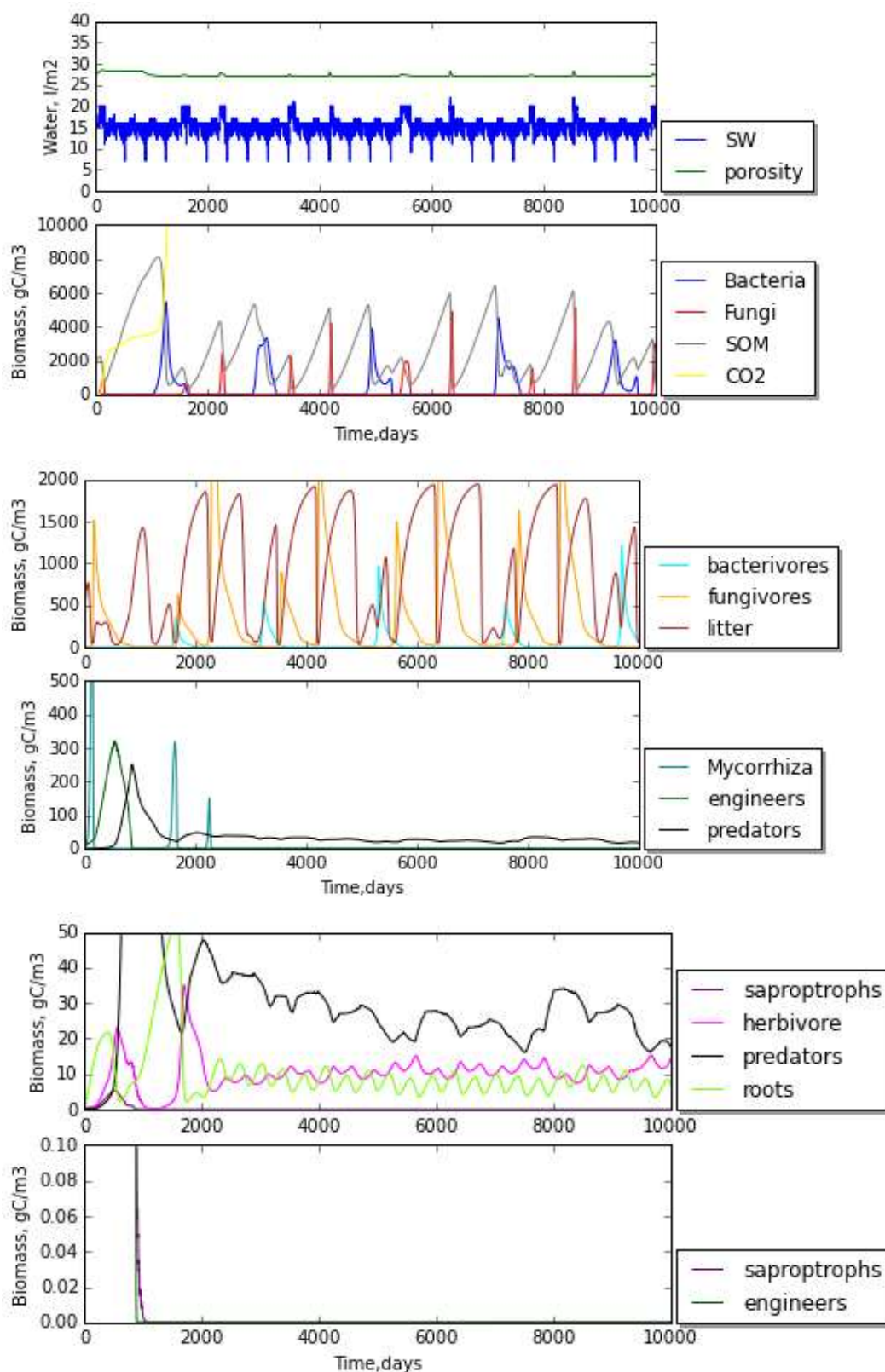
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**Model implementation:**

- The model was coded in python, using python(x,y) an open source scientific python environment
- The 'first final version' of the model code will be available online by end of April 2018 and will be open-source
- The number of input parameters is limited, and suggested values for dominant ecosystems will be supplied
- Model verification and sensitivity analyses has been performed. The model reacts as expected concerning leaving out classes of soil fauna, changing climate (t or precipitation), effect of engineers on structure and impact on hydrology and C-sequestration

**Model output**

In the following graphs a sample run of the model over 10.000 days is shown showing the patterns of the different faunal groups, the effects on soil water (SW) and soil organic matter (SOM). Note the difference in Y-axis, due to large differences in total biomass of the different micro-meso- and macro-faunal groups.



**Fig. 2.** KEYLINK model outputs of C-pools for a hypothetical soil over 10,000 days



**BioLink**

[www.bio-link.eu](http://www.bio-link.eu)

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**Take home message**

We hope to apply the KEYLINK model widely to all kinds of ecosystems and climates...preference for sites with data on litter, soil structure, soil fauna, SOM and driving variables climate and soil water. The code is quite simple, and python(x,y) is open source as is the KEYLINK model. Contact us for information or help.

