







## Soil Biodiversity and Ecosystem Services

# MEETING PROGRAMME and ABSTRACTS

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

> 4th Annual Meeting Sofia, 12-14 April 2016







### Dear Participants,

It is our pleasure to host the 4th Meeting of COST Action FP1305 BioLink in Sofia, Bulgaria, from 12th to 14th April 2016.

Following previous meetings in Reading, Krakow and Rome, the focus of this event will be the relationship of belowground biodiversity to ecosystem services provided by forest and tree crop ecosystems. In the last decades, we have all witnessed the enormous research effort directed at documenting biodiversity loss and elucidating its impact on ecosystem functioning, including services and goods that ecosystems provide. However, the majority of research has been focused on the aboveground biota and much less on the unseen soil organisms. Studying the underground biota and its complex relationships is far more difficult and challenging, and this meeting will give us an opportunity for interesting discussions sharing our knowledge and experience, and meeting new colleagues.

The objectives of the BioLink network are:

- To summarise current knowledge of belowground biodiversity in forest ecosystems across a management intensity gradient.
- To integrate new theoretical and technological advances in biodiversity research in the forest ecosystem research community.
- To provide a focal point for the dissemination of information about forest biodiversity and ecosystem function.
- To collate and exchange recent findings from experimental and observational studies of belowground biodiversity in perennial tree crops and simplified ecosystems.
- To compare experimental and model results from natural forests and tree crops concerning effects on soil biodiversity.
- To identify new cross-disciplinary research areas.

We hope you will all enjoy the meeting and the city.

From the organisers.



### Organising and scientific committee:

(alphabetically)

- Boyko GEORGIEV, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences
- Elena VANGUELOVA, Forest Research, Farnham, UK
- Gergana VASSILEVA, Institute of Biodiversity and Ecosystem Research, BAS
- Ivan YANCHEV, Institute of Biodiversity and Ecosystem Research, BAS
- Lyudmila LOZANOVA, Institute of Biodiversity and Ecosystem Research, BAS
- Margarita GEORGIEVA, Forest Research Institute, BAS
- Maria GLUSHKOVA, Forest Research Institute, BAS
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- Martin LUKAC, Reading University, Reading, UK
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- Radka FIKOVA, Institute of Biodiversity and Ecosystem Research, BAS
- Stela LAZAROVA, Institute of Biodiversity and Ecosystem Research, BAS
- Vlada PENEVA, Institute of Biodiversity and Ecosystem Research, BAS





### **Programme**

#### Park Hotel Moskva

### Day 1, April 12th (Tuesday) 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 Vanguelova and Douglas Godbold

- 10.00-10.30 **Kevin R. Butt** Earthworms and ecosystem services *University of Central Lancashire, Preston, UK*
- 10.30-11.00 **Lyubomir Penev** One Ecosystem of journals: Data and narrative-integrated publishing on Pensoft's ARPHA Publishing Platform *Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria*

#### 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 **Gergely Boros** The effects of forestry treatments on enchytraeid worms (Annelida, Oligochaeta) in a Hungarian sessile oak-hornbeam forest *Centre for Ecological Research, Institute of Ecology and Botany, Hungary*
- 11.50-12.10 **Francis ASHWOOD** Woodland restorationon landfill sites: Earthworm activity and ecosystem service provision *Forest Research, Alice Holt Lodge, Farnham, Surrey, UK*
- 12.10-12.30 **Vlada Peneva** Soil nematode diversity ecosystem functions, services and disservices *Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria*
- 12.30-12.50 **Diogo Neves Proença** Microbiomes of potato-cyst-nematodes isolates kept in laboratory conditions and from environment *CEMUC*, *University of Coimbra*, *Coimbra*, *Portugal*
- 12.50-13.00 **Isabel Abrantes** (short communication)

#### 13:00-14:00 Lunch





#### 14:00-15:30 Plenary Session Part 3

Session chaired by Annamaria Bevivino and Ivano Brunner

- 14.00-14.30 **Alessio Mengoni** (Invited speaker) The pan-bacteriome approach. A way to decipher the puzzling diversity and dynamics of bacterial community in soil

  Department of Biology, University of Florence, Sesto Fiorentino, Italy
- 14.30-14.50 **Manuel Fernández-López** Analysis of the rhizospheric microbial communities of *Quercus pyrenaica* along an altitudinal gradient *Soil Microbiology Dept.*, *Estacion Experimental del Zaidin*, *Granada*
- 14.50-15.10 **Taina Pennanen** Digging the mechanisms behind the growth of spruce role of diversity of ectomycorrhizal fungi *Natural Resources Institute Finland (Luke), Vantaa, Finland*
- 15.10-15.30 **Petr Baldrian** Forest microbiome habitat diversity, dynamics, and ecosytem functioning *Laboratory of Environmental Microbiology, Institute of Microbiology of the CAS, Praha, Czech Republic*

#### 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** Bacterial and fungal communities in rhizosphere of cork oak (*Quercus suber* L.): Linking microbiome and forest health *Biocant Park, Cantanhede, Portugal*
- 16.20-16.40 **Lars Vesterdal** Is tree species diversity or tree species identity the most important driver of European forest soil carbon stocks?

  Deptartment of Geosciences and Natural Resource Management, University of Copenhagen, Frederiksberg, Denmark
- 16.40-17.00 **Brynhildur BJARNADÓTTIR** The effect of deforestation on streamwater ecosystems in Iceland *University of Akureyri, Iceland*
- 17.00-17.20 **Dessie Assefa** Variability in fine root morphology, biochemistry, and litter quality indices of ten tropical tree species *Institute of Forest Ecology, BOKU, Vienna, Austria*
- 17.20-17.40 **Marc Goebel** Fine root production and phenology among 11 temperate, mature tree species in a common garden in Poland *Department of Natural Resources, Cornell University, Ithaca, USA*

18:00-20:00 Wine reception (Restaurant "Panorama", Park Hotel Moskva)



### Day 2, April 13th (Wednesday)

09.30-10.30 Networking and poster session **(Ground floor)** 

#### 10:30-11:00 Coffee break

#### 11:00-13:00 WGs sessions, Part 1

- WG1: Conference Room #5 (Ground floor)
- WG2: Conference Room #3 (Mezzanine floor)
- WG3: Conference Room #4 (Mezzanine floor)
- WG4: Conference Room #2 (Mezzanine floor)

#### 13:00-14:00 Lunch

#### 14:00-16:00 WGs sessions, Part 2

- WG1: Conference Room #5 (Ground floor)
- WG2: Conference Room #3 (Mezzanine floor)
- WG3: Conference Room #4 (Mezzanine floor)
- WG4: Conference Room #2 (Mezzanine floor)

#### 16:00-16.30 Coffee break

**16:30-17:15 MC Meeting** (Conference Room #5, Ground floor)

19:00-22:00 Conference dinner (Restaurant "Edno vreme", Borisova gradina PARK)

### Day 3, April 14th (Thursday)

#### 09.30-11.00 WGs sessions, Part 3

- WG1: Conference Room #5 (Ground floor)
- WG2: Conference Room #3 (Mezzanine floor)
- WG3: Conference Room #4 (Mezzanine floor)
- WG4: Conference Room #2 (Mezzanine floor)

#### 11:00-11:30 Coffee break

**11:30-12:00 Closing remarks** (Conference Room #5, ground floor)

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### (O-1) Earthworms and ecosystem services

Kevin Richard Butt<sup>1,2</sup> (Invited speaker)

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Earthworms form a major component of the soil fauna in a range of temperate and tropical ecosystems, particularly grassland and forests. They have been utilized by Man for millennia, although many of their positive actions were recognized only relatively recently (Darwin, 1881). This group are now fully considered to be ecosystem service providers and make contributions in a number of ways. Immediate thoughts may relate to pedogenesis itself, plus the maintenance of soil structure and regulation of water flow. Nevertheless, cycling of soil nutrients, assistance with primary production, potential climate regulation, pollution remediation and provision of cultural services are also worth investigation – and have been shown to be mediated by earthworms (Blouin et al., 2013). This presentation will draw upon selected findings from the scientific literature to illustrate some of these points and, where possible, utilize investigations of which the author has been a part. Development of some basic techniques, which have permitted these investigation to be undertaken, may also be explored.

Keywords: Burrowing, Casting, Nutrient cycling, Pedogenesis.

#### References:

Blouin M et al. (2013) Review of earthworm impacton soil function and ecosystem services. European Journal of Soil Science 64:161–182.

Darwin C (1881) The Formation of Vegetable Mould through the Action of Worms with observations on their habits (John Murray, London).





### (O-2) One Ecosystem of journals: Data and narrative-integrated publishing on Pensoft's ARPHA Publishing Platform

**Lyubomir Penev**<sup>1,3</sup>, Pavel Stoev<sup>2,3</sup>, Teodor Georgiev<sup>3</sup>, Benjamin Burkhard<sup>4</sup> (Invited speaker)

 $^{\rm 1}$ Institute for Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences;  $^{\rm 2}$  National Museum of Natural History, Bulgarian Academy of Sciences;

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One of the most worrying impediments in biological sciences is the prevailing publishing of scholarly articles in non-machine-readable formats such as paper or PDF. In the recent years, there is an increasing pressure for publishing biological content and data in formats that can be harvested by computers. This increases discoverability and enables re-use of data and reproducibility of research.

Pensoft's publishing platform ARPHA¹ was developed as a next generation collaborative environment supporting the full life cycle of a manuscript, from authoring through peer-review, publication and dissemination. Its ARPHA Writing Tool (AWT)² provides pre-defined, but flexible, article templates, including track change and comments tools, revision history, and online collaboration between authors and peers. This innovative workflow was pioneered by the following journals:

The **Biodiversity Data Journal**<sup>3</sup> (BDJ) invented data publication and re-use as an integral part of the research cycle. All data published in BDJ can be imported to and downloaded from the article text in tabular format (CSV). Specimen observation records can be imported into manuscripts from online resources (e.g., GBIF). Upon publication, occurrence data and taxon descriptions are also automatically exported in machine-readable Darwin Core Archives and become available for harvesting and indexing by aggregators.

The **One Ecosystem**<sup>4</sup> journal was launched recently as ecology and sustainability data journal, building on the experience and success of BDJ and with a special focus on the currently very prominent topic of *ecosystem services*. The journal welcomes contributions documenting also non-conventional research outcomes, including data, models, methods, workflows, results, software, perspectives, and policy recommendations in all branches of ecology and environmental science.

The **Research Ideas and Outcomes**<sup>5</sup> (RIO) journal focuses on publication of entire research cycles, starting from research ideas, grant proposals, PhD and PostDoc projects, data, software, posters, presentations, research articles, etc., across sciences and humanities. RIO provides several unique features<sup>6</sup>, for example three stages of public peer-review: (a) authororganized, pre-submission, (b) community-sourced, post-publication, and (c) journal-organised, post-publication (optional), and others.

<sup>1</sup> http://arphahub.com

<sup>&</sup>lt;sup>2</sup> http://arpha.pensoft.net

<sup>&</sup>lt;sup>3</sup> http://bdj.pensoft.net

<sup>&</sup>lt;sup>4</sup> http://oneecosystem.pensoft.net

<sup>&</sup>lt;sup>5</sup> <u>http://riojournal.com</u>

<sup>6</sup> http://rio.pensoft.net/about#Unique-Features



### (O-3) The effects of forestry treatments on enchytraeid worms (Annelida, Oligochaeta) in a Hungarian sessile oak-hornbeam forest

Gergely Boros, Bence Kovács, Péter Ódor

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The experiment investigates the effect of different forest management practices on forest site conditions, regeneration and biodiversity. The following treatments were carried out in a homogeneous stand of mature sessile oak – hornbeam forest using six replicates in a randomized complete block design: preparation cutting, gap creation, micro clear-cut, retention tree group within the clear-cut and control. Microclimate and topsoil variables will be measured during the experiment.

As belowground invertebrate indicators enchytraeid worms were monitored around permanent quadrates. 3 quantitative soil samples are taken in every quadrate (90 samples) 2 times per year with a soil corer. Samples were divided into 3 levels (0-4 cm, 4-8 cm, 8-12 cm) to estimate the vertical effect of the treatments. Each mature enchytraeid individuals were identified till species level. Before the treatments the original forest site conditions, enchytraeid diversity and density were surveyed for one year (2014). The treatments were carried out in January-February 2015.

One year after the treatments serious decrement of density was found in clear-cuts and retention tree group. The latter phenomena means that retention groups of 10-12 trees were not able to buffer the original assemblage for these small sized Annelids. In vertical distribution decrease of individuals were detected in the upper and middle layers. The species composition has improved for smaller geophages species (eg. *Achaeta*) compared to bigger size litter consumer species (eg. *Fridericia*).



### (O-4) Woodland restoration on landfill sites: Earthworm activity and ecosystem service provision

Francis Ashwood<sup>1</sup>, Kevin Butt<sup>2</sup>, Elena Vanguelova<sup>3</sup>, Kieron Doick<sup>4</sup>.

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The addition of composted greenwaste (CGW) into soil-forming materials during land reclamation may improve tree growth, alleviate certain negative soil properties and provide an effective waste management solution. CGW addition may also assist the establishment of sustainable earthworm populations, which in turn can further aid soil development through their burrowing and feeding activities. This presentation summarises the findings of a threeyear PhD project investigating CGW and earthworm interactions with trees on reclaimed land. A large-scale field experiment and a nursery-basedmesocosm experiment revealed the responses of the tree species Italian alder and Norway maple to CGW and earthworm addition in reclaimed soil. Findings revealed a synergistic effect of CGW addition and earthworm activity leading to significantly greater tree growth. CGW addition significantly increased levels of soil organic carbon and essential plant macro-nutrients, with earthworm activity increasing the accumulation of organic carbon into reclaimed soils. Additional laboratory-based research revealed the performance of four common UK earthworm species in reclaimed soil, and demonstrated that both CGW and the leaf litter of Italian alder and Norway maple can support earthworm establishment. These laboratory experiments demonstrated that the earthworms Aporrectodea longa and Allolobophora chlorotica are particularly suitable candidates for inoculation to reclaimed soil. A survey of a newly reclaimed site showed that natural colonisation of reclaimed land by earthworms can occur rapidly (within 2 years), where soil quality is given due consideration and legacy soil materials are stockpiled and applied following best practice guidance. This PhD therefore demonstrates methods for effectively improving woodland establishment and soil quality on reclaimed landfill, through CGW application and earthworm activity promoting soil development and encouraging tree growth.

Acknowledgments: This PhD project was part funded by the Forestry Commission's Thames Beat team.

Keywords: Earthworm, Reclamation, Composted greenwaste, Soil quality, Tree growth.



### (O-5) Soil nematode diversity - ecosystem functions, services and disservices

Stela Lazarova, Milka Elshishka, Alexander Mladenov, Vlada Peneva

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Nematodes are the planet's most abundant metazoans, major component of soil-inhabiting micro-fauna and soil food webs. They are both taxonomically and functionally diverse and play significant ecological role, not only as pests but also in organic matter transformation. In the last two decades many studies on nematode community structure in relation to environmental changes or disturbances showed that nematodes can be useful indicators of soil quality and ecosystem function. Their utility as biological indicators of soil health has been established by European scientists and later on explored by scientists worldwide. However, the need for considerable taxonomic skills and high quality microscopy hampered their use in practice which subsequently led to exploration of faster DNA-based techniques for studying nematode community structure.

This presentation will focus on the widely used nematode faunal analysis based on the functional guilds and maturity indices, and metabolic footprints. Contribution of nematodes to soil food webs and services through their metabolic and behaviour activities are considered.

Aknowlegements: The authors are grateful to BioLink COST FP1305 and ANIDIV2 projects for supporting this presentation.

Keywords: nematode diversity, soil food web, ecosystem function and services, environmental change, tree dominated ecosystems.



### (O-6) Microbiomes of potato-cyst-nematodes isolates kept in laboratory conditions and from environment

Diogo Neves Proença<sup>1,2</sup>, Isabel Luci Conceição<sup>3,4</sup>, Paula Vasconcelos Morais<sup>1,3</sup>

<sup>1</sup>CEMUC, University of Coimbra, Coimbra, Portugal <sup>2</sup>CESAM & Department of Biology, University of Aveiro, Aveiro, Portugal <sup>3</sup>Department of Life Sciences, University of Coimbra, Coimbra, Portugal <sup>4</sup>CFE, University of Coimbra, Coimbra, Portugal diogo.proenca@gmail.com

Potato is an important staple crop worldwide with an estimated production of 375 million tons every year. Portugal has a lower potato yield average comparatively to the average in European Union and may be attributed to the presence of plant-parasitic nematodes, for example, potato-cyst nematodes (PCN), Globodera spp. Recently, in some European collections, PCN isolates kept in lab cultures started to die and were not able to multiply after the first generation obtained in potato cultures in pots also shared by the Portuguese collections. This work is aimed to assess the microbiome of wild PCN and PCN maintained in laboratory collections. Cysts from Globodera spp. were extracted from soil infested with PCN. DNA was extracted from wild cysts (W-PCN), immediately after their isolation from the soil, and from cysts maintained in laboratory (L-PCN) in closed glass tubes, at room temperature, during 6 months. Molecular methods (DGGE and Illumina MiSeq) were used to assess the microbiomes (Bacteria, Archaea and Fungi) present in cysts. Sequence data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). DGGE profiles showed that the microbial community was different between wild and laboratory cysts. When comparing W-PCN with L-PCN, by the analysis of Illumina Miseq, it was possible to verify in L-PCN the increase of classes Gammaproteobacteria (30% to 60%, namely Acinetobacter) and Bacilli (8% to 20%, namely Bacillus and Staphylococcus) and a decrease in the percentage of microorganisms of the classes Actinobacteria (18% to 8%), Sphingobacteriia (7% to 1%), Betaproteobacteria (13% to 1%) and Alphaproteobacteria (20% to 7%). The number of bacteria and archaea involved in nitrification pathways in L-PCN was lower than in W-PCN. Moreover, we found the yeast Saccharomyces (30%) in L-PCN microbiome. The microbiomes of L-PCN and W-PCN are different and these differences might be involved in the loss of viability of the isolates in lab cultures. Saccharomyces was previously shown to kill nematodes and its presence in the L-PCN microbiome could also be related to the death of PCN maintained in lab conditions.

Acknowledgements: This work was supported by FCT, postdoctoral fellowship SFRH/BPD/100721/2014 of D.N.P. and project PTDC/BIA-BIC/3488/2012.

Keywords: Potato-cyst nematodes, Microbiome, Diversity, DGGE, Illumina, Globodera



### (O-7) The pan-bacteriome approach. A way to decipher the puzzling diversity and dynamics of bacterial community in soil

### **Alessio Mengoni** and Giovanni Bacci (Invited speaker)

Department of Biology, University of Florence, Sesto Fiorentino, 50019, Italy <u>alessio.mengoni@unifi.it</u>

Understanding changes in bacterial community structure over time is one of the major challenges in microbial ecology. The easy genome sequencing of bacterial isolates and metagenomic-based analyses of microbial communities have opened a large window over taxonomic and genome diversity of microorganisms in the environment. Indeed, environmental variations often affect taxonomic composition and taxa abundance in microbial communities [1], and may have strong effect on soil health and plant productivity. However, the successional dynamics of taxa in a community, as well as the large genome variability of soil microorganisms are challenging our ability to biologically interpret them. Conceptual frameworks have been proposed to help elucidating evolution of bacterial species and of its genome [2]. In particular, plant-associated bacteria were shown to have peculiar genome organization and evolution [3, 4], which is linked to ecological niche exploitation. Moreover, recently, we proposed an interpretative model of bacterial community dynamics, which consider common (core) and variable (accessory) taxa along a time series or following environmental changes in a community [5]. We tested this approach, by performing a longitudinal study to investigate both the amount and the structure of temporal changes of a soil bacterial community, challenged with Cd2+ as perturbing agent [5]. Results showed that core and accessory bacteriome represented roughly 35% and 50% of the taxa detected and were constituted by different bacterial phyla, suggesting that the pan-bacteriome model may be a useful approach to gain insight for inferring different functionalities of bacteria taxa.

Keywords: multipartite genome, soil, panbacteriome, pangenome, cadmium, metagenomics

#### References:

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### (O-8) Analysis of the rhizospheric microbial communities of *Quercus pyrenaica* along an altitudinal gradient.

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Functional and taxonomic rhizospheric microbial diversity for different states of a particular tree species forest is poorly understood. We used shotgun sequencing to analyze the microbial community composition in melojo-oak (Quercus pyrenaica Willd.) rhizospheric soil for three different situations along an altitudinal gradient: a) a low altitude, non-optimal site for forest maintenance; b) an intermediate altitude, optimal site for forest; and c) a high altitude, expansion site but without a real forest. We observed that, at each altitude, the same microbial taxa appear both in the taxonomic analysis of the whole metagenome and in the functional analysis of the methane, sulfur and nitrogen metabolism. Although there were no major differences at the functional level, there were significant differences at the phylogenetic level between the rhizospheres of forest and the highest, expansion site. Proteobacteria and Actinobacteria were the most differentially abundant phyla in forest soils compared to the expansion site rhizhosphere. Moreover, phyla Verrucomicrobia, Bacteroidetes and Nitrospirae were more highly represented in non-forest rhizosphere. Our study shows that differences of the rhizospheric microbial communities of the same tree species are due to relative abundance of each taxon. Moreover, these differences are indirectly dependent on the existence of forest canopy and altitude, which modifies the soil biogeochemistry.

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Keywords: metagenomics, melojo-oak, microbial functional diversity, biogeochemical cycles, rhizosphere metabolism



### (O-9) Digging the mechanisms behind the growth of spruce - role of diversity of ectomycorrhizal fungi

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The means by which trees establish and utilize fungi is intriguing given the lack of host specificity shown by many fungal species. We aimed to assess the mechanisms behind the previous observations on the positive relationship between ectomycorrhizal (EM) fungal species richness and Norway spruce stand productivity. We studied how susceptibility to EM infection and the composition and functionality of the associated EM fungal communities relate to host genotype of young seedlings and their long-term growth performance. We found that Norway spruce genotype partly controlled the EM colonization of seedlings. However we found no relationship between the host future growth performance and the established EM communities or the functional capacity of single ectomycorrhizas; seedlings representing contrasting future phenotypes were equally colonized by EM fungi and the potential exoenzymes activities varied only according to the colonizing EM fungal species, not between host geno- or phenotypes. Nevertheless, the short root architecture, which was found to be a moderately heritable trait, varied consistently between seedlings of contrasting future phenotypes. One year old seedlings known to show fast growth in a later stage had sparse and widespread rootlets compared to seedlings representing the stunted future phenotypes. Norway spruce does not seem to show strong genetic signal for withinpopulation selection towards its EM fungi at the species level. The superior growth of some spruce phenotypes may be a consequence of resource allocation and optimal root structuring in the juvenile stage rather than the extent of colonization by EM fungi. We suggest that root physiological factors may subsequently lead to a higher capacity for symbiotic interactions in heterogeneous forest soil. An adequate and versatile means of nutrient acquisition is an important factor enabling fast growth, but might also provide the basis for positive feedback via enhanced mutualistic fungal interactions.

Keywords: Ectomycorrhizal fungi, diversity, Picea abies, roots, exoenxyme

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### (O-10) Forest microbiome – habitat diversity, dynamics, and ecosytem functioning

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Forest ecosystems are of global importance as recent C sinks and pools. However, their behaviour in the future is largely unpredictable due to complexity of their functioning involving trees as dominant primary producers, microorganisms as major decomposers and other organisms. Despite intensive research, the role of microorganisms at the ecosystem level is not fully appreciated. This is mainly due to simplistic approaches that often target only selected habitats, such as the soil or tree roots. In reality, the complexity of the ecosystem is much higher and habitats are often unique with specific properties. The microbiomes of tree or plant leaves and living tissues, roots, deadwood, leaf and root litter, mycelial mats in soils, tree-associated invertebrates, rhizosphere and bulk soil as well as weathered mineral horizons are each highly specific and respond to the chemistry, nutrient availability, and drivers of temporal dynamics. Recent papers make it possible to provide a first view of the composition of microbial communities inhabiting these individual niches, allow us to formulate the potential major processes driving their existence and functioning. It is also possible to outline the model of their dynamics on the scales ranging from day/night and annual cycles, to decomposition of dead organic matter of various recalcitrance and ecosystem development itself. The microbial communities inhabiting soil, plant litter, rhizosphere and roots of forest plants, deadwood or aboveground plant tissues differ tremendously in the drivers of their assembly and consequently in diversity and composition, functioning and dynamics in time which is specific for each habitat. Due to this, to achieve the understanding of the microbial contribution to the functioning of the ecosystem, the analysis has to be done on the "ecosystem microbiome" level considering the fact that ecosystem-level processes, such as, e.g., disturbance or stand development typically involve all habitats.





### (O-11) Bacterial and fungal communities in rhizosphere of cork oak (Quercus suber L.): Linking microbiome and forest health

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Oak forest health is a major concern due to the socioeconomic, environmental and biodiversity importance of these trees in the Northern hemisphere. Triggered by complex interactions between biotic and abiotic factors, oak forest has been declining [1]. Belowground solutions to aboveground problems [2] need to be explored by new multifaceted perspectives and cutting-edge approaches. Therefore forest soil biodiversity is of utmost importance and key in developing new strategies to address this issue. The role of tree-microbiome interactions has been highlighted as significant to the overall system stability and plays an essential role in tree homeostasis [3,4], mainly in the rhizosphere [5].

In this study, rhizosphere microbiome of healthy and declined cork oaks was analyzed by 16S and ITS2 amplicon pyrosequencing. The preliminary results of bacterial and fungal community characterization showed significant differences between the two health conditions. These results support the hypothesis that healthy trees contain different associated microorganisms compared with declined trees, motivating further studies to clarify and highlight the role of microbiome on forest health. The ongoing project aims to study the tree rhizosphere microbiome using the cork oak forest as a decline model. Bacterial and fungal communities will be analyzed in healthy and declined trees by metagenomics approaches and correlated with temporal-spatial factors. Furthermore, root microbiome of different tree decline stages will be assessed and correlated with forest decline progression. Results derived from this project will provide new insights into the definition of forest health microbiome, unraveling potential microbes with an important role in tree growth, health and productivity. With this knowledge, new forest management strategies may be developed such as the use of probiotic microbial consortia for forest regeneration, disease suppressive soils to control phytopathogens or microbial markers for monitoring forest health.

Acknowledgments: We acknowledge António Grilo from Biocant's Microbiology Laboratory and Mariana Ribeiro Telles from APFC for their cooperation and support in the collection of samples and Cátia Pinto from Genomics Unit at Biocant for her help and critical review.

Keywords: Forest Health, Soil Biodiversity, Rhizosphere Microbiome, Next Generation Sequencing, Cork oak

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### (O-12) Is tree species diversity or tree species identity the most important driver of European forest soil carbon stocks?

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Studies of functional species diversity have reported positive effects for aboveground carbon (C) sequestration, but the question remains whether higher soil C stocks could also result from belowground niche differentiation including more efficient root exploitation of soils. We studied soil C stocks in tree species diversity gradients within the FunDivEurope project to explore biodiversity-ecosystem functioning relationships in six European forest types in Finland, Poland, Germany, Romania, Spain and Italy. In the Polish forest type the sampling was extended to include subsoils.

We found consistent but modest effects of species diversity on total soil C stocks (forest floor and 0-20 cm) across the six European forest types. Carbon stocks in the forest floor alone and in the combined forest floor and mineral soil layers increased with tree species diversity. In contrast, there was a strong effect of species identity (broadleaf vs. conifer) and its interaction with site-related factors. Within the Polish forest type we sampled soils to 40 cm and found that species identity was again the main factor explaining total soil C stock. However, species diversity increased soil C stocks in deeper soil layers (20-40 cm), while species identity influenced C stocks within forest floors. Root biomass increased with diversity in 30-40 cm depth, and a positive relationship between C stocks and root biomass in the 30-40 cm layer suggested that belowground niche complementarity could be a driving mechanism for higher root C input and deeper distribution of C in diverse forests.

We conclude that total C stocks are mainly driven by tree species identity. However, modest positive diversity effects were detected at the European scale, and stronger positive effects on subsoil C stocks in Poland were associated with higher subsoil root biomass. Targeted selection of tree species would be a stronger forest management approach for C sequestration than increasing tree species diversity per se.

Keywords: Soil carbon, tree species diversity, tree species identity, roots, European forest types



### (O-13) The effect of deforestation on streamwater ecosystems in Iceland

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Forests provide many important ecosystem services. The interaction between water and forests is one of the fundarmental services that forests provide. Changes in catchment vegetation can have large impacts on stream ecosystems and this study deals with the interaction of forests and stream ecosystems. The study took place in S-Iceland. Measurements were done on eight streamwater ecosystems, four of them running through a birch forest while four of them were running through a degraded eroded land. We measured the biomass of the ecosystem surrounding the streams as well as the transport of terrestrial organic matter into the streams. We also measured the decomposition of litter in the streams using the litter bag method (fine and coarse mesh bags). The invertebrate fauna found within the coarse bags was also studied.

The results show that the biomass production within the forest ecosystems was 13.7 times higher than on the eroded land. However the transport of terrestrial organic matter in the streams was only 2,8 times higher than in the eroded land. This indicates that much of the organic matter that was found in the streams of the eroded land, had been transported over a long distance, preferably by wind.

No significant difference was detected between the two ecosystems when looking at the decomposition of litter in the fine mesh bags but the decomposition rate was much higher (94%) in the birch ecosystem when looking at the coarse mesh bags. This indicates a more important role of invertibrates in the decomposition process within the forest ecosystem.

These results shows how important it is to include different stream types when terrestrial-aquatic interactions are studied.



### (O-14) Variability in fine root morphology, biochemistry, and litter quality indices of ten tropical tree species

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Fine roots (<2 mm in diameter) of trees and shrubs play an important role in the acquisition of soil resources and are major flows of carbon into the soil. However, little is known about their morphological indices in relation to their chemical composition and carbon investment to biomass production. The aim of this study was to test whether root traits varied with species and correlate with biochemistry via proxy carbon fractionations and their response to carbon costs to build dense root systems. Root morphology, root chemistry, litter quality indices, and amount of glucose needed to construct a gram of fine root biomass production and the consequent CO2 evolution were investigated on fine roots of ten tropical tree and shrub species collected in northern highland of Ethiopia. Fast growing species exhibited higher specific root length (SRL), specific root area (SRA), specific root volume (SRV) but lower root tissue density (RTD) than slow growing species. The carbon content of fine roots was below the expected 50% of the dry mass. All species were characterized by high values of acid insoluble fractions (AIF). The carbon content, AIF, and lignocellulose index were higher for slow growing species. Root tissue density showed a strong positive correlation with AIF and C content whereas SRL, SRV are negatively correlated with AIF and C content. The cost to construct a gram of root biomass and the amount of CO<sub>2</sub> released during production were significantly higher in slow growing species than fast growing species. Therefore, we conclude that, despite differences in morphological traits and chemical composition between species, AIF, RTD, and C cost to fine roots are highly correlated irrespective of species.

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Keywords: Fine toots, root morphology, biochemistry, litter quality index, carbon cost



### (O-15) Fine root production and phenology among 11 temperate, mature tree species in a common garden in Poland

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Repeated, non-destructive observations of fine root growth provide insights into seasonal growth dynamics, belowground carbon allocation and resource acquisition. Our objectives were to explore the extent to which seasonal growth patterns of fine roots (< 1 mm in diameter) were similar across species and years as a means to understand belowground dynamics in a temperate forest ecosystem. Our a priori prediction was that deciduous species would have season-specific root production, while the evergreen species would have more continuous fine root production. We used minirhizotrons to collect 6 years of observations on fine roots (1st and 2nd - order roots; <1 mm diameter) of 11 tree species growing in monoculture plots within a common garden.

Our results indicate similar timing of fine root growth can occur across tree species of diverse phylogeny and leaf phenology. Root production peaked between July and October for all species for six years. The timing of the peaks of fine root production varied significantly for only 4 species; in other words, the peaks occurred at the same time for a given year in 7 species. Total annual fine root length production was positively associated with total rainfall of both the current and previous year across species, and the relationship was not influenced by whether the plants were angiosperms or gymnosperms. We concluded that the relatively synchronous fluctuation in peak periods of growth across multiple years for 11 species suggests a large influence of environmental conditions on seasonal root growth. This was supported by the link of total root production to annual rainfall. The linkage of actual year total root length production with previous-year rainfall underscores the importance of lags in biological responses and the importance of long-term studies.



## (P-1) Gene expression pattern in above-ground olive tissues upon root colonization by a beneficial bacterium and a pathogenic fungus using a split-root system

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Pseudomonas fluorescens PICF7 is an indigenous inhabitant of olive roots, displays endophytic lifestyle and exerts effective biocontrol against Verticillium wilt of olive caused by the soil-borne fungus Verticillium dahliae. Previous studies have demonstrated that colonization of olive roots either by strain PICF7 or by V. dahliae triggers differential aboveground transcriptomic changes, mostly related to defense responses to (a)biotic stresses. Some of these responses are common in both interactions. An olive split-root system was developed in order to examine within a 14- day interval the expression pattern of selected genes in above-ground tissues when both microorganisms were spatially separated (i.e. inoculated in different compartments of the split-root system) and sequentially applied (first PICF7, and one week later the pathogen). The final aim is to assess whether PICF7 is able to set up a systemic effective response in the host prior to be challenged by the pathogen. Defense-related genes identified in PICF7- and/or V. dahliae-olive root interactions (i.e. CO-MT, PAL, ACO, CAT, WRKY, 14-3-3 and BRU1) were selected to evaluate by RT-qPCR their expression patterns in aerial tissues when: PICF7 was alone in one root compartment (days 1-14), the pathogen was added to the other compartment (days 7-14), and the pathogen was alone (days 7-14). Differential responses were found depending on the tested gene. Thus, the expression pattern of some genes was not affected regardless the microbial input (e.g. a 14-3-3 protein coding gene). Some showed opposite expression patterns related to the time PICF7 or V. dahliae were inoculated (e.g. a WRKY transcriptional factor). Finally, others only responded upon pathogen's presence (e.g. BRU1, a brassinosteroid-regulated protein and CO-MT, involved in phenylpropanoid biosynthesis). Ongoing experiments are evaluating whether these expression patterns are similar when the PICF7 and the pathogen are not spatially separated.

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Keywords: Verticillium dahliae, Pseudomonas fluorescens PICF7, systemic responses

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### (P-2) Stress response and metabolic potential of halotolerant fungi

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Salinity is one of the most important abiotic stress with a negative impact on the growth and development of microorganisms. The negative effect of salinity on density, diversity and metabolic activity of bacteria is relatively well understood (Szymańska et al., 2014; 2016), while research on fungi are still under investigation. Fungi associated with plants (e.g. endophytic and mycorrhizal) can significantly reduce plant stress at saline areas (Hrynkiewicz et al., 2015). Since salt stress can decrease growth of mycelium and inhibit formation of conidia, sclerotia and germination of spores, their impact on plant growth can be significantly diminished. Understanding of the range of tolerance and metabolic properties of halotolerant fungi can increase potential of selected strains in their application in the future.

The main objective of this study was to analyze tolerance to salt stress and metabolic potential of selected halotolerant fungal strains. Investigated fungal strains were isolated from fruit bodies collected under black alder (*Alnus glutinosa* L.) at saline area in Słonawy (northern Poland) and identified based on ITS region. Our results revealed significant correlation between analysed fungal species and their tolerance to increasing concentrations of sodium chloride and potential for utilization of different substrates.

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Keywords: salt stress, metabolic activities, halotolerant fungi

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Szymańska S et al. (2016) Metabolic potential and community structure of endophytic and rhizosphere bacteria associated with the roots of the halophyte *Aster tripolium* L. Microbiological Research 182: 68-79.



### (P-3) Soil microbial community in a temperate old-growth forest

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Effect of spatial variation in vegetation and abiotic environment on soil microbial community was studied in an old-growth forest in the reserve Dobroč (central Slovakia). Data on vegetation, microclimate, soil properties and microbial activity were collected on two linear transects crossing both the core of the reserve and the buffer zone.

The presented results demonstrated mutual links between the aboveground and belowground components of a forest ecosystem. Microclimate seems to affect soil microbiota less than interactions with vegetation. Microbial activity was shown to be strongly affected by the availability of organic and mineral resources in the topsoil, but spatial distribution of resources was linked with tree distribution. A positive correlation between ground-vegetation species richness and diversity and microbial functional diversity was confirmed, but there are no signs of enhanced microbial activity under high understory plant diversity, as both positive and negative effects on the activity of functional microbial groups were observed. It seems that soil microbiota prefers diverse ground-layer vegetation, but not necessarily diverse tree vegetation. We observed similar responses to the proximity of beech and spruce on one side, and noble hardwoods and fir on the other side what is unexpected in the case of silver fir. The effect of trees cannot thus be simplified to conifers vs. broadleaves contrast. Redundance analysis of microbial data identified potassium content, plant richness and influence of fir as the drivers of functional group composition.

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Keywords: microbial activity, soil functional diversity, plant diversity, natural forest



### (P-4) Vegetative and edaphic control on soil C storage in altitudinal forests of Himalayan Bhutan

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The vulnerability of Himalayan ecosystems under the present global climate change scenario is receiving much attention because of its direct link with the ecological and socio-economic conditions of the vast area of South Asia. The mountainous forest ecosystems of the eastern Bhutan Himalayan regions are susceptible to similar impacts. The forest ecosystems have potential of positive feedback to elevated atmospheric  $CO_2$  by fixing C in biomass and soil, however, the response of climate change on soil C stock in these densely vegetation cover is largely unknown. We investigated above and belowground tree biomass, litterfall, soil physical and microbial properties in relation to soil C stocks in two forests of sub-alpine coniferous, dominated by hemlock (*Tsuga dumosa*) and temperate mixed broadleaved, dominated by oak (*Quercus lanata & Quercus griffithii*) at altitudes of 3300 m and 2300 m respectively. We found that C storage differ significantly along vertical distribution across the forest types however, the influence of vegetaion cover on soil C was inconsistent, rather local climate induced soil and site properties were seemed to be predominant factors.

Keywords: Altitudinal forest, fine root biomass, soil C, climate change



### (P-5) Belowground diversity and metabolic potential of bacteria associated with halophyte Salicornia europaea

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Salinity is an abiotic factor that affects the population structures of all living organisms. Salicornia europaea L.belongs to the group of halophytes characterized by the highest salt tolerance in the world (Szymańska et al. 2014). We have investigated the plant-microbial interactions of S. europaea (Herbaceae) as potential mechanisms responsible for the high tolerance of halophytes to saline conditions. The abundance, community-level physiological profiles and diversity of microorganisms associated with the plant were determined for endophytes from the roots, rhizosphere, and salt-affected soil. Above analysis were performed for microorganisms co-existing with the roots of halophytic plant Salicornia europaea growing at two saline sites in central Poland: a salty meadow in the vicinity of a soda factory (anthropogenic salinity) and an area affected by natural brine (landscape park).

Well-adapted groups of endophytic halotolerant bacteria associated with this plant can be a source of unique bacterial strains useful for a subsequent applications in the improvement of plant adaptation to saline soils. However, only detailed investigations (e.g., physiological and molecular characterization) of individual microorganisms with high precision and in controlled environmental conditions can explain basic causal mechanisms of their effects on plants (Hrynkiewicz and Baum, 2012).

Acknowledgements: This research was financially supported by a grant from the National Science Centre (Poland) (DEC-2012/07/B/NZ9/01801).

Keywords: salt stress, halotolerant bacteria, metabolic activities

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### (P-6) In vitro screening for Trichoderma strains with the potential to control Armillaria species causing tree root diseases

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Armillaria species cause soil-borne root diseases to almost all woody plants including both crop and forest trees, therefore, there is an emerging need for environment-friendly biocontrol solutions of the problem. *Trichoderma harzianum* was found to suppress the pathogen in tea stem sections buried in the soil (Otieno et al. 2003), however, *Trichoderma* spp. were found to be ineffective for *Armillaria* root rot control in peach (Schnabel et al. 2011).

Twelve Trichoderma strains were selected from the Szeged Microbology Collection (SZMC). All strains derived from Hungarian soil samples and identified by barcoding-based sequence analysis of the internal transcribed spacer region region performed with TrichOkey2.0 at www.isth.info. The 12 Trichoderma strains represented species T. asperellum (2), T. harzianum (5), T. atroviride (2), as well as T. virens, T. gamsii and T. hamatum (1-1 each). The Trichoderma strains were confronted in all combinations with 11 Armillaria strains representing A. ostoyae (6), A. gallica (2), A. cepistipes (2), A. mellea (2), Armillaria sp. (6), and A. borealis (1) on potato dextrose agar plates. Armillaria strains were inoculated with agar plugs (5 mm in diameter) cut from the edge of 2-week-old colonies 1.5 cm from the center of PDA plates, while the inoculation of Trichoderma strains was performed after 14 days of incubation in a similar way to a position 3 cm apart from the inoculation position of the Armillaria strains. After further 10 days of incubation, Biocontrol Index (BCI) values were calculated based on image analysis of plate photos performed according to Szekeres et al. (2006). Results revealed that the examined Trichoderma strain efficiently restricted colony growth of the Armillaria strains and in many cases they were able to overgrow and intensely produce conidia on the surface of Armillaria colonies. The results suggest that Trichoderma strains have the potential to control Armillaria root diseases in crop and forest trees.

Acknowledgments: This study was supported by the Hungarian Government and the European Union within the frames of the Széchenyi 2020 Programme (GINOP-2.3.3-15-2016-00006).

Keywords: *Armillaria*, root disease, *Trichoderma*, biocontrol, *in vitro* antagonism References:

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## (P-7) Plant-fungal interactions in alpine ecosystems: environmental drivers and the influence of plant host genetic variation on mycorrhizas across elevation and latitudinal gradients

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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. Despite their importance, alpine ECM fungi are poorly known and the majority remain undocumented and undescribed. Baseline diversity data on these fungal communities and their environmental drivers are needed in the face of future climate changes.

We aim to: 1) Study the community composition and structure of mycorrhizal fungi associated with four alpine plants -- Betula nana, Dryas octopetala, Salix herbacea and Bistorta vivipara -- along elevation gradients in the Alps; 2) Investigate the effect of biotic (host identity, genetic variability and distribution) and abiotic (elevation and plant and soil chemistry) factors on their diversity and distribution; and, 3) Study the ECM communities associated with Betula pendula and its hybrids with B. nana.

We will sample the mycorrhizal communities in roots and in soil associated with these plants with different distribution patterns in the Alps and at different elevations. Moreover, where *B. nana* and *B. pendula* co-occur (hybrid zone) mycorrhizas from both the parents and the hybrids, and leaf material to analyse across a gradient of host genotypes, will be collected. We will complement our root analyses with the collection of fruiting bodies and related specimens from various Fungaria to help with the identification of fungi. Plant and soil chemistry will be measured and all environmental variables available recorded.

We will generate baseline diversity and distribution data of ECM fungal communities and identify their biotic and abiotic drivers. We also expect to identify fungal species or species assemblages of conservation concern in the Alps.

Acknowledgments: The David and Claudia Harding Alpine Plant Conservation and Research Programme. Spatial analysis team at RBGK.

Keywords: mycorrhizal fungi, European Alps, host genetic variation, elevation gradients, symbiosis.



### (P-8) Mycorrhizal enzyme activity profiles at the tree line

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At a tree line site (1700m) and a lower elevation site (1100m) in the Austrian Alps, Potential enzyme activities were determined using the high-throughput photometric and fluorimetric 96-well black microplate assays in 2014 and 2015. Four enzyme activities were measured on mycorrizal and non-mycorrhizal root tips: β-glucosidase (BG, which hydrolyses cellobiose into glucose), N-acetyl- glucosaminidase (NAG, which breaks down chitin), acid phosphatase (AP, which releases inorganic phosphate from organic matter), and leucine-amino-peptidase (LAP, which breaks down polypeptides) at tree line (*Picea abies*, *Pinus mugo* and *Rhododendron ferrugineum*) site and *Picea abies* of lower elevation site.

The results indicated that the absolute levels of activity of each of the enzymes were similar, but between morphotypes the levels of activity varied several fold for all enzymes between years. In 2015, thirteen ectomycorrhizal morphotypes from *Picea abies* at tree line and lower the elevation were determined and 8 ectomycorrhizal morphotypes from *Pinus mugo*. Averaged across all morphotypes of ectomycorrhizal species no significant differences were shown in any of the enzymes between tree species or elevation except that  $\beta$ -glucosidase activity was significant higher in *Pinus mugo*. On the fine roots of *Rhododendron ferrugineum* the activity of N-aceytylglucosaminadase, acid phosphatase, and leucine aminopeptidase was significantly lower compared to the ectomycorrhizal morphotypes of the tree roots.

Keywords: mycorrhizal, enzyme activity, tree line



### (P-9) Comparison of roots traits under different tree species as influenced by soil type in forest ecosystems in Bulgaria and the UK

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Forests play a vital role in carbon stocking and cycling. Fine roots (<2 mm) are very dynamic component of ecosystems and contribute to the element cycling and accumulation in soils. Although the research of belowground root traits in forest ecosystems is well developed, there is still not enough data in terms of different tree species and the direct influence on the soil environment. The aim of present research is to compare the root biomass, root morphological parameters, root chemistry and carbon accumulation in forest ecosystems dominated by Faqus sylvatica and Pseudotsuga menziezii on similar soil types in Bulgaria and sets of Fagus sylvatica, Quercus robur and Pinus silvestris stands on two contrasting soil types in the UK. The root biomass of four main European tree species Fagus sylvatica, Pinus silvestris, Quercus roburand Pseudotsuga menziezii was sampled in forest sites (6 sites in the UK and 4 in Bulgaria) in order to identify the differences between species, and within and between vegetation zones in order to study the relationships between root biomass and the climatic, site and stand factors. The root sampling was performed by sequential coring technique method, root morphological characteristics by WinRhizo system, root biomass and necromass by separating live with dead roots, drying to constant mass and weighing, root C and N via C and N analyser and root chemistry after digestion and ICP analysis. Present paper outlines the preliminary results obtained and creates basis for further discussions and comparisons.

Acknowledgments: The authors are grateful to BioLink COST FP1305 project for supporting the study which was done during the STSM of the first author.



### (P-10) Comparison of several arbuscular mycorrhizal fungi and sweet marjoram (*Origanum majorana* L.) symbiotic associations in heavy metal polluted soil

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Mycorrhizal fungi participated in plant nutrient assimilation, root development and metal absorption from the soil depending on the resistance and stress tolerance of the involved strain. The overview of several mycorrhizal and marjoram symbiotic associations represented the effect of the studied stress over different strains and their interactions with the plants. We tested four mycorrhizal isolates, derived from different soils: Claroideoglomus claroideum (Cc1), Rhizophagus clarum, Claroideoglomus claroideum (Cc2), Funneliformis mosseae. In order to get insight into the role of mycorrhizal symbiosis in protecting Origanum majorana L. against the excess of metals (Cd and Pb), we investigated growth, uptake and distribution of heavy metals in the plant parts, mycorrhizal colonization status, glomalin- related soil proteins and acid phosphatase activity (in root and soil). Pb and Cd partitioning in nonmycorrhizal plants were soil>shoots>root while in symbiotic associations variants changed to soil>root>shoots. Bio concentration factor (BCF) and translocation factor (TF) values confirmed by the less heavy metals shoot uptake in inoculated than control plants. The root dry weight increased in plants inoculated by C. claroideum strain (Cc1), which is isolated from industrially metal-contaminated sites. The highest shoot biomass correlated with the percentage of mycorrhization, relative mycorrhizal dependency, glomalin production and acid phosphatase activity was determined by C. claroideum (Cc2) and Funneliformis mosseae. Both strains derived from natural metalliferous sites. The findings in this study are essential to get the most benefits of mycorrhizal association in unfavorable conditions connected with plant development and herbal products free of harmful ingredients.

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Keywords: Arbuscular mycorrhizal fungi, Origanum majorana L., heavy metals



### (P-11) Preliminary studies of ectomycorrhizal fungal communities in protected and managed mixed coniferous forests

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The main objective of presented studies is to compare above- and below-ground community structure of ectomycorrhizal fungi in continental mixed coniferous forests (Querco roboris-Pinetum), at three pairs of forest stands. In each pair, one forest stand is located within the forest reserve (forest reserves "Bażantarnia", "Olbina" and "Czaplowiza"), while the second forest stand is a mature managed forest (in terms of forest association as much as possible comparable to the forest reserve). Our investigation is carried out using traditional fruiting bodies inventories and molecular identification of ectomycorrhizal morphotypes. Preliminary result indicate that quantitative below-ground community structure of ectomycorrhizal fungi (species richness) do not differ between protected forests and managed forests, but higher aboveground species richness of ectomycorrhizal fungi was noted in managed forests. However individual pairs of stands are differentiated in respect of species composition and abundance and furthermore type of the forest management can significant influence to qualitative community structure of fungi. This appears to be due greater volume of dead wood in protected forest in comparison to the managed forests. Dead wood provides specific ecological niches allowing for the development the fruit bodies of some specialized ectomycorrhizal fungi (e.g. Tomentella, Pseudotomentella).

Keywords: soli fungal community, ectomycorrhizal fungi, species richness, forest management type, coniferous forests

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### (P-12) Belowground diversity as important factor for maintaining ecosystem functions of mountain spruce forests

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Management effect on forest ecosystem functions is one of the key problems of sustainable forestry. Unfortunately, the number of studies dealing with the relationship between belowground diversity and ecosystem functions of differently intensively managed forests is still relatively low. New project within COST Biolink started in winter 2015 in the Giant Mts (Czech Republic), aiming to compare the belowground diversity of roots and fungi in natural and managed mountain Norway spruce forests (alt. about 1200 m a.s.l.) and managed spruce forests on the site of the indigenous beech forest stands (alt. 800 m a.s.l.). The total of 18 permanent research plots (6 per each type) were established to study fungal diversity in the soil (Illumina sequencing), diversity of fungal symbionts of spruce ectomycorrhizae (morphotyping and Sanger sequencing) and diversity of fungal fruiting bodies. Simultaneously, the production of ectomycorrhizal mycelia (mesh bags) and fruiting bodies, as well as fine root status from soil cores will be estimated. The assessment of natural regeneration, herbaceous vegetation cover and tree crown response to environmental stress enable us to estimate and predict the rate of forest ecosystem function performance related to belowground diversity. Due to combination of the climatic factors and forest naturalness we supposed the lowest fungal diversity in managed forests in high altitude and the highest belowground production in low altitude managed forests.

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Keywords: belowground diversity, roots, fungi, ectomycorrhiza, forest management



### (P-13) Dynamics of soil carbon processes following forest disturbance by clear cut harvest and stem girdling

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Trees drive forest soil carbon (C) dynamics. Decomposition of soil organic matter (SOM) is an important ecosystem function and depends on e.g. the availability of belowground and aboveground C inputs, microbial biomass in soil, and soil microclimatic conditions. Trees therefore heavily influence soil C processes both indirectly by regulating soil moisture and temperature (e.g. water uptake by roots, crown shading), and directly through C inputs into the soil via above-ground litter, root exudates and root turnover. However, abrupt and substantial changes to these influences occur during the wide-spread mortality associated with forest disturbance, and subsequently a range of important driving factors are quickly reconfigured. In order to study how forest disturbance affects the respective biotic and abiotic factors driving soil C processes, and to investigate their respective influences separately, we established a forest disturbance experiment in a montane beech forest located in the Austrian Alps. The experiment includes stem-girdling, clear-cutting and woody debris addition treatments and started in spring 2015. Immediately after clear-cutting soil temperature significantly increased compared to control and girdling treatments, whereas changes in soil moisture were of minor importance. However, woody debris addition at the clear cuts dampened extreme temperatures via shading. Neither soil microbial biomass, dissolved organic C and dissolved nitrogen (N), C and N content, pH, and root bio- and necromass, nor the decomposition of SOM (measured as microbial C mineralization at 10 °C;  $R_{10}$ ) and its temperature sensitivity ( $Q_{10}$ ) changed in the first weeks post-disturbance. Since total soil CO<sub>2</sub> efflux showed no response to the treatments, we assume mainly a temperature related increase in microbial respiration and/or root respiration from stored carbohydrates to maintain total CO<sub>2</sub> efflux at pre-disturbance levels.



### (P-14) Soil nematode assemblages from different habitats from North Pirin Mountain: assessment of ski runs impact

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Soil nematode assemblages from different habitats (mountain pine, Macedonian pine and spruce forests, subalpine meadows and ski runs – bare and with grasses) were studied in the frame of a PhD project in order to assess changes caused by ski runs on various community parameters. Herein, we present the results on the structural and functional diversity of nematode assemblages at the level of Macedonian pine forest area (2050 m a.s.l.) that includes 15 sampling sites (6 forest fragments) and ski runs (5 with and 2 without grass). Several community parameters were analysed: relative abundance, diversity and maturity indices, trophic and life strategies (c-p) functional group.

Nematode assemblages of bare runs were characterised by very low abundance and generic richness, dominated by nematodes of c-p 4 functional group, all trophic groups present with plant feeders being very rare; ski runs with grasses supported the most abundant and divers nematode assemblages, dominated by omnivorous nematodes and bacterial feeders, c-p 2 and c-p 4 functional groups prevailed; the Macedonian pine fragments had relatively balanced trophic structure, with higher proportion of predators and very small part of plant feeders. Two bare ski runs with strongly degenerated soil nematode assemblages had higher diversity and maturity four years later.

In general, the distribution of functional groups showed specific pattern for different habitats and years of sampling with omnivorous nematodes being the dominant group in all types of habitats except in forest fragments where the predators prevailed. With few exceptions the group of persisters (c-p 4 and c-p 5) prevailed in all sites including the bare ski runs caused by the high abundances of *Aporcelaimellus* spp.

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### (P-15) Diversity of endosymbiont bacteria associated with Xiphinema americanum-group parasitic nematodes

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The associations of invertebrates with microorganisms are of great importance to their ecology and biology. Recent technological advances in molecular studies have given an insight into the microbiomes associated with various nematodes and provided insight into their functional role.

Xiphinema americanum-group consists of plant parasitic nematodes having a global distribution with some species transmitting nepoviruses that cause substantial economic damage to a wide range of crops. A main characteristic of this group is the presence of obligate intracellular endosymbiont bacteria that have been identified as belonging to the Verrucomicrobia division. They are considered an integral component of the *Planctomycetes-Verrucomicrobia-Chlamydiae* (PVC) superphylum containing seven subdivisions with Xiphinematobacter symbionts, assigned to subdivision 2 (Schlesner et al., 2006).

The objective of this study was to characterise the genetic diversity of endosymbiont bacteria from 22 *X. americanum*-group populations collected from six continents using 16S ribosomal DNA. Using a conservative value of < 97 % sequence similarity, our data indicated the presence of nine phylotypes of *Xiphinematobacter* of which six have previously not been reported. The phylotypes appear to be grouped based on the geographic origin of the nematode populations with two of them found in Europe only; four were restricted mainly to North America and three showed a greater geographical variation. The *Xiphinematobacter* sequences acquired during this study help to inform the taxonomy of Verrucomicrobia, however further investigations are needed to explain their functional role.

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### (P-16) Fungal endophytes from the common yew tree (*Taxus baccata*) produce antimicrobial metabolites

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Endophytic fungi are a group of highly diverse microorganisms that reside within plant tissues without causing diseases, moreover, they can even be beneficial to their host and produce a large variety of biologically active secondary metabolites.

In this study, samples of *Taxus baccata* (the common yew tree) were collected from the Botanical Garden of the University of Szeged, Hungary during the late spring of 2015. Three-three parts of ten individual plants were sampled. Plant pieces were washed and surface-sterilized with 70% aqueous ethanol and sodium hypochlorite solutions to kill epiphytic microorganisms, and placed in 3 replicates onto the surface of plates with Potato Dextrose Agar (PDA) and Rose Bengal Agar (RBA) media supplemented with ampicillin (50 µg/ml). Fungal growth was regularly monitored during the incubation period of 10 days at 25 °C. Finally, 200 isolates were recovered, purified and deposited at the Szeged Microbiology Collection (SZMC). Morphology- and culture-based identification of the isolated strains was confirmed by DNA sequence-based tools. The taxonomic diversity of the isolates was remarkably high: they represented mainly the genera *Alternaria*, *Aspergillus*, *Fusarium*, *Cladosporium*, *Rhizoctonia* and *Trichoderma*.

For the investigation of their biologically active metabolites, endophytic isolates were precultured on the surface of PDA plates. Agar plugs of these cultures were transferred into Erlenmeyer flasks containing potato dextrose broth and incubated in a rotary shaker (150 rpm) at 25 °C for 14 days. Cultures were filtered (0.45  $\mu$ m) and extracted sequentially with equal volume of hexane, chloroform and ethyl acetate. The organic phases were evaporated to dryness, resolved in 2 ml extraction solvent and tested in microdilution plate and agar diffusion assays against bacterial and fungal strains, respectively.

Results of this study provide a good basis for the discovery of new potential antimicrobial compounds.

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Keywords: common yew, Taxus baccata, endophytic fungi, antimicrobial metabolites



### (P-17) Atlas of Woody Plant Roots - Morphology and Anatomy with Special Emphasis on Fine Roots

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Tree roots are hosts for symbiotic mycorrhizal fungi and exude substances that affect whole belowground community, creating a unique and very complex biome - rhizosphere. Research of belowground diversity and processes conducted in natural stands where multiple tree and shrub species occur together is inevitably dealing with a problem of a tree root identification in heterogeneous samples. Due to lack of tree root identification, information on effects of tree root diversity on different aspects of belowground ecology is critically missing. Molecular tools for tree root identification are still under-developed, costly and more or less nonquantitative. Having in mind the procedure for sample preparation for any kind of root analysis, which includes cleaning and sorting of roots, a two-step identification approach, starting with morphology and continuing with anatomy was used in Atlas of Woody Plant Roots. Atlas is bound in a binder that allows further species to be added later to existing twelve temperate species. Morphological part deals with general aspects of root morphology, followed by morphological key for the selected species and plates with morphological descriptions accompanied by detailed photos. Anatomical part starts with list of characteristics in secondary xylem, bark and primary tissues, with descriptions (altogether 320 features), useful for identification, where peculiarities of root anatomy are emphasized. List of characteristics is followed by coded description of anatomical features of roots of selected tree species and plates with photos of typical anatomical structure and descriptions of the most characteristic features. At the end, a chapter on anatomical identification of roots thinner than 1 mm is presented, discussing problems related to identification of these roots. Atlas was published by Silva Slovenica Publishing Centre and is available free of charge thanks to EUFORINNO FP7 Capacities Project (REGPOT no. 315982).



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### (P-18) Arbuscular and ectomycorrhizal root colonization in soil exposed to extreme freezing temperatures

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The widely accepted theory by D.J. Read explains the dominance of mycorrhiza types in global vegetation zones in terms of the most limiting nutrient, which is determined by climatic factors – not as much directly, but through soil formation in each zone. However, temperature and water regimes may have also direct roles in the success of arbuscular mycorrhizas (AM) and ectomycorrhizas (EM); we hypothesize that AM are more tolerant to drought than EM, and EM are more tolerant to low temperatures and frost. Here, the focus is in the tolerance of mycorrhizal propagules in soil to very low temperatures.

We collected and homogenized surface soil from sites with diverse EM and AM vegetation in Eastern Finland in late autumn. Soil batches were exposed to a range of temperatures: + 5 °C (control), -12 °C, -25 °C, -48 °C and -130 °C. Seeds of silver birch (*Betula pendula*), grey alder (*Alnus incana*) or white clover (*Trifolium repens*) were sown in the treated soils. Plants were grown in similar, favourable conditions in a growth room. Mycorrhizal colonization rates were determined after 11 weeks.

EM were not affected by the treatments significantly in either birch or alder. Arbuscule formation in clover was not significantly affected, but vesicle formation was reduced as well as the occurrence of spores and hyphae in roots. The occurrence of AM hyphae was reduced also in alder.

In conclusion, EM propagules were able to survive even the lowest temperature without any reduction in the ability to colonize roots, as expected. The reduced vesicle, spore and hypha formation by AM may be either due to delayed colonization because of mortality of vegetative mycelium, or the survival of only some species.





### (P-19) High-throughput analysis of bacterial communities in differently treated construction wood samples

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Different treatments of construction wood are commonly used in the construction industry in order to reduce the rate and effects of wood biodegradation. These treatments inhibit or slow down the decomposition driven mainly by fungi, since they play a crucial role in this process. However, less is known about the influence of these treatments on wood inhabiting bacteria. Norway spruce (Picea abies) is commonly used in construction throughout Europe and was therefore chosen for this experiment. Bacterial communities among untreated and treated spruce samples were compared. The two treatments included thermal modification and chemical treatment with an alkaline copper quaternary (ACQ) - Silvanolin©. Samples were exposed outdoors and collected after one and two years. Wood sawdust was obtained by drilling into the samples and the total DNA was extracted from 50 mg of wood sawdust using a commercial kit. Bacterial V4 16S rDNA was amplified with primers F515 and R806 (Caporaso et al., 2011) that were both modified to include Illumina adapter. Furthermore, Nextera® indices were added in a separate PCR in order to enable sample multiplexing. The amplicon based sequencing was carried out on the Illumina MiSeq platform (300bp pairedend sequencing). Open source scripts and pipelines were used for data analysis. Preliminary results indicate a significant shift of bacterial communities in ACQ treated spruce, most notably with a significant increase in relative abundance of Methylobacteriaceae.

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