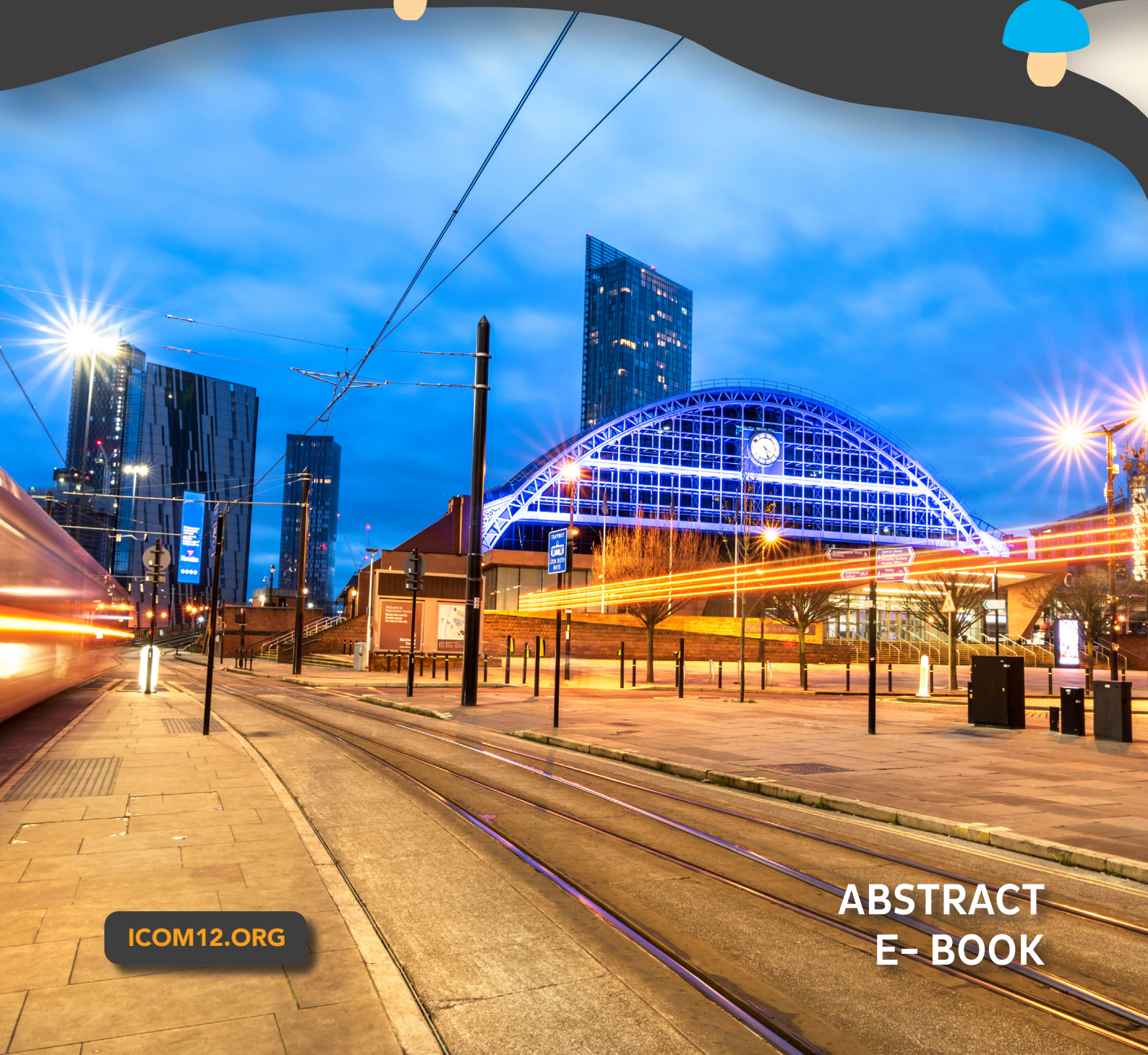




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**ABSTRACT  
E-BOOK**

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# **Mycorrhizas as Drivers of Interaction Networks**

**LT-001**

## **Mycorrhizas as Drivers of Interaction Networks**

### **EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND NITROGEN LEVELS ON MICRONUTRIENT UPTAKE AND WATER USE EFFICIENCIES OF TEFF GENOTYPES UNDER IRRIGATION**

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**Introduction/Aim:** Excessive application of nitrogen fertilizer during teff cultivation leads to progressive soil contamination in the long term and increases production costs. An alternative to reduce over fertilization is to enhance nutrient availability using arbuscular mycorrhizal fungi (AMF) that promote nutrition, crop growth and development.

**Materials and Methods:** combination of two teff genotypes, AMF and nitrogen levels were evaluated in field condition with factorial randomized complete block design.

**Results:** Our findings revealed that the combination of genotype zezew with AMF at 138 kg ha<sup>-1</sup> N increased above ground biomass yield by 1530.68%, grain yield by 321.71 %, nitrogen uptake in straw by 887.34 % nitrogen uptake in grain by 608.17%, zinc uptake in straw by 785.71%, zinc uptake in grain by 655.56%, iron uptake in straw by 3120% iron uptake in grain by 3225%, water use efficiency by 404% as compared to control respectively. The highest agronomic use efficiency(AUE) (32.95 kg kg<sup>-1</sup>) and apparent recovery efficiency(ARE) (31.23%) was recorded from the genotype zezew with AMF and at 46 kg ha<sup>-1</sup> N.

**Discussion:** enhanced Zn and Fe uptake under Zezew genotypes and AMF increases the nutritional quality of the crop on nutrient deficient soils. The combined application of Zezew teff genotypes with AMF and N fertilizer increased yield, nutrient uptake and water use efficiency compared to the control treatment.

**Conclusions:** Symbiosis of AMF with teff genotypes not only yield and nutrient uptakes, but also improved nutritional quality of the crop which eventually human health implication.

**Keywords:** Iron, Zinc, Nutrient uptake, teff genotype, use efficiency

**LT-003**

**Mycorrhizas as Drivers of Interaction Networks**

**ARBUSCULAR MYCORRHIZAL FUNGI ORCHESTRATE THE CIRCADIAN DYNAMICS OF RHIZOSPHERE MICROBIOME STRUCTURAL AND FUNCTIONAL INTERACTION NETWORKS**

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**Introduction/Aim:** Synchronized regulation of rhythms in symbiosis is crucial to coordinate dynamics in interactions throughout the day and night for all the partners. Circadian clocks are endogenous timing mechanisms that orchestrate gene expression and phenotypic rhythmicity of organisms. Plants, AMF and their associated microbiota have coevolved forming a unit termed the plant holobiont. Previously, we showed the arbuscule abundance exhibits a strong circadian rhythm which reflects the dynamics of nutrient exchange in AM symbiosis. Because the arbuscular mycorrhizal (AM) symbiosis mediates much of the nutritional flux between the plant and soil microbes, it likely represents the backbone of the plant holobiont driving daily fluctuations in the plant holobiont interaction networks. However, the consequences on rhizosphere microbiome structure and function remain a black box. We investigated circadian rhythms in rhizosphere microbiome structure and function, and their regulation by AM symbiosis.

**Materials and Methods:** Using a taxonomically and functionally defined diverse rhizosphere community, we tested the effects of the circadian regulation of the symbiosis between plants and AMF on rhizosphere microbe community structure and functioning. We used phenotype profiling, metabarcoding and metatranscriptomics.

**Results:** We found that AMF dramatically changed circadian-regulated structural and functional networks of the rhizosphere community. The circadian change of arbuscule abundance was linked to the periodic fluctuations of rhizosphere microbiome structure and the genes networks expressed by the rhizosphere community.

**Discussion:** AM symbiosis fundamentally influence the circadian interactions networks of rhizosphere microbiome.

**Conclusions:** Our study highlights the strong role played by the AM symbiosis in the orchestration of rhizosphere circadian-driven interaction networks.

**Keywords:** Circadian clock, Holobiont Chronobiology, Arbuscular mycorrhiza, Plant holobiont, Microbiome

**LT-004**

**Mycorrhizas as Drivers of Interaction Networks**

**STRUCTURAL ARCHITECTURE OF ECTOMYCORRHIZAL-BACTERIAL INTERACTIONS VIEWED BY HIGH RESOLUTION ELECTRON TOMOGRAPHY**

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**Introduction/Aim:** Ectomycorrhizal fungi associate with ~60% of terrestrial forests, offset ~25% of yearly CO<sub>2</sub> emissions and engage in complex multipartite communities with plants and bacteria. We recently determined ectomycorrhizal-microbial associates which positively promote plant development using soil core amplicon sequencing, transcriptomics and greenhouse recapitulation experiments (Berrios, 2023, Curr. Biol.). However, it remains uncertain how species interactions occur at the sub-cellular level and how contacts may dynamically remodel during community formation.

**Materials and Methods:** Here, we apply cryogenic electron tomography (cryo-ET) and light microscopy to explore the structural mechanisms of inter-species interaction during tri-partite community formation using field-derived fungal and microbial isolates.

**Results:** These approaches spotlight cellular wall and membrane architecture at microbial contact points in a species dependant manner.

**Discussion:** Ongoing investigations seek to assess how symbiotic architectures mediate nutrient and macromolecule exchange within soil communities.

**Conclusions:** Using cryogenic electron tomography we observe sub-cellular depictions of ectomycorrhizal fungi and bacteria from soil isolates to investigate the structural mechanisms of species contact.

**Keywords:** ectomycorrhiza, bacteria, microscopy, symbiosis

LT-005

## Mycorrhizas as Drivers of Interaction Networks

### SHARED BUDDIES: ECTOMYCORRHIZAL FUNGAL COMMUNITIES IN SEEDLINGS OF TWO IMPORTANT NORTH AMERICAN NATIVE FORESTRY SPECIES

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**Introduction/Aim:** Wildfires have increased in scale and severity globally. They can result in contiguous patches of tree mortality, hindering seedling regeneration and ecosystem recovery. One of the reasons for this sluggish recovery is the severe reduction of belowground ectomycorrhizal fungi (EcMF). Resprouting trees may facilitate post-fire recovery by maintaining EcMF inoculum in the soil. Thus, resprouting plants and their mycorrhizas can influence seedling establishment and performance, either positively or negatively. The aim of this study was to determine if seedlings of a resprouting species (*Quercus gambelii*) share EcMF with a non-resprouting species (*Pinus ponderosa*).

**Materials and Methods:** To achieve this, both plant species were cultivated in pots with soil from a mix forest. After ten months, the abundance and diversity of EcMF in their roots were analyzed.

**Results:** We observed that *P. ponderosa* showed higher colonization (98.9%) than *Q. gambelii* (79.6%). EcMF communities differed significantly between species. Only three out of the 11 identified EcMF species were common to both forestry species, including the most abundant ones: *Peziza* sp. 1 (~70%) and *Tomentella* sp. (15-20%). In *Q. gambelii*, the relative abundance of all the other EcMF was very low, while in *P. ponderosa* only *Rhizopogon* sp. (~12%) was nearly as abundant as *Tomentella* sp.

**Discussion:** Despite their abundance in western US forests, this is the first time that the composition of EcMF communities were simultaneously described in *P. ponderosa* and *Q. gambelii*, which exhibit distinct post-wildfire behaviors.

**Conclusions:** Our findings suggest that resprouting species may facilitate conifer establishment after severe wildfires by providing compatible EcMF inocula.

**Keywords:** Ectomycorrhizal fungi, *Pinus ponderosa*, *Quercus gambelii*, Resprouting species, Wild-fire

LT-006

## **Mycorrhizas as Drivers of Interaction Networks**

### **ECTOMYCORRHIZAS AND ENDOPHYTES: A NEGLECTED DUAL ECOLOGICAL NICHE?**

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**Introduction/Aim:** Ectomycorrhizal fungi (EcMF) are key symbionts in many ecosystems, particularly temperate ones, as they associate with a wide variety of tree species providing them nutrients among other things. Recent studies suggest that some EcMF are also capable of colonizing the roots of plants that do not form ectomycorrhizae, without apparent damage: we therefore call them endophytes. However, this second ecological niche has received little attention to date.

**Materials and Methods:** To assess the extent of this dual ecological niche, we sampled healthy roots of non-EcM plants (42 species, 17 families) in various temperate ecosystems across France and sequenced their mycobiota. Furthermore, we developed a fluorescence in situ hybridization (FISH) protocol to visually confirm endophytism in *Russula* spp.

**Results:** We identified EcM taxa in all species studied (but not all individuals), in variable proportions between sites and plant species. Although ubiquitous, bipartite network analyses reveal patterns of specialization in non-EcM plant and EcMF interactions. We observed an overabundance of EcMF of the *Russula* genus in sites producing fruiting bodies and confirmed that *Russula* spp. are endophytic in the roots of non-EcM plants by FISH and confocal microscopy.

**Discussion:** Proximity to EcM tree species seems to favor the colonization of non-EcM plants, suggesting that endophytism is a secondary niche for EcMF as observed in other EcM genera (e.g., *Tuber* spp.).

**Conclusions:** Our results further confirm the dual EcM/endophytic ecological niche in EcMF and support the "waiting room" hypothesis which stipulates that endophytism would have enabled the transition between saprotrophic (ancestral state that can persist vestigially) and ectomycorrhizal lifestyles.

**Keywords:** ectomycorrhiza, endophytism, *Russula*, metabarcoding, FISH microscopy



LT-007

## Mycorrhizas as Drivers of Interaction Networks

### ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI IN BIOGENIC VOLATILE-MEDIATED COMMUNICATION DURING HERBIVORE ATTACK ON TOMATO PLANTS

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**Introduction/Aim:** Plants damaged by insect herbivores (i.e., emitters) emit a complex blend of biogenic volatile organic compounds (bVOCs) to which neighboring plants (i.e., receivers) react by priming their defenses and becoming more resistant to herbivores, a phenomenon termed plant communication. This process has been documented in a wide variety of plant species, but its context-dependency remains underexplored. We aim to understand how associations with beneficial organisms such as arbuscular mycorrhizal fungi (AMF) may enhance plant communication.

**Materials and Methods:** In a controlled greenhouse experiment, we paired emitter and receiver tomato plants *Solanum lycopersicum* in a crossed factorial design to examine the effects of AMF-plant associations on bVOC-mediated communication in response to herbivory by the generalist chewing insect *Spodoptera exigua*. Both emitter and receiver plants, grown in individual pots, were subjected to either the absence or presence of mycorrhizal inoculum, thus effectively teasing apart mycorrhizal effects on the emission and reception components of signaling.

**Results:** Receiver plants inoculated with AMF whose partner (emitter) was subjected to herbivory had minimal induced resistance (i.e. reduced leaf damage and herbivore mass gain).

**Discussion:** Instead, our results show that herbivory induced plant-plant communication through bVOCs may play an important role in promoting stronger AMF associations (i.e. greater root mycorrhization and production of extra-radical hyphae).

**Conclusions:** Overall, our study contributes insights into the mycorrhizal mechanisms that underlie plant communication in response to herbivory.

**Keywords:** arbuscular mycorrhizal fungi, multi-trophic interactions, plant-plant communication, herbivory, volatile communication

LT-008

## **Mycorrhizas as Drivers of Interaction Networks**

### **MAXIMUM ENTROPY NETWORKS SHOW THAT PLANT-ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ASSOCIATIONS ARE ANTI-NESTED AND MODULAR**

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**Introduction/Aim:**In the last 15 years there have been various applications of network theory to the modelling of the associations between fungi and their host plant communities. Many applications have used a bipartite description similar to that of plant-pollinator networks, in which one layer of nodes is the plants and the other layer the fungi. Many metrics have been used to describe the structure of this type of networks and their relation to stability and dynamics.

**Materials and Methods:**Most applications have relied on null models from algorithms that randomly rewire the observed connections in terms of who is connected to whom, but subject to some basic features such as the observed number of connections to each species. Here, we review the applications to Arbuscular Mycorrhizal Fungi, highlight previous findings and present a novel application of a new generation of network models that relax some of the very limiting assumptions of traditional null models.

**Results:**We show the implications of the new models in terms of new results, which in some cases reverts previous conclusions, and the potential of new applications for example to weighted networks.

**Discussion:**We discuss the implication of these patterns for stability, especially in terms of how perturbations may propagate through the network, and the role of AMF species identity, which is critical to how these networks respond to perturbations such as species removal.

**Conclusions:**Our results show that in many cases AMF-plant associations tend to be anti-nested (plant-specific) but either modular, with a core-periphery structure, or both, depending on the assemblages analysed.

**Keywords:** Plant-Arbuscular Mycorrhizal Fungi (AMF) associations, bipartite Networks, Maximum entropy, Plant specific AMF

LT-009

## **Mycorrhizas as Drivers of Interaction Networks**

### **ARBUSCULAR MYCORRHIZAL FUNGI-MEDIATED UPTAKE OF TREE-DERIVED NUTRIENTS BY CROPS – RESULTS FROM AN ON-FARM AND MESOCOSM EXPERIMENT**

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**Introduction/Aim:** Trees within farmers' fields can enhance crop productivity through nutrient inputs but the role of arbuscular mycorrhizal (AM) fungi in mediating the uptake of tree-derived nutrients by crops from beyond crops' rooting zones is unclear.

**Materials and Methods:** On-farm, we set up root and AM fungi exclusion and control plots around *Faidherbia albida* trees and used the <sup>15</sup>N natural abundance technique to determine the magnitude of AM fungi-mediated uptake of tree-derived nitrogen by maize plants from beyond their rooting zones. In mesocosms, with maize plants in in-growth cores, we assessed tree-to-maize versus soil-to-maize nutrient transfer and the role of tree-maintained versus maize-associated AM fungi by applying <sup>15</sup>N and <sup>33</sup>P tracers via tree stem injection or root-free soil labeling compartments.

**Results:** With our on-farm experiment, we show that one third of tree-derived nitrogen in maize leaves was attributed to AM fungi-mediated nitrogen uptake from beyond maize plants' rooting zones and two thirds to nitrogen from tree leaf litter. Results from our mesocosms provide neither evidence nor disproval for tree-to-maize nutrient transfer via AM fungi. In the absence of trees, maize-associated AM fungi mediated uptake of nutrients from beyond maize plants' rooting zones.

**Discussion:** It remains unclear whether crop- and tree-associated AM fungal mycelia interlink to further enhance crops' access to nutrients. Yet, regardless of whether it is tree-maintained and/or crop-associated AM fungi, our results demonstrate that AM fungi can bridge the rooting zones of trees and crops.

**Conclusions:** Hence, managing for AM fungi can enhance the positive effect of trees in farmers' fields on crop nutrition.

**Keywords:** Agroforestry, common mycorrhizal networks, *Faidherbia albida*, maize, nutrient transfer, <sup>15</sup>N/<sup>33</sup>P isotope tracing

LT-010

## **Mycorrhizas as Drivers of Interaction Networks**

### **EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON BIOLOGICAL ACTIVITY AND BIOCHEMICAL PROPERTIES OF SOIL UNDER AGROFORESTRY CONDITIONS IN WESTERN INDIA**

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**Introduction/Aim:** Rajasthan's arid soils with high salinity and low organic matter content provide farmers low yields despite high costs for agricultural inputs, causing them to search for other options. In this context, Arbuscular Mycorrhizal Fungi (AMF) have great potential reducing fertilizer use by mediating soil nutrient cycles. Prior research indicate that AMF has various effects on soil enzyme activities. However, not yet reported are quantitative information on microbial biological and chemical soil properties for pearl millet, grown within agroforestry system in drought areas.

**Materials and Methods:** Field experiment on pearl millet (*Pennisetum glaucum*) was conducted at AUJ in July 2023 (Kharib season) within agroforestry conditions. Five different biofertilizer inoculums with two different field plots (random plot design), well-watered (WW, four monthly waterings) and drought (DW, one-time monthly watering) were used. Harvesting and soil samples analysis after 3-months as per standard methods.

**Results:** We investigated changes in soil biochemical properties related to C, N, and P cycling with biofertilizer inoculation. The field of pearl millet inoculated with *G.intraradices* showed highest MBC content of 1185.2 mg·kg<sup>-1</sup> (WW) and 1095.4 mg·kg<sup>-1</sup> carbon under DW field-conditions. In terms of MBN content, inoculation with *G.intraradices*+*Gl.fasciculatum* returned values of 1356.9 mg·kg<sup>-1</sup> (WW) and 1317.1 mg·kg<sup>-1</sup> under DW conditions.

**Discussion:** Maximum catalase activity was found in soils inoculated with *G.intraradices* alone (72.9%) whilst it was lowest (32.8%) in WW soils treated with *G.intraradices*+*Gl.fasciculatum*. Contrasting to control, urease, dehydrogenase, and microbial populations have all improved in the AMF-inoculated fields.

**Conclusions:** The findings of this study will significantly impacting the use of AMF in sustainable agriculture.

**Keywords:** Pearl millet, Arbuscular mycorrhizal fungi (AMF), Soil Microbial Carbon, Soil Microbial Nitrogen, Soil enzyme activity,

P-001

## **Mycorrhizas as Drivers of Interaction Networks**

### **ARBUSCULAR MYCORRHIZAL FUNGAL INOCULATION OF CROPS DRIVES TROPICAL AMF COMMUNITY DIVERSITY, INTERACTION NETWORK SIZE, CONNECTIVITY AND ROBUSTNESS**

Yuli Marcela Ordoñez<sup>1</sup>, Lucas Villard<sup>2</sup>, Isabel Cristina Ceballos Rojas<sup>1</sup>, Soon Jae Lee<sup>2</sup>, Ian Robert Sanders<sup>2</sup>, [Alia Rodriguez Villate](#)<sup>2</sup>

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**Introduction/Aim:** There is much interest in inoculating crops with arbuscular mycorrhizal fungi (AMF) to improve crop yield. However, there is very little known about how such practices affect the diversity and community structure of the pre-existing AMF community. This is important as inoculation effects on crop growth can either be direct, or indirect, by how the rest of the AMF community structure is affected. Here we show how inoculation with different AMF siblings of the same species greatly alters native AMF communities in what could be a beneficial way.

**Materials and Methods:** We inoculated cassava in conventional cassava farming with genetically variable *Rhizophagus irregularis*. We performed amplicon sequencing to study inoculation effects on in-root AMF community diversity and structure.

**Results:** Closely related *R. irregularis* isolates had markedly different effects on alpha and beta diversity of AMF communities as well as their phylogenetic relatedness. Most strikingly, inoculation with AMF enhanced interaction networks among native AMF taxa, resulting in more connected and robust networks of these communities composed entirely of positive rather than negative interactions among taxa.

**Discussion:** Our results are surprising because inoculating a crop with AMF represents a potential perturbation of the microbial community, including the community of native AMF. It is entirely unexpected that such a perturbation would lead to stronger positive interactions among a greater number of native AMF taxa.

**Conclusions:** Our study demonstrates that AMF inoculation strongly affects local AMF communities. However, rather than disrupting those communities, AMF inoculation drives more robust and resilient interactions among AMF taxa in community networks.

**Keywords:** competition, coexistence, AMF networks, interaction networks, inoculation, agriculture

P-002

## **Mycorrhizas as Drivers of Interaction Networks**

### **INVESTIGATING THE DEVELOPMENT OF EXTRARADICAL MYCELIUM AND COMMON MYCORRHIZAL NETWORKS OF ARBUSCULAR MYCORRHIZAL FUNGI IN MICROCOSMS**

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**Introduction/Aim:** A feature of mycorrhizal fungi, including arbuscular mycorrhizal fungi (AMF), is their ability to develop a large extraradical mycelium very efficient in absorbing mineral nutrients from the soil. These extraradical hyphae may simultaneously colonize adjacent plants of the same or different species, leading to common mycorrhizal networks (CMNs). CMNs are suggested to play important roles in ecosystems, from capture and distribution of nutrient resources to transmission of defense signals. There are few studies with regard to the ability of AMF developing extraradical hyphae and their rate of spread, eventually leading to the CMN formation in grasslands.

**Materials and Methods:** We set up a microcosm, containing a central plant of *Trifolium repens* inoculated with a single AM-fungus surrounded by a simplified plant community composed by representatives of three functional groups -graminoids, forbs, legumes- common in grasslands. Plants were grown on a sterile substrate and separated by a 20-micron mesh to avoid root-root contact.

**Results:** After 3 and 5 months, plants were harvested and mycorrhization levels were evaluated in the central and surrounding plants. *Rhizophagus irregularis* was much more efficient, in terms of percentage and timing of colonization of surrounding plants, compared to *Gigaspora margarita*.

**Discussion:** This allows *R. irregularis* to be the most suitable for future experiments.

**Conclusions:** This experimental set up can be used to assess the rate of extraradical mycelium and CMN formation and the preferential colonization of host plants by different AMF, and to test biotic/abiotic factors affecting this ability, which may be a preferable trait for selecting suitable AMF inoculants for inter/mixed cropping systems.

**Keywords:** extraradical hyphae, AM fungi, common mycorrhizal network, grassland, host specificity, microcosms

P-003

## **Mycorrhizas as Drivers of Interaction Networks**

### **CHARACTERISATION OF ARBUSCULAR MYCORRHIZA-MEDIATED INTRA- AND INTER-PLANT DEFENCE PRE-ACTIVATION AND PRIMING RESPONSES IN DAUCUS CAROTA.**

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**Introduction/Aim:** Majority of land plants form a symbiosis with mycorrhizal fungi. Furthermore, arbuscular mycorrhizal fungi (AMF) create a subterranean network of inter-connected plant root systems, thus forming common mycelial network (CMN) that is proposed to function not only as a provider of essential mineral nutrients, but also as an information superhighway by transferring diverse chemical signals between plants.

**Materials and Methods:** We used in vitro *Daucus carota* hairy-root cultures inoculated with AMF *Rhizophagus irregularis* to examine the preactivation or priming of systemic as well as inter-plant defense responses by determining the activity of biotic stress response marker genes before and after a pathogen attack.

**Results:** AMF-colonization facilitated pre-activation of several plant defence genes not only in *D. carota* hairy roots which were treated with bacterial flg22 or wounded but also in non-elicited receiver *D. carota* which were connected with the elicited sender via AMF in dual compartment Petri dish assay. Furthermore, we observed JA and SA marker gene priming in receiver roots after *Fusarium sporotrichioides* infection which was dependent on AMF colonization and inter-plant connection.

**Discussion:** To compliment or molecular data, we have established *D. carota* - *Fusarium sporotrichioides* pathoassay to determine pathogen performance in roots receiving inter-plant signals from elicited neighbours. Preliminary results indicate that CMN mediated signals modulate receiver plant tolerance to *F. sporotrichioides*.

**Conclusions:** Our data support the hypothesis that AMF can mediate inter-plant signals involved in root defence activation and priming upon fungal pathogen attack.

**Keywords:** common mycelial network, inter-plant signals, plant-fungi interactions, plant defence priming, *Daucus carota*, *Rhizophagus irregularis*

P-004

## **Mycorrhizas as Drivers of Interaction Networks**

### **DRIVERS OF VARIATION IN ECTOMYCORRHIZAL FUNGAL EXPLORATION TYPES**

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**Introduction/Aim:** Ectomycorrhizal fungi (EMF) are a ubiquitous guild of tree-associated microbial symbionts found across the globe, but we know surprisingly little about EMF trait distributions and implications for forest functioning. EMF attributes of mycelium development related to hydrophobicity and rhizomorph production influence fungal foraging and resource acquisition and can be represented by 'exploration types'.

**Materials and Methods:** Here, we combined datasets of EMF species from root tips and soils from long-term forest inventory plots across the continent of Europe to model the biological, chemical, and physical predictors of EMF exploration type distributions. EMF were sampled from root tips and soils, and full length fungal ITS amplicons were sequenced, clustered into operational taxonomic units, and assigned to EMF genera, species, and exploration types. We used in situ observations of forest properties and processes as covariables in network analyses, linear regressions, and partial least squares regression models to understand potential drivers of EMF exploration types and feedbacks with forest tree properties/processes.

**Results:** We show that high biomass, rhizomorph producing EMF are more dominant in soils than roots, and that medium-distance fringe EMF on living roots are strongly negatively linked to plant pathogens. Medium distance-fringe EMF were also tightly linked to forest tree growth and foliar nutrition. In contrast, low biomass, non-rhizomorph producing contact-type EMF were not linked to tree development but co-varied strongly with soil chemical and physical characteristics.

**Discussion:** EMF traits related to exploration types therefore demonstrate significant niche differentiation across the continent of Europe.

**Conclusions:** EMF exploration types have vast implications for forest productivity and biogeochemistry.

**Keywords:** Forest Ectomycorrhizal fungi, Mycorrhizal fungi Exploration type, mycorrhizal foraging strategy, rhizomorphs, Mycorrhizal fungi data modelling.



P-005

### **Mycorrhizas as Drivers of Interaction Networks**

#### **INTERACTIVE EFFECTS OF NITROGEN FORMS AND ARBUSCULAR MYCORRHIZAL FUNGI ON THE GROWTH OF SEVERAL INVASIVE AND NATIVE PLANTS**

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) can help plants acquire nitrogen and it prefers for ammonia more than nitrate. In addition, different plants have different preference for soil nitrate and ammonia. However, the interactive effects of nitrogen forms and AMF on plant growth and nutrient uptake are still unclear.

**Materials and Methods:** We conducted a common garden experiment of three factors (AMF, nitrogen form and nitrogen level). Two invasive plants of the family Asteraceae, *Bidens Pilosa* (also called *Bidens alba*) and *Chromolaena odorata*, and two native plants of Asteraceae, *Eclipta prostrata* and *Emilia sonchifolia*, were selected in this study.

**Results:** Our results showed that soil nitrogen forms and levels had significant effects on plant-AMF symbiosis, and the effects of nitrogen addition and AMF on growth and nutrient uptake were different between invasive and native plants. The symbiotic benefit of AMF at low nitrogen level was significantly lower than that at high nitrogen level. The symbiotic benefit of invasive plants was greater under single form nitrate or ammonia rather than the native plants.

**Discussion:** AMF might promote the invasion of invasive plant *B. Pilosa* under soil ammonia dominant condition with high nitrogen availability. The interaction between native plants and AMF was better under the condition of relatively balanced nitrogen forms in the soil, while the invasive plants obtained greater benefits from mycorrhizae under nitrate or ammonia alone.

**Conclusions:** This study improves the understanding of the enhanced mutualisms hypothesis of invasive plants in the context of nitrogen form and level.

**Keywords:** arbuscular mycorrhizal fungi, exotic invasive plants, mycorrhizal response, nitrogen form, nitrogen level

P-006

### **Mycorrhizas as Drivers of Interaction Networks**

#### **UNVEILING THE DIVERSITY AND SPECIFICITY OF ORCHID MYCORRHIZAL FUNGI ASSOCIATIONS: INSIGHTS FROM GEOGRAPHIC REGIONS AND METHODOLOGICAL COMPARISONS**

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**Introduction/Aim:** Orchids are threatened due to their great ornamental and medicinal values, and their seed germination is entirely dependent on compatible orchid mycorrhizal fungi (OMF). However, understanding the diversity and specificity of orchid-fungus associations is crucial for understanding the ecology of these symbiotic relationships and for orchid conservation purposes.

**Materials and Methods:** Here, we present results demonstrating the diversity of OMF isolates obtained from four orchid species, *Anacamptis morio*, *Dactylorhiza sambucina*, *Orchis mascula* and *Platanthera bifolia* growing in the Czech Republic, Sweden and Italy. These orchids are known to associate with *Ceratobasidiaceae* and *Tulasnellaceae* fungi, showing variation in the specificity of orchid-fungus associations. The orchid roots were screened for OMF using culture-dependent (isolation from intracellular hyphae coils) and culture-independent (Next Generation Sequencing of root sections) techniques.

**Results:** The culture-independent method revealed greater fungal diversity than the culture-dependent one, but despite the lower detection, the fungal strains isolated were in majority the abundant OMF in adult roots.

**Discussion:** The poster presents a comparison of the diversity and specificity of orchid-fungal associations across different geographic regions and orchid taxa obtained by these two methodologies and compares their efficiency for OMF detection.

**Conclusions:** The scientific outcomes can provide deeper insights into the functional implications and evolutionary dynamics of these symbiotic relationships.

**Keywords:** orchid mycorrhizal fungi, fungal diversity, terrestrial orchids, mycorrhizal specificity, orchid conservation

P-007

## **Mycorrhizas as Drivers of Interaction Networks**

### **CO-INOCULATION OF ARBUSCULAR MYCORRHIZAL FUNGI AND DARK SEPTATE ENDOPHYTES: EFFECTS ON PLANT GROWTH AND THE RHIZOSPHERE**

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) play vital similar roles in the nutrient absorption of plants, by extending the action of the root system. However, there is limited understanding of the diversity of DSE fungi and their ecological functions in ecosystems where they coexist with AMF. Unlike the obligate AMF symbiosis, DSE can grow independently from a host plant and switch to saprotrophic nutritional modes. This work is aimed at gaining a preliminary understanding of the interactions between AMF and DSE, and extend their potential use in future field applications.

**Materials and Methods:** We examined the impact of single and combined inoculation with the AMF *Rhizoglyphus irregularis* and the DSE *Cladosporium endophyticum* aff. on rosemary (*Rosmarinus officinalis*) and leek (*Allium porrum*) potted plants (n=10) subjected to nutrient-stress by evaluating physiological parameters. The microbial community structure of bulk soil was analysed by DNA extraction and amplification of prokaryotic 16S and fungal ITS2 rRNA.

**Results:** Our results showed that AMF inoculation improved the yield of both rosemary and leek, while DSE inoculation promoted leek growth but reduced rosemary development.

**Discussion:** However, the AMF/DSE combined inoculation resulted in higher rosemary growth than when AMF was inoculated alone. Cell counts indicated that the extent of AMF colonisation was reduced in the combined AMF/DSE inoculation of rosemary, pointing to some degree of fungal competition in this crop.

**Conclusions:** Such detrimental/synergistic dual effect of combined inoculation was not observed with leek, but in this case a higher yield was obtained when DSE was inoculated alone compared to AMF.

**Keywords:** Microbial inoculants, nutrient-stress, plant-microbe interactions, mutualism

P-008

## **Mycorrhizas as Drivers of Interaction Networks**

### **AM HYPHAL HIGHWAY IS NOT ENOUGH TO SECURE EFFICIENT ORGANIC N UTILIZATION IN STERILE SOIL**

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**Introduction/Aim:**Hyphae of arbuscular mycorrhizal (AM) fungi in soil associate with a specific microbiome, which appears particularly important for acquisition of nutrients from organic compounds. To test if the AM fungi carry their beneficial companions to soil patches containing such resources, we devised a pot experiment and manipulated microbial inputs into a bacterial-free plant-AM fungus-soil system.

**Materials and Methods:**Experimental pots, each including one <sup>15</sup>N-labelled chitin and one mineral nitrogen (N) enriched patch, were gamma irradiated after their assembly, and provided with monoxenically produced AM fungal inoculum, surface sterilized *Andropogon gerardii* seeds, and size-fractionated soil microbial inoculum (1, 2, 5 or 1000 micrometer or no microbes) and grown under sterile water and air supply for 9 weeks.

**Results:**We encountered very little <sup>15</sup>N transfer from the chitin patches to the plants, irrespective of the microbial diversity treatment. AM hyphal foraging preferences assessed by quantitative PCR indicated that exploration of the mineral N patches was more effective than that of the chitin patches.

**Discussion:**Our results demonstrated that, upon absence of mineralizing microbes in the organic nutrient patches, such patches appeared less attractive for the AM fungi as compared to the mineral N patches. AM fungal hyphae obviously were ineffective in priming mineralization of organic N even if provided with complex soil microbiomes at a distance from the patches.

**Conclusions:**It seems that chitin-enriched patches only become attractive for the AM fungi after chitin mineralization by competent microbes. Such microbes appear, however, not to be effectively transported to spatially restricted organic resources in soil via AM hyphal pathways.

**Keywords:** Chitin, nitrogen, sterile microcosms, microbial diversity gradient, arbuscular mycorrhizal hyphae, *Rhizophagus irregularis*

P-009

### **Mycorrhizas as Drivers of Interaction Networks**

#### **MICROBE TREE METABOLITE INTERACTIONS IN THE SOIL - PHYLLOSHERE CONTINUUM OF POPLAR TREE: WHEN MICROBES REWIRE POPLAR ROOT EXUDATE AND METABOLOME**

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**Introduction/Aim:** Trees are associated with a broad range of microorganisms colonizing their tissues. The early dynamics of the assembly of the microbiota from the root to shoot axis and how it is linked to root exudates and metabolite contents remain unclear.

**Materials and Methods:** Sterile poplar cuttings of *Populus tremula x tremuloides* clone T89 were planted in natural or gamma-irradiated soils. Bulk and rhizospheric soils, root and shoot tissues were collected from day 1 to day 30 to track fungal and bacterial communities by DNA metabarcoding. Root exudates, root and shoot metabolites were analyzed by gas chromatography-mass spectrometry.

**Results:** Root microbial colonization massively modifies the composition of root exudate, root and shoot metabolomes. Root exudation is dynamic over time, as are the rhizospheric, root and shoot microbiomes. Roots and shoots were first colonized by saprophytic microorganisms and later transitioning to endophytes and symbionts. They were first colonized by the same microorganisms that are later replaced by habitat-specific taxa, indicating soil is a reservoir of microorganisms for shoot colonization.

**Discussion:** Lipid and defense metabolites are exclusively detected in root exudates in absence of microorganisms, suggesting an active consumption by microbes or negative feedback on their production. Modification of root and shoot metabolites can be seen as indicators for microbial community establishment. Specifically, microbial colonization predominantly instigated alterations in primary metabolism in roots, while in shoots, it primarily influenced defense metabolism.

**Conclusions:** This study highlights the profound impact of microbial interactions on metabolic pathways of plants, shedding light on the intricate interplay between plants and their associated microbial communities.

**Keywords:** *Populus tremula x tremuloides*, microbiota, root exudate, metabolomics

P-010

### **Mycorrhizas as Drivers of Interaction Networks**

#### **INVESTIGATION OF ARBUSCULAR MYCORRHIZA-MEDIATED SYSTEMIC AND INTER-PLANT DEFENCE RESPONSES IN *MEDICAGO TRUCATULA***

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**Introduction/Aim:**Arbuscular mycorrhizal fungi (AMF) are capable of forming common mycelial network (CMN) to connect multiple plant root systems underground. However, the functions of CMN in inter-plant signalling and defence responses against pests and pathogens remain largely elusive with very few empirical studies. Here, we wish to test the hypothesis that AMF mediates inter-plant signals involved in plant defence activation.

**Materials and Methods:**To investigate the hypothesis, two *Medicago truncatula* (R108) plants were interconnected with *Rhizophagus irregularis* or grown without AMF. Only inter-plant signal senders were stimulated by bacterial flagellin and/or mechanical wounding while receiver plants were left untreated. We tested the expression of several plant defence marker genes (e.g. MtLOX2, MtLOX4, MtVSP1, MtPAD4, MtBGL, MtLYK5) in signal sender as well as receiver leaves 4h and 24h after application of the stimuli.

**Results:***M. truncatula* colonization with AMF reduced marker gene induction after flg22 and wounding in systemic leaves of sender plants at 4h and 24h. Interestingly, AMF-colonized receiver plants also displayed decreased marker gene activation upon interplant signals from elicited senders compared to sender plants that were not connected with receivers via AMF.

**Discussion:**When using *Daucus carota* in a similar experimental setup, we identified opposite effect on *D. carota* defence marker expression, suggesting a species-specific effects of AMF-connected receiver responses. Furthermore, decreased *M. truncatula* response to AMF-connected and defence-elicited neighbours appears to translate into enhanced susceptibility fungal pathogen *Botrytis cinerea*.

**Conclusions:**Our results support the hypothesis that CMN may mediate inter-plant signals that could either weaken or the strength receiver plant defense responses in species-specific manner.

**Keywords:** plant defence, inter-plant signals, common mycelial network, pathogen resistance, *Medicago truncatula*, *Rhizophagus irregularis*

**P-011**

## **Mycorrhizas as Drivers of Interaction Networks**

### **ECTOMYCORRHIZAL FUNGI CAN SUPPRESS GROWTH OF PHYTOPHTHORA**

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**Introduction/Aim:** Ectomycorrhizal fungi (EM) have been reported to promote resistance of tree seedlings to soil-borne pathogens. Among these Phytophthoras are the most destructive plant pathogens, causing more than 66% of all fine root diseases of woody plants.

**Materials and Methods:** Five taxa of Phytophthoras originally isolated from forest soils in the Czech Republic, where grown together with seven species of ectomycorrhizal fungi from Slovenian or Austrian forests, in in vitro challenge experiments. The fungi were grown on agar plates of either V8 medium or MMN medium. The radial growth of the fungi was determined over several weeks.

**Results:** As expected Phytophthoras grew fastest on the V8 medium, whereas the ectomycorrhizal fungi grew fastest on the MMN medium. In the challenge experiments, the outcome was dependent on the taxa of Phytophthora and EM fungus, and the medium used. On the V8 medium the faster growing Phytophthora overgrew all of the EM fungi, whereas on the MMN medium many of the EM fungi suppressed the growth of Phytophthora. However, one EM fungus *Lactarius aurantiacus* suppressed the growth of all Phytophthoras except the fastest growing *Phytophthora plurivora* on both media.

**Discussion:** The outcome of the challenge experiments was dependent upon Phytophthora and EM fungal taxa, but also the nutritional status of the cultures.

**Conclusions:** The ability of EM fungi to suppress growth of Phytophthoras is taxa dependent. Some EM fungi for example *Lactarius aurantiacus* can suppress the growth of Phytophthoras.

**Keywords:** Phytophthora, Ectomycorrhizal fungi, Suppression, Growth media, In vitro

P-012

## **Mycorrhizas as Drivers of Interaction Networks**

### **SPATIO-TEMPORAL CHANGES OF VIRUS CONCENTRATION IN ARBUSCULAR MYCORRHIZAL FUNGI INOCULATED GRAPEVINE**

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**Introduction/Aim:**Arbuscular mycorrhizal fungi (AMF) have beneficial effect on grapevine health. However, in the context of virus infection, scale of its impact remains ambiguous. To investigate whether mycorrhizal fungi induce changes in grapevine - virus system, virus quantitation was performed.

**Materials and Methods:**Two types of AMF inoculums were used on grapevine plants infected with grapevine rupestris stem pitting associated virus (GRSPaV), solely or in combination with other viruses. Sampling was done multiple times for root, petiole, and leaves of different maturity, followed by RNA extraction, and qPCR virus quantitation. Delta-delta Ct method was used for statistical analysis obtained by normalizing with treatment containing only GRSPaV without AMF.

**Results:**K-means clustering for temporal changes in virus relative quantity grouped roots and young (non-developed) leaves in distinct clusters. Repeated measures ANOVA revealed significant difference in early sampling points for root and young leaves based on AMF inoculum with mycorrhizae increasing GRSPaV quantity in roots for all virus combination, while young leaves showed opposite effect regardless of virus composition. Virus quantity was unaffected in remaining grapevine tissues.

**Discussion:**Seasonal variations followed normal GRSPaV distribution with greater extremes in AMF treated plants. Increase in GRSPaV quantity in root is possibly caused by induced root formation and nutrient supply supported via newly established symbiosis. Changes in virus quantity for developing leaves of AMF plants toward later sampling time points suggests virus translocation in a source-sink manner from root to young leaves.

**Conclusions:**AMF induced short-term changes of GRSPaV concentration depending on tissue type, with greatest influence on roots.

**Keywords:** Grapevine tissue, grapevine rupestris stem pitting-associated virus, Rhizophagus irregularis, Funneliformis mosseae, Funneliformis caledonium



P-013

### **Mycorrhizas as Drivers of Interaction Networks**

#### **ARBUSCULAR MYCORRHIZAL MEDIATED INTERPLANT SIGNALING IN MEDICAGO TRUNCATULA RESISTANCE TO FUNGAL PATHOGENS BOTRYTIS CINEREA AND FUSARIUM SPOROTRICHOID**

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**Introduction/Aim:** Plant colonization with arbuscular mycorrhizal fungi (AMF) is known to induce plant systemic resistance (ISR) against pathogen attack aboveground. Moreover, AMF can form common mycelial network (CMN) which inter-connects neighboring plants. However, the potential effects of CMN-mediated inter-plant signals remains to be investigated along with molecular mechanisms involved. Here we test the hypothesis whether AMF can function as a potential transmitter of biotic stress signals between plants to confer resistance to two economically important fungal pathogens.

**Materials and Methods:** We tested a range of known plant defence elicitors – flg22, infiltration of bacterial suspension (*Pantotea*, *Erwinia*, *Pseudomonas*), mechanical wounding and their combinations for the capability to induce systemic acquired resistance (SAR) in *Medicago truncatula* plants. Next, we colonized two *M. truncatula* with *Rhizophagus irregularis* for the establishment of CMN. We elicited immune responses in the sender plant and applied *Botrytis cinerea* or *Fusarium sporotrichoides* on the naïve receiver plants to measure necrotic lesions or infection zones. We compared the results with AMF colonized plants where the CMN was interrupted as well as AMF-free control plants.

**Results:** We discovered that wounding and flg22 application generates CMN-dependent interplant signal which confers resistance to *F. sporotrichoides* in the receiver plants. The resistance phenotype was abolished when CMN was interrupted and not observed in AMF-free plants. Surprisingly, biotic stress elicitation in the sender plant induced increased susceptibility to *B. cinerea* in AMF connected receiver plants.

**Discussion:** Our results support the hypothesis that CMN can mediate inter-plant signals which confer resistance or susceptibility to pathogen attack.

**Conclusions:** We are further analyzing receiver plant metabolome and transcriptomic responses to understand the molecular mechanisms involved in interplant signal generation and decoding in the receivers.

**Keywords:** inter-plant signals, mycorrhiza, plant-fungi interactions, plant defense, fungal pathogens

**P-014**

## **Mycorrhizas as Drivers of Interaction Networks**

### **THE IMPACT OF ORGANIC SOIL AMENDMENTS AND AMF-INOCULANTS ON NEMATODE COMMUNITIES IN APPLE ORCHARDS**

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**Introduction/Aim:** Nematodes are versatile, but there is limited understanding of the impacts that the application of bioinoculants may have on their composition and diversity. This study aims to better understand the impacts of organic amendments and AMF products on the nematode dynamics of apple orchards under organic and integrated management.

**Materials and Methods:** To assess treatment effects, the environmental DNA of soil obtained during spring and fall (2021) was assessed by 18S rDNA metabarcoding of nematodes.

**Results:** Both organic- and integrated- orchards contained higher nematode diversity during spring than in fall, although the diversity in organic orchards was generally higher. During spring for both orchards, the nematode richness was significantly reduced by treatments, including their effect on nematode composition. The seasonal effect was further revealed by differences in the proportion of families ( Rhabditidae and Tylenchidae) and their corresponding trophic groups.

**Discussion:** The observed seasonal variations are likely attributed to favorable conditions like precipitation and warm weather which facilitate decomposition to support bacterivorous nematodes during spring, whereas during fall more plant roots enrich root-feeding nematodes, like family Tylenchidae. The reduction in community diversity was likely due to the dominance of specific nematode trophic- or family- groups after field treatment.

**Conclusions:** We showed that nematode trophic guilds varied between seasons with just a few orders dominating the different guilds. Therefore, the dynamics in soil nematode functioning can be anticipated by identifying dominant nematode taxa. Our study provides insights into the effects of bioinoculants and bioextracts on the nematode composition in soils managed under different agroecosystems.

**Keywords:** Nematode community, Arbuscular Mycorrhiza Fungi (AMF), Organic soil amendments, 18S rDNA metabarcoding, Bioinoculants

P-015

## **Mycorrhizas as Drivers of Interaction Networks**

### **ARBUSCULAR MYCORRHIZAL-HERBIVORE INTERACTIONS AND THE COMPETITIVE RELEASE OF SUBDOMINANT TALLGRASS PRAIRIE SPECIES**

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**Introduction/Aim:** Plant-microbial-herbivore interactions play a crucial role in the structuring and maintenance of plant communities and biodiversity. In grasslands, herbivores can greatly influence the survival, growth, and reproduction of plants. However, few studies examine interactions of above- and belowground grazing and AM mycorrhizal symbiosis on plant community structure.

**Materials and Methods:** We established experimental mesocosms containing eight tallgrass prairie grass and forb species in native prairie soil, maintained with and without mycorrhiza, with and without soil nematodes, and with and without grasshoppers. We examined independent and interacting effects of above- and belowground herbivores on AM symbiosis and plant community structure, and potential influences of mycorrhizal responsiveness of host plants on herbivory tolerance and concomitant shifts in plant community composition.

**Results:** We observed interactions between AM fungi and both above- and belowground herbivores, while herbivore effects were additive. The dominance of mycorrhizal-dependent C4 grasses in mycorrhizal mesocosms was increased by grasshopper herbivory but reduced by nematode herbivory. C3 grasses exhibited competitive release in the absence of mycorrhiza, but this was reversed with grasshopper herbivory. Forbs showed species-specific responses to both mycorrhiza and herbivores.

**Discussion:** Large positive loadings for mycorrhizal-dependent grasses and forbs indicate AM responsiveness of plant species is integral to community structure. Further, mycorrhiza has stronger effects on communities not subjected to aboveground herbivory. Above- and belowground herbivory had additive effects on plant community dynamics.

**Conclusions:** Our research indicates that AM symbiosis is the key driver of dominance of C4 grasses in tallgrass prairies, with foliar and root herbivory being two mechanisms for maintenance of plant diversity.

**Keywords:** Herbivory, Responsiveness, Plant-herbivore, mesocosms

P-016

## **Mycorrhizas as Drivers of Interaction Networks**

### **CHARACTERIZATION OF THE ROLE OF POPLAR TERPENES IN TREE-FUNGI INTERACTIONS**

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**Introduction/Aim:** Plant terpenes, serving as organic compounds and secondary metabolites, exert a significant influence on both plant development and plant-microbe interactions. Among these,  $\beta$ -amyrin, a pentacyclic triterpene, is acknowledged as part of the terpenes involved in plant defense mechanisms. However, the precise role of  $\beta$ -amyrin in reconciling defense mechanisms with symbiotic interactions is still unclear. Our study focuses on unravelling the role of  $\beta$ -amyrin in the context of ectomycorrhiza and endophytic symbiosis.

**Materials and Methods:** Transgenic poplars were produced with enhanced (OE) or suppressed (KO) expression levels of a gene encoding  $\beta$ -amyrin synthase. Root growth and development, as well as root colonisation by the ectomycorrhizal fungus "*Laccaria bicolor*" and the endophytic fungus "*Hyaloscypha* (synonymous: *Cadophora*) *finlandica*" were evaluated in the study.

**Results:** Our preliminary results indicate that overproduction of  $\beta$ -amyrin does not impact root development. No significant differences were observed among the tested poplar lines regarding the rate of ectomycorrhiza (EcM) formation with "*L. bicolor*" and the presence of the endophyte "*H. finlandica*" in to the roots. Nevertheless, we showed that a high-level of  $\beta$ -amyrin inhibits "*H. finlandica*" coil formation. This suggests that  $\beta$ -amyrin could potentially hinder endophyte fungus penetration and limit its colonization to the root cell apoplast.

**Discussion:** Our results will provide a valuable data to understand the function of  $\beta$ -amyrin in plant immunity and its participation in symbiotic relationships.

**Conclusions:** Our findings will deepen our understanding of the role of tree terpenes in the regulation of ectomycorrhizal and endophytic interactions.

**Keywords:** Ectomycorrhiza, endophytes,  $\beta$ -amyrin, terpene

P-017

## **Mycorrhizas as Drivers of Interaction Networks**

### **HOW DO ARBUSCULAR MYCORRHIZAL FUNGI (AMF) FROM DIFFERENT SOIL LEGACIES AFFECT INTERACTIONS BETWEEN PLANTS AND ROOT-FEEDING NEMATODES?**

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**Introduction/Aim:** Soil legacies are changes in soil properties left by previous plants and can influence subsequent plant growth. This study investigates how soil legacies, particularly plant-induced changes in arbuscular mycorrhizal fungi (AMF) communities, affect plant-belowground herbivore interactions.

**Materials and Methods:** In a plant-soil feedback experiment, riverbank soil was first conditioned by plants of either *Centaurea jacea* (native) or *C. stoebe* (range-expanding) plants. Subsequently, in the feedback phase, new *C. jacea* plants were grown in the presence or absence of the root-feeding nematode *Meloidogyne hapla*, in sterile riverbank soil inoculated with soil microbes (AMF, soil bacteria (Mi), AMF+Mi, or none) isolated from the soils conditioned by either native or range-expanding plants.

**Results:** AMF and AMF+Mi from *C. stoebe*-conditioned soil increased *C. jacea*'s tolerance to high densities of the root-feeding nematodes whereas plant resistance was unaffected by any soil treatment. Mi alone reduced plant biomass regardless of root-feeding nematode presence and soil-conditioned sources, but AMF mitigated this effect. AMF colonization (25-50%) was positively associated with plant biomass ( $p < 0.05$ ; for *C. jacea*-conditioned soil).

**Discussion:** Only AMF from *C. stoebe*-conditioned soil improved *C. jacea*'s tolerance, indicating plant species-specific effects in the impact of AMF-mediated soil legacies on plant-root-feeding nematode interaction. The effects of AMF colonization on plant biomass were not linear, indicating a complex relationship between AMF colonization level and plant growth.

**Conclusions:** This study sheds light on the plant species specificity of AMF-soil legacy effects on plant-root-feeding nematode interactions, emphasizing the need to further explore the role of AMF community shifts in driving soil legacy effects on plant defenses.

**Keywords:** arbuscular mycorrhizal fungi, root-feeding nematodes, soil legacies, tolerance

P-019

## **Mycorrhizas as Drivers of Interaction Networks**

### **TREE MYCORRHIZAL TYPES AND THEIR MIXTURE ALONG TREE DIVERSITY GRADIENTS SHAPES THE SOIL FUNGAL COMMUNITIES IN FOREST ECOSYSTEMS**

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**Introduction/Aim:** Extensive research has elucidated the underlying processes of plant-soil microbe associations and their immediate interaction with arbuscular mycorrhizal and ectomycorrhizal fungi. However the understanding of interactions among tree species with similar and different mycorrhizal types, as well as mycorrhizal type mixtures along tree diversity levels, remains scarce. This study investigates the interactive effects of mycorrhizal types their mixture and tree diversity on the soil fungal richness and community composition in their rooting zone

**Materials and Methods:** The study was performed at the MyDiv tree diversity experimental platform, where arbuscular (AM) trees, ectomycorrhizal (EcM) trees, and mixtures of AM and EcM trees were planted in monocultures, two-species plots and four-species mixtures. Soil samples were collected from the rooting zones of eight target tree species and the fungal communities were assessed by paired-end Illumina sequencing of the fungal internal transcribed spacer region.

**Results:** We observed significant effects of tree mycorrhizal type mycorrhizal type mixtures, and their interaction with increasing tree diversity on fungal alpha-diversity patterns and fungal trophic guilds. Overall, the fungal community composition was shaped by tree mycorrhizal type, mycorrhizal type mixture and their interactions along with tree diversity gradient. Consistent effects were observed for fungal trophic modes, except for symbiotic-saprophytic communities.

**Discussion:** Our findings give valuable insights into the complex interplay among tree mycorrhizal types, mycorrhizal type mixtures, and tree diversity gradients in shaping soil fungal communities

**Conclusions:** This enhances our understanding of forest ecosystem dynamics and their ecological roles in forest soil health and fertility.

**Keywords:** arbuscular mycorrhiza, ectomycorrhiza, Illumina sequencing, trophic guilds

P-020

## **Mycorrhizas as Drivers of Interaction Networks**

### **μCT-IMAGING THE IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI ON ROOT-SOIL CONTACT AND SOIL-PLANT HYDRAULICS UNDER DROUGHT**

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**Introduction/Aim:** Although Arbuscular Mycorrhizal Fungi (AMF) are assumed to play a pivotal role in plant response to drought, studies investigating the impact of AMF on root-soil contact and soil-plant hydraulics, especially under contrasting soil textures, are still lacking. We hypothesized that 1) AMF extend root-soil contact and hence limit the drop in matric potential across the rhizosphere, especially in drying soil, 2) AMF enhance soil-plant hydraulics and plant water status during soil drying, 3) the impact of AMF on soil-plant hydraulics is more pronounced in coarse textured soils.

**Materials and Methods:** To test these hypotheses, we measured the relation between transpiration rate, soil and leaf water potential of tomato with reduced mycorrhiza colonization (RMC) and the corresponding wild type (WT) under contrasting soil textures. In a follow up experiment, we utilized synchrotron-based X-ray computed microtomography to image extraradical mycelium and to investigate the interplay of AMF and soil textures on root-(mycelium-)soil contact.

**Results:** Root-mycelium-soil contact was substantially greater than root-soil contact of the host plant. Furthermore, AMF enhanced the soil-plant hydraulic conductance of the WT during soil drying. In contrast, soil plant hydraulic conductance of the RMC declined more abruptly as soil dried.

**Discussion:**

**Conclusions:** We conclude that AMF enhance the effective root-soil contact and thus maintain the hydraulic continuity between root and soil. This reduces the drop in matric potential across the rhizosphere and enhances soil-plant hydraulic conductance of tomato under drought stress. Our findings provide new avenues towards a mechanistic understanding of how the cosmopolitan AMF confer drought tolerance to host plants in variable soil textures.

**Keywords:** Soil drying, CT-Imaging, water potential, root-soil contact, soil-plant hydraulics

P-021

## **Mycorrhizas as Drivers of Interaction Networks**

### **PLANT GENETIC EFFECTS ON THE SPATIAL HETEROGENEITY OF MAIZE MYCORRHIZOSPHERE MICROBIOTA**

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) extend extraradical hyphae (ERH) beyond the rhizosphere into the hyphosphere, accessing nutrient patches unreachable for roots. AMF rely on interactions with other microbes in the hyphosphere and on their hyphal surface (hyphoplane) to mineralize soil organic phosphorus. However, whether roots and hyphae assemble distinct bacterial and fungal communities and to what extent soil fertilization and plant genotypic variations affect those communities is largely unknown.

**Materials and Methods:** We investigated the bacterial and fungal (BF) communities in root- and hyphae-associated compartments sampled from four genotypically distinct maize lines grown under varying phosphate and nitrogen fertilization in three field studies. To obtain the hyphoplane from loamy field soil, we developed a half-automated sieving and sucrose centrifugation method.

**Results:** The BF communities inhabiting the root endosphere and rhizosphere differed remarkably in diversity and structure from those found in the hyphoplane and hyphosphere. Soil fertilization, and to a lesser extent plant genotypic changes, affected the BF communities throughout all compartments. The knock-out of Pht1;6 affected the bacterial community composition at the hyphoplane more significantly than the other plant genetic changes, but did not alter the ERH length.

**Discussion:** Although further mechanistic investigations are needed, our study contributes to characterizing hyphoplane fungi and bacteria and provides a list of BF genera that could play a role in hyphoplane communities assembly and be relevant for arbuscular mycorrhizal symbiosis.

**Conclusions:** We conclude that plant genetic changes and signaling are transmitted to communities assembled by hyphae.

**Keywords:** hyphoplane, hyphosphere, microbial communities, mycorrhizal P uptake, extraradical hyphae



**P-023**

**Mycorrhizas as Drivers of Interaction Networks**

**ECTOMYCORRHIZAL SYMBIOSIS AND THEIR RELATION TO HOST PLANT PERFORMANCE AND GAS EMISSIONS FROM SOIL**

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**Introduction/Aim:** Mycorrhizal fungi are key drivers of nutrient cycling and biological carbon fixation across global ecosystems through their interactions with growing and decaying flora and their associated microbiomes. These organisms form a vast subterranean hyphal network using carbon as their building and develop specialized symbiotic interfaces with their host plant for nutrients and carbon exchange. Arbuscular mycorrhiza (AM) and ectomycorrhiza (EcM) are the two globally dominant and widespread types of mycorrhizal symbiosis, and the most studied ones. While our understanding of the role of these biotrophic symbionts in the global carbon cycle is still developing, it is becoming evident that they are more vital than previously thought.

**Materials and Methods:** Herein, we conducted well-designed in vitro and mesocosm experiments aimed at characterizing and deciphering the impact of different ectomycorrhizal fungal species isolated from poplar roots and rhizosphere on CO<sub>2</sub> and volatile organic compounds (VOCs) emissions from soil and plant health and development.

**Results:** Preliminary results from these experiments showed that different EcM fungal species significantly improved plant functional traits associated with soil carbon sequestration, including enhanced root system, and increased above ground plant biomass.

**Discussion:** In addition, inoculation of plants with a synthetic community of four EcM fungi resulted in a significant change of VOC profiles.

**Conclusions:** Current experiments are carried out using a multiplexer automated soil CO<sub>2</sub> Flux System to determine the impact of this community on soil CO<sub>2</sub> fluxes.

**Keywords:** Ectomycorrhizal symbiosis, beneficial fungi, Poplar, volatile organic carbon

P-024

## **Mycorrhizas as Drivers of Interaction Networks**

### **CHITOOOLIGOSACCHARIDE PERCEPTION IS REQUIRED FOR ARBUSCULAR MYCORRHIZAL ASSOCIATIONS IN *MEDICAGO TRUNCATULA***

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**Introduction/Aim:** Plants engage in symbiotic relationships with arbuscular mycorrhizal fungi (AMF), utilizing signaling molecules like chitooligosaccharides (COs) and lipo-chitooligosaccharides (LCOs) to facilitate nutrient acquisition. In *Medicago truncatula*, COs, specifically CO4-CO8, induce symbiosis signaling through receptor-like kinases MtCERK1, LYR4, and LYK8, forming a crucial receptor complex. This complex contributes to calcium oscillations essential for symbiotic outcomes.

**Materials and Methods:** *Medicago truncatula* YC3.6 and Calcium imaging

**Results:** In a recent study, we generated a *lyk8/cerk1* double mutant, which exhibited a complete absence of arbuscular mycorrhizal fungal colonization. Moreover, calcium oscillations triggered by COs were entirely abolished in this mutant. Intriguingly, while CO-triggered calcium oscillations were affected in the *lyk8/cerk1* mutant, those induced by LCOs remained unaffected.

**Discussion:** The result highlights the specific engagement of the LYK8/CERK1 receptor complex, its pivotal role in the regulatory dynamics of arbuscular mycorrhizal associations.

**Conclusions:** Chitooligosaccharide perception is required for arbuscular mycorrhizal associations in *Medicago truncatula*

**Keywords:** *Medicago truncatula*, Arbuscular mycorrhizal fungi, Chitooligosaccharides, Lysin motif receptor-like kinase

P-025

## **Mycorrhizas as Drivers of Interaction Networks**

### **TRANSCRIPTOMIC AND METABOLOMIC PLANT RESPONSES TO INTER-PLANT SIGNALS MEDIATED BY COMMON MYCELIAL NETWORK**

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) colonize multiple root systems, forming common mycelial network (CMN) as wired connection between plants. However, the role of CMN in transferring signals from plant-to-plant remains poorly understood and characterised. Here we investigate plant responses to signals received via CMN from neighbours that have been challenged with plant defence elicitors

**Materials and Methods:** *Medicago truncatula* plants were inoculated with *Rhizophagus irregularis* to establish CMN which was left intact or interrupted mechanically. Sender plants were challenged by wounding and immunogenic peptide flg22. Receiver plant leaves were collected for characterisation of transcriptomic (RNA-seq) and metabolomic (GS-MS & HPLC-MS) responses

**Results:** We identified more than 4800 DEGs in naïve plants responding to signals from flg22-treated and wounded neighbour sender plants and dependent on intact CMN. These included transcription factors, receptor-like kinases and plant defence genes involved in salicylic and jasmonic acid signalling pathways along many other biological functions. Furthermore, we identified markedly different volatile and metabolite profiles of *Medicago* plants that share intact AMF-connection with biotic stress stimulated signal senders. These involved increased production of plant volatiles methional, 2,4-heptadienal and decrease in 2-amino-4-methylpentamine as well as changes in flavonoid and phenol lipid pathways.

**Discussion:** Plant transcriptome and metabolome demonstrates significant responses to neighbour stress signals and depend on intact CMN between plants. This has profound implications for plant defence against future attack from pests and pathogens.

**Conclusions:** Our data support the hypothesis that CMN mediates inter-plant signals which induce pronounced and complex changes in receiver plants prior to exposure to secondary infections or pest attack.

**Keywords:** common mycelial network, inter-plant signals, plant transcriptome, pathogen resistance, *Medicago truncatula*, *Rhizophagus irregularis*

P-026

## **Mycorrhizas as Drivers of Interaction Networks**

### **ESSENTIAL CRITERIA FOR FEREMYCORRHIZAL SYMBIOSIS**

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**Introduction/Aim:** Feremycorrhiza (FM) is a symbiosis between the Australian native fungus *Austroboletus occidentalis* and diverse plant species. We clarify and emphasize that feremycorrhiza, meaning 'nearly mycorrhiza' (i.e. not mycorrhiza, but closely related to ectomycorrhiza), is a suitable term for this novel symbiosis.

**Materials and Methods:** A fungus must satisfy all of the following three criteria to be categorized as feremycorrhizal:

**Results:** 1) phylogeny - belonging to an ectomycorrhizal (ECM) lineage, 2) physiology - possessing the key hallmarks of ECM fungi, including the lack of invertase (sucrose-hydrolyzing) genes and limited saprotrophy as indications of facultative biotrophism, and 3) function - promoting plant growth and nutrition, while the root colonization feature is absent.

**Discussion:** Another newly discovered ECM-related symbiosis, Guapiroid ECM, may represent an earlier stage in the evolution of feremycorrhiza from ECM within the genus *Austroboletus*, wherein the fungus (*Austroboletus festivus*) has already lost the capacity to form Hartig nets but can still colonize roots. The hexose-limited saprotrophism and the loss of robust saprotrophic activities in the feremycorrhizal fungus would only be evolutionarily advantageous if the fungus had transitioned into a biotroph (i.e. if the fungus had already secured a carbon source from host plant), similar to ECM fungi, and would not confer an adaptive advantage for a free-living saprotroph in soil. Hence, ectomycorrhiza-to-feremycorrhiza evolution is a more likely scenario than feremycorrhiza-to-ectomycorrhiza evolution.

**Conclusions:** The advent of two distinct and novel ECM-related symbioses within the fungal genus *Austroboletus* testify to the dynamic interactions between genetic, environmental, and ecological factors defining the symbiotic relationships between fungi and plants.

**Keywords:** Feremycorrhiza, Nearly Mycorrhiza, Ectomycorrhiza, Appropriate Terminology, Symbiosis

P-027

## **Mycorrhizas as Drivers of Interaction Networks**

### **CROSS-KINGDOM NUTRIENT EXCHANGES IN THE PLANT-ARBUSCULAR MYCORRHIZAL FUNGI-BACTERIA CONTINUUM**

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**Introduction/Aim:** Mycorrhizal symbioses are pervasive interkingdom interactions established between land plants and soil fungi. Among them, the association between plants and arbuscular mycorrhizal (AM) fungi is well acknowledged for its dramatic impacts on plant performance and ecosystem functioning. Only recently, AM fungi-associated bacteria have been identified as cooperative actors which participate in the two-partner symbiosis; the bacteria which colonize the hyphosphere (the thin soil area surrounding the extraradical hyphae) are named hyphospheric bacteria to distinguish them from bacteria living inside the AM hyphae.

**Materials and Methods:** We develop the novel concept of plants-AM fungi-bacteria (PAMFB) continuum to summarize the current advances and discuss future perspectives in soil microbiology.

**Results:** First, we describe the top-down carbon flow and the bottom-up mineral (especially phosphorus and nitrogen) flow in the PAMFB continuum as well as how AM fungi-bacteria interaction influences biogeochemical cycling of nutrients (e.g., carbon, phosphorus and nitrogen). Second, we discuss how AM fungi interact with hyphospheric bacteria or endobacteria to regulate the nutrient exchanges between plants and AM fungi, and the possible molecular mechanisms underpinning this continuum.

**Discussion:** We focus on future perspectives for studies on the hyphosphere to facilitate applications of AM fungi and hyphospheric bacteria in the current context of sustainable agriculture.

**Conclusions:** We present the most recent advances in our understanding of how plants, AM fungi, and their associated bacteria exchange host plant-derived C and soil-derived minerals. These exchanges provide energy to the heterotrophic partners and economically convenient nutrients to plants.

**Keywords:** Carbon flow, nutrient flow, hyphosphere, hyphospheric bacteria, regulatory processes

P-028

### **Mycorrhizas as Drivers of Interaction Networks**

#### **IMPACT OF DOUBLE ROOT SYMBIOSIS (ARBUSCULAR MYCORRHIZA AND NODULATION) ON NUTRIENT DISTRIBUTION IN CEREAL CROP-LEGUME INTERACTION**

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**Introduction/Aim:** Intercropping cereal crops with legumes offers an agroecological solution to improve sustainability and environmental impacts. Roots of cereal crops and legumes could be connected by a network formed by the mycelium of arbuscular mycorrhizal fungi (AMF). Legumes also form nodules with rhizobial bacteria. In these mutualistic symbioses, nutrients are exchanged between plants and root microorganisms.

**Materials and Methods:** *Medicago truncatula* and *Sorghum bicolor* were used. The fungal and rhizobial partners were *Rhizophagus irregularis* and *Sinorhizobium medicae*. Pairs of plants were planted into microcosms, with three compartments; two root hyphal compartments are separated from a hyphal compartment by an air gap. Gene regulation of plant and symbionts was studied in roots and nodules through transcriptomic analysis. The 3D repartition of AMF in the root system was assessed after root transpiration. Nutrient exchanges were studied through stable isotope tracing.

**Results:** Our results give new insights in the functioning and physiology of plant/microbe and plant-plant interaction. Presence of one or two root symbiosis modifies the carbon investment in the network by each plant, and the nitrogen reward and its redistribution. Benefits is root symbiosis dependent. The fungal gene expression revealed a fine tuning in both plants depending on the presence of Rhizobial symbiosis. Plant gene expression depends on the N:P content.

**Discussion:** The legume and the cereal crop nutrition are modulated when one or two root symbiosis are formed.

**Conclusions:** Our study open the question on the engineering of mycorrhizal fungi and rhizobia to modulate the functioning of mycorrhizal roots and nodules and to modify nutrient fluxes in intercropping.

**Keywords:** arbuscular mycorrhiza, nodulation, nutrient distribution, crop-legume interaction

P-029

## **Mycorrhizas as Drivers of Interaction Networks**

### **CLARIFYING THE DEFINITION OF COMMON MYCORRHIZAL NETWORKS**

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**Introduction/Aim:**The current use of the term ‘common mycorrhizal network’ (CMN) stipulates a direct, continuous physical link between plants formed by the mycelium of mycorrhizal fungi. This means that a specific case (involving hyphal continuity) is used to define a much broader phenomenon of hyphae interlinking among roots of different plants.

**Materials and Methods:**We propose a more inclusive definition of the CMN as a network formed by mycorrhizal fungi among roots of different plants, irrespective of the type of connection or interaction, and not limited to direct hyphal linkages.

**Results:**This approach leads to a hierarchy of concepts surrounding common mycorrhizal networks, where the specific case of hyphal continuity is denoted as CMN-HC.

**Discussion:**By contrast, the broader concept of common fungal networks (CFN) is introduced as well, capturing the case where fungi in addition to mycorrhizal fungi are involved, or rather, not specifically excluded.

**Conclusions:**This hierarchy is supported by the current reality of research, as shown in a systematic mapping exercise for arbuscular mycorrhizal fungi.

**Keywords:** AM fungi, common mycorrhizal network, grassland, common fungal networks, hyphal continuity

P-030

## **Mycorrhizas as Drivers of Interaction Networks**

### **ASSESSING THE DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGAL SPORE ASSOCIATED FUNGI AND BACTERIA USING METAGENOMICS AND CULTURING**

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**Introduction/Aim:**Arbuscular Mycorrhizal Fungi (AMF) cultivate internal and external microbiomes that include bacteria and non-AMF fungi. The AMF microbiome harbors bacteria with genes for solubilizing phosphorous, fixing nitrogen, producing phytohormones, and more, indicating that these bacteria influence the plant host. To utilize the benefits of AMF in agriculture, understanding the diversity, assembly, and function of the AMF microbiome is key.

**Materials and Methods:**AMF spores were obtained directly from sorghum field soil in Arizona and Georgia, and from derived pot cultures. Fungal and bacterial amplicon sequences were obtained from popped and un-popped AMF spores to assess the diversity and variability of internal and external microbial associates at a single spore level. Further culturing was performed to isolate spore-associated bacteria and non-mycorrhizal fungi for whole genome sequencing and further experimentation. Additionally, scanning electron microscopy was used to visualize the spore surface.

**Results:**Culturing results indicate that viable bacteria and non-mycorrhizal fungi are on the surface of AMF spores. Preliminary microscopy results show bacteria adhering to the surface of AMF spores.

**Discussion:**Here we present one of the first robust studies of endophytic bacteria and fungi inhabiting the spores of AMF. It is increasingly clear that the mycorrhizal symbiosis encompasses fungal, bacterial, and plant interactions. Understanding the complex ways AMF interact with other microbes, especially in agricultural systems, will facilitate improvements to farming practices that foster healthy mutualistic microbial communities.

**Conclusions:**The results of this study will provide identification of putative important internal and external fungal and bacterial partners of sorghum-associated AMF for future functional studies.

**Keywords:** spores, microbiome, metagenomics, endobacteria, interactions



**P-031**

## **Mycorrhizas as Drivers of Interaction Networks**

### **SPATIAL STRUCTURE OF SOIL MYCORRHIZAL COMMUNITIES IN A DISTURBED XERIC SHRUBLAND**

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**Introduction/Aim:** Mycorrhizal communities are known to influence aboveground vegetation dynamics and ecosystem functioning. However, at small scales (a few meters), their variability and spatial distribution are poorly understood. In this case study, we aimed to 1) explore the spatial structure and patchiness of soil mycorrhizal communities, 2) assess the effect of soil properties on the heterogeneity of mycorrhizal communities, and 3) determine their relationship with aboveground vegetation.

**Materials and Methods:** We applied spatially intensive sampling in a xeric shrubland in Mexico to describe the spatial variability of mycorrhizal fungi. The aboveground vegetation was characterised by patches of arbuscular mycorrhizal plants, with ectomycorrhizal trees embedded within the vegetation matrix. In a 50x30 m plot, a 5 m soil sampling grid was established, representing 60 samples. Trees and shrubs inside the plot were mapped in relation to the sampling grid. Soil mycorrhizal communities were determined by DNA metabarcoding and supplemented by measurements of soil physical and chemical properties.

**Results:** Our results indicate spatial aggregation of mycorrhizal species richness and community composition, with distinct patchiness observed between mycorrhizal groups. The dissimilarity of mycorrhizal communities was poorly explained by soil chemistry but responded to aboveground vegetation distribution.

**Discussion:** Our findings suggest that at a small scale, with low variability in soil properties, mycorrhizal communities are spatially structured by the occurrence and proximity to their plant symbionts. Disturbances in vegetation structure might result in changes to mycorrhizal communities and their functioning within ecosystems

**Conclusions:** At a small scale, soil mycorrhizal fungi are not randomly distributed in soils but are aggregated in patches.

**Keywords:** arbuscular mycorrhizal fungi, ectomycorrhizal fungi, distribution pattern, local scale, spatial

P-032

## **Mycorrhizas as Drivers of Interaction Networks**

### **INFLUENCE OF ANNUAL GRASS INVASION ON BIOCRUSTS AND MYCORRHIZAL ABUNDANCE IN A SEMI-ARID GRASSLAND**

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**Introduction/Aim:** Networks of mycorrhizal fungi may facilitate the distribution of resources from biocrusts to plant roots in semiarid grasslands. Biocrust cover has decreased with invasion by the annual grass, *Ventenata dubia*. Invasion by *V. dubia* has unknown effects on arbuscular mycorrhizal (AM) fungi. In a field survey and glasshouse experiment, we investigated how biocrusts influence the abundance of viable AM fungal propagules during *V. dubia* invasion.

**Materials and Methods:** We established field plots at three sites in the Palouse Prairie bioregion that varied in relative cover of native and invasive plants. Native plots had <5% relative cover of *V. dubia*, Transition plots contained a mixture of native and invasive cover, and Invaded plots had >30% relative cover of *V. dubia*. We collected soil samples from below biocrusts and bare soil in each plot, near native bunchgrass *Pseudoroegneria spicata* and invasive grass *V. dubia*. Samples were used as inocula in a greenhouse experiment. After harvest, fungal colonization was quantified in roots.

**Results:** In Native plots, the soil beneath biocrusts had a higher abundance of AM fungi compared to bare soil. Unexpectedly, increasing invasion of *V. dubia* did not impact the abundance of AM fungi.

**Discussion:** Our results suggest that biocrusts create a micro-niche for mycorrhizal fungi in Native plots. Also, there is a lag between shifts in the plant community and the abundance of viable AM propagules in invaded soil.

**Conclusions:** A better understanding of the interactions among native plants, biocrusts, and AM fungi will be important for mitigating the impacts of invasive species and informing grassland restorations.

**Keywords:** arbuscular mycorrhizal, biocrusts, invasion, grassland, disturbance

## Mycorrhizas as Drivers of Interaction Networks

### **TUBER MAGNATUM ENDOPHYTICALLY COLONIZES ROOTS OF HOST AND NON-HOST PLANTS**

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**Introduction/Aim:** *Tuber magnatum* is one of the most precious and appreciated truffles all over the world. It is an ectomycorrhizal fungus related to specific broadleaf trees. Nevertheless, its mycorrhizae have rarely been found in natural environment, even in productive sites, or only obtained under controlled conditions.

Recent studies have described the endophytic behavior of some *Tuber* spp. with non-ectomycorrhizal plant species that share their habitat. Similarly, in this work we investigated the endophytic hypothesis between *T. magnatum* and non-host species by applying microscopy and molecular approaches.

**Materials and Methods:** Three natural *T. magnatum* productive areas were selected (Italy). *Tuber magnatum* fruiting points were localized through ascoma collection, analyzed by PCR with *T. magnatum* specific primers on soil samples and georeferenced. The roots of 70 non-host plants surrounding fruiting points and three *T. magnatum* spore-inoculated poplar seedlings were analyzed. The roots of the plants were first surface sterilized, and their DNAs amplified with *T. magnatum* specific primers.

**Results:** *Tuber magnatum* was detected inside 12 wild species and in all poplar seedlings. Later, the positive samples were analyzed with the FISH (Fluorescent In Situ Hybridization) technique using specific probes to confirm the presence of *Tuber magnatum* mycelium inside the roots.

**Discussion:** The dualism ectomycorrhizae-endophitism of *T. magnatum* mycelium lifestyle may be probably involved in the adaptation to different soil conditions, heat stress and water scarcity and it can represent an adaptation of the white truffle life cycle.

**Conclusions:** This study suggests possible future applications of non-host species into the *T. magnatum* cultivation.

**Keywords:** endophytism, Italian white truffle, non-host plants, FISH

P-035

### **Mycorrhizas as Drivers of Interaction Networks**

#### **BENEFICIAL BACTERIA FOR THE MYCORRHIZATION OF CORYLUS AVELLANA L. AND QUERCUS PUBESCENS WILLD. WITH TUBER MELANOSPORUM VITTAD. IN GLASSHOUSE CONDITION**

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**Introduction/Aim:**The black truffle (*Tuber melanosporum* Vittad.) is a valuable ectomycorrhizal fungus that forms symbiotic relationships with a variety of host plants. The diverse soil microbial community, which includes Mycorrhizal Helper Bacteria (MHB), influences its development and may improve mycorrhizal symbiosis. This study investigates how co-inhabitant bacteria affect the mycorrhization of *Quercus pubescens* Willd. and *Corylus avellana* L. with *T. melanosporum*.

**Materials and Methods:**The trial was designed as a completely randomized block factorial experiment with six different treatments: the five bacteria selected for the experiment with the truffle inoculum (*Pseudomonas fluorescens*- *T. melanosporum*, *Pseudomonas jessenii*- *T. melanosporum*, *Priestia megaterium*- *T. melanosporum*, *Paenibacillus polymyxa*- *T. melanosporum*, and *Serratia marcescens*- *T. melanosporum*) and the control inoculated only with *T. melanosporum*.

**Results:**After eight months, roots of *C. avellana* inoculated with *P. megaterium* showed a significant increase in shoot height and mycorrhization percentage (73%) compared to the control (60%). On *Q. pubescens*, the strain *P. jessenii* produced statistically higher values for mycorrhizal percentage (69%) than the control (52%), as well as a significant increase in the average values of the number of root tips, projected and surfaced area.

**Discussion:**According to the results, two distinct bacterial strains induced mycorrhization in the two plant hosts. This interesting aspect could be attributed to plant species having different nutritional element requirements in the presence of different MHB.

**Conclusions:**This work reports that the use of environmentally friendly natural microbial inocula, such as MHB, may represent a potential alternative to fertilizers, as well as the possibility of selecting the best fungal-bacterial-plant combination for mycorrhizal optimization.

**Keywords:** ectomycorrhizal symbiosis, black truffle, Mycorrhizal Helper Bacteria, hazelnut, downy oak

P-036

## **Mycorrhizas as Drivers of Interaction Networks**

### **ARBUSCULAR MYCORRHIZAL FUNGI ALTER OXIDATIVE STRESS RESPONSE IN VIRUS INFECTED GRAPEVINE**

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**Introduction/Aim:**Limited and inconsistent data on interactions between plants, virus and arbuscular mycorrhizal fungi (AMF) are available. In this study we addressed AMF potential to modify oxidative stress responses caused by virus infection in grapevine as a model woody plant.

**Materials and Methods:**Greenhouse experiment was set up with 15 treatments in total. Grapevine was treated with two types of AMF inocula and infected with four different virus combinations, including their respective untreated controls. Lipid peroxidation (LPO), superoxide dismutase (SOD), guaiacol and ascorbate peroxidase (GPOD, APX), glutathione reductase (GR), polyphenol oxidase (PPO), ascorbic acid (AsA) and proline were measured in mature leaf tissue five months after AMF inoculation.

**Results:**AsA and SOD responded to AMF inoculation most clearly and reached significantly lower values in AMF-plants compared to non-AMF plants. Proline increased significantly in plants treated with one type of AMF inoculum and in specific virus combinations. APX, PPO and GPOD variations depended to a lesser extent to AMF presence and more on combination of viruses.

**Discussion:**Virus infection causes oxidative stress in grapevine, which can potentially be alleviated by mycorrhiza-induced resistance. AMF application caused overall reduced AsA and SOD in mycorrhized plants, indicating a stronger struggle with viruses in AMF-free grapevine. Higher proline in plants inoculated with one AMF inoculum type and in specific virus combinations point to AMF and virus composition as important factor in this tripartite interaction.

**Conclusions:**AMF induces significant modifications in oxidative status of grapevine coping with virus infection, elucidating poorly understood interactions of plants, virus and AMF.

**Keywords:** arbuscular mycorrhizal fungi, grapevine viruses, ascorbic acid, superoxide dismutase, proline

P-037

## Mycorrhizas as Drivers of Interaction Networks

### TOWARDS A RELEVANT EXPERIMENTAL MODEL OF AM FUNGAL HYPHOSPHERE

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**Introduction/Aim:**Hyphosphere of arbuscular mycorrhizal (AM) fungi harbors microbial communities assumed to play important role in plant holobiont functioning. Yet our knowledge about complex interaction in this soil zone is still in infancy. To establish realistic experimental model of the hyphosphere and deepen understanding of the processes in this zone, *de novo* isolation and functional characterization of bacterial strains from this elusive space have been undertaken.

**Materials and Methods:**Bacteria were isolated from root-free compartments enriched either with chitin or phytate, identified by 16S sequencing, and tested for their capacity to degrade chitin, phytate, and proteins. Pairwise interactions between selected isolates were examined on Petri plates, and their behavior within AM fungal hyphosphere is assessed *in vitro*.

**Results:**Most cultured bacteria belonged to genera of *Streptomyces*, *Pseudomonas*, and *Sphingomonas*, with several other genera represented as well. Preliminary results indicate lack of strong antibiosis between the isolated strains. Testing their behavior in the presence of AM fungus is currently ongoing.

**Discussion:**Molecular profiling of the root-free patches, which were used for bacterial isolation, indicated that *Sphingomonas* was one of the genera strongly associated with AM fungus. Interaction of this particular genus with other bacteria and with the AM fungus may thus be of exceptional significance for the functioning of AM fungal hyphosphere.

**Conclusions:**Although preliminary tests are still ongoing, the results obtained to date promise establishment of a relevant experimental model of AM fungal hyphosphere. The bacterial isolates could be provided either alone or in synthetic communities to address several open questions in AM fungal hyphosphere ecophysiology.

**Keywords:** arbuscular mycorrhiza, hyphosphere, microbiome, model, diversity, nutrient

P-038

## **Mycorrhizas as Drivers of Interaction Networks**

### **HOW DO PLANTS, MYCORRHIZAL FUNGI AND SOIL MICROBES INTERACT TO PROMOTE CARBON AND NUTRIENT CYCLING?**

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**Introduction/Aim:** Phosphorus plays a critical role in facilitating plant growth and Arbuscular Mycorrhizal Fungi (AMF) provide an alternative avenue for phosphate absorption in soil. The hyphosphere, characterized by the presence of AMF hyphal exudates, serves as a focal point for the mobilization of organic phosphate. Understanding the ecological roles of the hyphosphere microbiome is crucial to elucidate the broader impact of these interactions on plant nutrition.

**Materials and Methods:** We will employ in vitro culture system with bi-compartmental petri plate and *Rhizophagus irregularis* to investigate the effect of variable Pi concentrations on hyphal exudates and impact of these exudates on soil microbial community. Further in vivo characterizations of the hyphosphere microbiome will be performed using compartmentalized microcosms in broad bean and maize using metabolomics, metabarcoding and metagenomics.

**Results:** We will present first results from the experiments on how hyphosphere microbiome responds to changes in Pi availability. We expect that increasing Pi would alter the hyphal exudate composition and dependency of plants on hyphosphere microbiome.

**Discussion:** Clarifying the ecological functions of the hyphosphere microbiome in mobilizing phosphate in variable Pi concentration will provide fresh insights into its impact on plant nutrition performance. AMF along with the associated microbiome can promote plant growth by enhancing the uptake of otherwise inaccessible soil nutrients.

**Conclusions:** This study will further our knowledge on the tripartite interaction between plants, AMF and the hyphosphere microbiome facilitating phosphorus cycling in the plant-soil system.

**Keywords:** Mycorrhiza, phosphate, Hyphosphere, micorbes.

P-039

## **Mycorrhizas as Drivers of Interaction Networks**

### **DISENTANGLING THE CONTRIBUTION OF MYCORRHIZAL FUNGI ON SOIL ORGANIC CARBON IN A SIMULATION MODEL**

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**Introduction/Aim:** Mycorrhizal fungi (MF) is the important medium that not only allow the quick input of photosynthate into soils but also influence the decomposition of soil organic carbon (SOC). Much research has focused on the role of mycorrhizal fungi in the plant growth and soil carbon dynamic. However, the contribution of different pathway to soil carbon buildup was nondescript.

**Materials and Methods:** Here, we use a modelling approach, which emphasizes the feedback caused by nutrient supply between mycorrhizae and plants, to assess the contribution of the MF to belowground carbon changes.

**Results:** We found that the aboveground and belowground carbon stocks of the ecosystem showed contrasting responses to mycorrhizal colonization. The increased nitrogen provided by MF decreased plant C/N ratio and caused a 17% increase in photosynthate, and ultimately contributed up to 7.17% increase in soil organic carbon storage. The directly involved of MF in carbon decomposition caused SOC decrease. With the prosperity of saprotrophic microorganisms and the increase of extracellular enzymes, MF contributes -10.61% to the SOC storage, and contributes nearly -29% in particulate organic carbon pool. The most powerful pathway contributing to mineral-associated organic carbon (MOC) is recalcitrant necromass, which contributed up to 30.63% of MOC storage and 18.28% SOC accumulation.

**Discussion:** Different pathway of MF mediated to SOC was distinguished in our model. Overall, although the colonization of MF caused a 13.68% decrease in SOC carbon storage, but contribute to a 10.1% increase in the proportion of MOC pool.

**Conclusions:** Specifically, our model provides a way to explicitly account for mycorrhizal pathways in influencing SOC simulation.

**Keywords:** mycorrhizal fungi, carbon stabilization, carbon stock, model simulation, plant nitrogen absorption



P-040

## Mycorrhizas as Drivers of Interaction Networks

### COMPARISON OF ECTOMYCORRHIZATION ABILITY OF *CANTHARELLUS ANZUTAKE* AMONG SIBLING CROSSING STRAINS

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**Introduction/Aim:** *Cantharellus anzutake* is an ectomycorrhizal fungus that can be grown on *Pinus densiflora* and *Quercus serrata* seedlings under laboratory conditions. We established spore isolates of C-23 strain that fruited in pot culture. The established monokaryotic strains were crossed to produce dikaryotic strains. It was concluded that *C. anzutake* has a bipolar mating system. The aim of this study was to verify the genetic effects of *C. anzutake* on the ectomycorrhizal formation on pine hosts and fructification.

**Materials and Methods:** We tested in vitro ectomycorrhizal synthesis in the parental strain C-23 and nine crossed dikaryotic strains all sharing a common monokaryotic strain with five replicates (pine seedlings). After 4 months, all seedlings were checked for the mycorrhizal status and transplanted into culture bottles filled with 250 mL of soil for another 4 months, and were transplanted into 1L bottles for 8 months. These were measured for the stem length, stem base diameter, and, number of leaves, fine root tips and ectomycorrhizal root tips. Then the mycorrhization rate (number of ectomycorrhizal root tips/ number of fine root tips) was calculated.

**Results:** All pine seedlings showed ectomycorrhizal formation with all *C. anzutake* strains tested. The number of ectomycorrhizal root tips and the mycorrhization rate differed significantly among the strains. So far, a total of nine fruiting bodies occurred in the four strains that showed higher mycorrhization rates.

**Discussion:** These results suggest that the quantitative characteristics of ectomycorrhization are controlled by the genetic backgrounds of *C. anzutake* strains.

**Conclusions:** Such quantitative characteristics of ectomycorrhization could control the fructification.

**Keywords:** ectomycorrhiza, ectomycorrhizal seedlings, *Cantharellus*, pot culture, dikaryotic strains

P-041

## **Mycorrhizas as Drivers of Interaction Networks**

### **UNDERGROUND INTERNET: ROLE OF MYCORRHIZAL NETWORK IN MEDIATING INTER-PLANT SIGNALS AND DEFENCE AGAINST PATHOGENS**

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**Introduction/Aim:** Majority of land plants form symbiotic associations with mycorrhizal fungi which extend the physiological surface of plant roots. In addition, arbuscular mycorrhizal fungi (AMF) interconnect multiple plants underground while forming common mycelial network (CMN). CMN is hypothesised to function as information superhighway in transferring diverse signals from plant-to-plant in response to biotic stress stimuli.

**Materials and Methods:** To address this hypothesis, we used AMF *Rhizophagus irregularis* to interconnect two *Medicago truncatula* plants and explored the effect of known plant defence elicitors on pathogen tolerance of AMF-connected inter-plant signal receivers. Moreover, we analysed *Medicago* leaf metabolites (HPLC-MS) and emitted volatiles (GS-MS) together with RNA-seq data to compare responses of the inter-plant signal receivers before and after the pathogen attack.

**Results:** AMF-colonization status modulates both intra- and inter-plant responses to flg22 and wounding compared to AMF-free plants. Moreover, we identified markedly different volatile, metabolite and transcriptional profiles of *Medicago* plants that share intact AMF-connection with biotic stress stimulated signal senders compared to plants with interrupted AMF-connection. Furthermore, this manifested in altered plant susceptibility to pathogenic fungi *Botrytis cinerea* and *Fusarium sporotrichoides*.

**Discussion:** Future experiments will explore small RNAs as candidates for CMN-mediated inter-plant signals and associated molecular mechanisms in receiver plants. Furthermore, an ongoing project tests our working hypothesis in other economically important crop and tree species.

**Conclusions:** Our data support the hypothesis that CMN mediates interplant signals which may modulate receiver plant susceptibility or tolerance to pathogenic fungi in species specific manner and highlights plant molecular mechanisms that may be involved in this phenomena.

**Keywords:** common mycelial network, inter-plant signals, plant defence, pathogen resistance, *Medicago truncatula*, *Rhizophagus irregularis*

P-053

## **Mycorrhizas as Drivers of Interaction Networks**

### **EXPLORING THE SECRETS OF HYPHOSPHERE OF ARBUSCULAR MYCORRHIZAL FUNGI: FROM ECOLOGICAL PROCESSES TO CROPPING SYSTEM**

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**Introduction/Aim:** Most plants have a hyphosphere, the thin zone of soil around extraradical hyphae of arbuscular mycorrhizal (AM) fungi, which extends beyond the rhizosphere. This important interface has critical roles in plant mineral nutrition and water acquisition, biotic and abiotic stress resistance, mineral weathering, the formation of soil macroaggregates and aggregate stabilization, carbon (C) allocation to soils, and interaction with soil microbes.

**Materials and Methods:** We reviewed recent research advances in the hyphosphere of AM fungi and critically appraised the important findings related to the hyphosphere processes, including physical, chemical, and biological properties and functions.

**Results:** We highlight the ecological functions of AM fungal hyphae, which have profound impacts on global sustainability through the biological cycling of nutrients, C sequestration in soil, the release of greenhouse gas emissions from soil, and the diversity and dynamics of the microbial community in the vicinity of the extraradical hyphae.

**Discussion:** As a critical interface between AM fungi and soil, hyphosphere processes and their important ecological functions have begun to be understood and appreciated and are now known to be implicit in important soil processes. Recent studies provide new insights into this crucial zone and highlight how the hyphosphere might be exploited as a nature-based solution through the understanding of interactions with the microbiome and the impacts on key processes governing resource availability, to increase the sustainability of agriculture and minimize its environmental impact.

**Conclusions:** Uncovering hyphosphere chemical and biological processes and their subsequent agricultural, ecological, and environmental consequences is a critical research activity.

**Keywords:** Arbuscular mycorrhizal (AM) fungi, Hyphosphere, Extraradical hyphae, Physiochemical process, Biological interaction, Nutrient cycling

## **Mycorrhizas as Drivers of Interaction Networks**

### **WHEN PLANTS GROW ON OTHER PLANTS: CAN MYCORRHIZAL FUNGI DRIVE DIRECT PLANT-PLANT INTERACTION NETWORKS IN TROPICAL FORESTS?**

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**Introduction/Aim:**Plants growing on other plants, the so-called epiphytes, represent no less than 10% of the land flora. While most epiphytes have long been considered to lack mycorrhizal associations, the Orchidaceae family (which represents ca. 80% of epiphytic species) consistently associates with saprotrophic-mycorrhizal fungi including on tropical tree bark. Yet, the distribution and ecological functions of these fungi in epiphytic habitats are still poorly understood.

Because orchids rely on their mycorrhizal fungi for their so-called symbiotic germination, we studied the relationship between the distribution of these bark fungal communities and that of epiphytic orchids on different tree species in a lowland tropical forest.

**Materials and Methods:**We used massive sequencing of fungal eDNA to identify (i) the bark-dwelling fungal communities of 13 tree species, and (ii) the fungi associated with the mycorrhizal roots of one orchid species which shows a strong and significant preference for one tree species. Finally, (iii) we isolated fungi from mycorrhizal roots for in vitro germination experiments.

**Results:**Results show that (i) bark-dwelling fungal communities vary according to tree species in the forest. The host-specific orchid species displays (ii-iii) specific interactions with three mycorrhizal fungi in its range, with clear temporal turnover between seedlings and adults.

**Discussion:**Seedlings-associated mycorrhizal partners are preferentially associated with the host-tree bark and may therefore spatially constrain seedling establishment in the habitat.

**Conclusions:**This study reveals for the first time that mycorrhizal fungi could drive host-epiphyte interactions in understudied tropical ecosystems and provides some insight on the effect of mycorrhizal fungi on plant ecological networks.

**Keywords:** Bark fungi, environmental DNA, epiphytic mycorrhizas, host specificity, orchid mycorrhizal

fungi, spatial distribution

# Ecosystem Functioning

O-001

## Ecosystem Functioning

### FACILITATIVE AND COMPETITIVE INTERACTIONS BETWEEN MYCORRHIZAL AND NON-MYCORRHIZAL PLANTS IN AN EXTREMELY PHOSPHORUS-IMPOVERISHED ENVIRONMENT

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**Introduction/Aim:** Non-mycorrhizal cluster-root-forming species enhance the phosphorus (P) acquisition of mycorrhizal neighbours in severely P-impooverished megadiverse systems. However, the effects on the facilitator and whether the facilitation is reciprocal remain unknown. Do mycorrhizal plants facilitate the defence of non-mycorrhizal plants against soil-borne pathogens in return for and via their symbiosis?

**Materials and Methods:** We characterised growth and defence-related compounds in *Banksia menziesii* (non-mycorrhizal) and *Eucalyptus todtiana* (ectomycorrhizal) seedlings grown either in monoculture or in mixture in a multifactorial glasshouse experiment involving ectomycorrhizal fungi and native oomycete pathogens.

**Results:** *Banksia menziesii* facilitated the growth of *E. todtiana*, which had a negative effect on that of *B. menziesii*. Furthermore, *E. todtiana* also activated a salicylic-acid mediated defence response in roots of *B. menziesii*, but only in the presence of ectomycorrhizal fungi. Roots of *B. menziesii* had higher levels of phytohormones (salicylic and jasmonic acids, jasmonate-isooleucine and 12-oxo-phytodienoic acid) than *E. todtiana*. However, *B. menziesii* also induced a shift in the defence strategy of *E. todtiana*, from defence-related secondary metabolites (phenolics and flavonoids) toward induced phytohormone response pathways.

**Discussion:** We conclude that ectomycorrhizal fungi play a vital role in the interactions between mycorrhizal and non-mycorrhizal plants in a severely P-impooverished environment, by introducing a competitive component within the facilitation interaction between the two plant species with contrasting nutrient-acquisition strategies.

**Conclusions:** This study sheds light on the interplay between beneficial and detrimental soil microbes that shape plant-plant interactions in severely nutrient-impooverished ecosystems.

**Keywords:** defence responses, facilitation, phytohormones, plant interactions

O-002

## Ecosystem Functioning

### MYCORRHIZAL SYMBIOSIS IN THE FORM AND FUNCTION OF PLANTS

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**Introduction/Aim:**The relationship between mycorrhizal symbiosis and the form and function of plants has traditionally been explored through above- and below-ground traits aimed at capturing survival and adaptive strategies. However, this relationship has primarily been examined indirectly by including mycorrhizal symbiosis within root-trait gradients. In this study, we investigate whether mycorrhizal symbiosis, with its critical activities in defence, detoxification, and nutrition for plant survival and adaptation, can directly influence plant form and function.

**Materials and Methods:**We conducted pairwise and gradient-wise analyses, considering collaboration, conservation, and plant size, using a comprehensive set of plant mycorrhizal traits and matching above and belowground plant traits. Our approach involved regression and ordination techniques, integrating categorical and numerical traits.

**Results:**Our results reveal correlations between mycorrhizal symbiosis and all examined gradients, varying based on the plant mycorrhizal trait employed. Interestingly, we observed contrasting patterns among different mycorrhizal types concerning aboveground traits (such as size and conservation gradients), while effects among types were more homogeneous with belowground traits.

**Discussion:**Mycorrhizal associations significantly influence plant morphology, physiology, and development, with their presence across most sections of the gradients suggesting a multifunctional role in plants, both nutritional and non-nutritional. This finding aligns with their ancient and diverse coevolution.

**Conclusions:**Integrating plant mycorrhizal traits into the broader framework of plant form and function provides valuable insights into plant resilience and adaptation to past, present, and future environments. This holistic approach enhances our understanding of plant responses to environmental challenges and opportunities for adaptation.

**Keywords:** mycorrhizal traits, functional ecology, mycorrhizal types, leaf economic spectrum, root economic spectrum



O-003

### Ecosystem Functioning

#### MYCORRHIZAE AND SOIL ECOSYSTEM FUNCTIONS: A TEST OF THE MAINTENANCE OF MYCORRHIZA-SOIL FUNCTION RELATIONSHIPS IN AGRICULTURAL COVERS

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**Introduction/Aim:**AM fungi may enhance most soil ecosystem functions, but agricultural management practices may alter their abundance and/or functioning and thereby their relationships with soil functions. We predicted that AM abundance in agricultural covers would relate negatively to soil functions associated to plant productivity and soil conservation due to the negative influence of fertilization and soil disturbance on mycorrhizal associations.

**Materials and Methods:**We sampled 50 sites in central Mexico including annual cropping, blackberry, avocado and forest (reference) covers and measured mycorrhizal abundance and indicator properties of C sequestration, fertility, aggregation, water retention, cycling, population regulation, and plant productivity. We used structural equation models and regressions to test the relation between AM abundance in soil and roots and indicators of those functions.

**Results:**Overall AM root colonization was a better predictor of soil functions than biomass in soil and had negative relations with fertility, cycling, C sequestration, and productivity, and positive relations with population regulation. When analyzed per cover, AM colonization had mostly negative relations with soil functions in avocado and positive in forest whereas in annual cropping and blackberry most relations were not significant.

**Discussion:**AM colonization 1) promoted plant productivity in annual cropping and forest soil, and population regulation in avocado and forest soil, and 2) was negatively related with C sequestration, fertility, cycling, and productivity in avocado.

**Conclusions:**Our predictions were in general supported, relations between AM abundance and soil functions were mostly negative in agricultural covers. Population regulation-AM colonization was the relation best preserved and blackberry the cover maintaining least altered functional relations.

**Keywords:** agronomic management, colonization, forest, soil ecosystem functions, multifunctionality

O-004

## Ecosystem Functioning

### DIGGING INTO THE "GADGIL EFFECT": A NEW EXPERIMENT TO UNRAVEL THE COMPETITIVE BALANCE BETWEEN FUNGAL GUILDS AND FOREST NUTRIENT CYCLING

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**Introduction/Aim:**In forest soils ectomycorrhizal (EM) and saprotrophic (ST) fungi interact and compete for nutrients. This competitive interaction may result in suppression of organic matter decomposition, a phenomenon known as the "Gadgil-effect". However, the mechanism by which ST and EM fungi interact in C-cycling remains controversial and poorly understood. Our novel double-controlled experiment, aims to unravel the interactions between ST and EM fungi under different forest and soil conditions to unravel the mechanism behind the Gadgil effect.

**Materials and Methods:**In late-summer 2023, we installed an experiment in forests in southern and eastern England on sandy and clayey soils, experiencing N deposition or N limitation. Plots (2x2m) were excavated (1m) to exclude EM fungi, with control plots allowing for new roots and EM ingress complete exclusion. Several measurements were made in the soil including litter decomposition, CO<sub>2</sub> evolution and fungal community analysis.

**Results:**Decomposition rates varied between substrates introduced in litter-bags and trenching treatments. Soil respiration and fungal communities also varied with notable differences between the two study locations.

**Discussion:**Our findings suggest that the competitive dynamics between EM and ST fungi may be impacted by existing root-stored carbohydrates supporting EM fungi post-root severance or might not align with the expected "Gadgil-effect". Notably, the variation between locations indicates the significant role of local environmental conditions, such as soil and forest type along with nitrogen availability, in modulating soil carbon-cycling.

**Conclusions:**Our experiment is providing new insights into the complex interactions between EM and ST fungi under varying environmental conditions and their collective impact on organic matter decomposition.

**Keywords:** Gadgil effect, litter decomposition, fungal guilds, nutrient cycling

O-005

## Ecosystem Functioning

### CONTRIBUTION OF MYCORRHIZAL FUNGI TO SOIL ORGANIC CARBON STABILIZATION

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**Introduction/Aim:** Soils, the world's primary terrestrial carbon (C) reservoir, play a central role in climate change mitigation. Yet, predictions remain largely uncertain due to gaps in our understanding of the complex interplay between plants and microorganisms and its impact on soil C cycling. Most plants live in symbiosis with mycorrhizal fungi, with plants providing assimilates in exchange for nutrients. This makes mycorrhizal fungi an important C sink in soils. However, the fate of fungal-C and its contribution to various soil organic matter fractions remains unclear.

**Materials and Methods:** To estimate the contribution of mycorrhizal fungal residues to particulate (POM) and mineral-associated organic matter (MAOM) in a temperate forest, we informed Bayesian mixing models with <sup>13</sup>C and <sup>15</sup>N natural abundance of POM and MAOM sources, i.e. leaves, roots, arbuscular mycorrhizal hyphae, ectomycorrhizal and saprotrophic sporocarps. To confirm the model output, amino sugars were measured as proxies for fungal residues in POM and MAOM.

**Results:** Plant litter constituted the main source of POM (>56%), while MAOM predominantly originated from fungal residues (>64%). Amino sugars confirmed the high contribution of fungi to MAOM.

**Discussion:** Systems dominated by ectomycorrhizal trees showed higher contribution of plant litter to POM compared to those with arbuscular mycorrhizal trees. Furthermore, simulated N deposition reduced the plant contribution to POM and MAOM in systems with arbuscular mycorrhiza indicating accelerated organic matter cycling compared to ectomycorrhizal systems.

**Conclusions:** Our results suggest fungal, not plant residues, as the main source of MAOM in temperate forests and highlight the critical role of mycorrhizal types for soil C stabilization.

**Keywords:** Mineral-associated organic matter, Particulate organic matter, Stable isotopes, Soil organic matter dynamics

O-006

## Ecosystem Functioning

### DOES FOREST AGE AFFECT ROOT-ASSOCIATED FUNGAL CARBON FLOW, SOIL ORGANIC MATTER TURNOVER AND NITROGEN CYCLING?

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**Introduction/Aim:** In boreal forests, ectomycorrhizal (ECM) fungi impact both build-up and decomposition of soil organic matter (SOM) while providing trees with nitrogen (N), but during succession, primary production is increasingly limited by N-availability. Working across a 158-year-old chronosequence, we hypothesise that a) co-metabolic priming of SOM decomposition, via ECM exudation, and inorganic-N cycling are dominant processes in younger forests, while exoenzymatic SOM degradation becomes increasingly important in older stands, and that b) the recalcitrance of ECM mycelial necromass increases with stand age.

**Materials and Methods:** We analysed fungal and prokaryotic community composition within different soil layers, and quantified genes coding for N transformations indicating loss of mineral N and fungal and bacterial abundance to examine the genetic potential for inorganic-N cycling and fungal:bacterial ratios, respectively. We also analysed aliphatic and aromatic acids in soil solution to quantify labile carbon fluxes, and determined mass loss and chemical transformation of ECM mycelia incubated *in situ*.

**Results:** Fungal and prokaryotic communities differed significantly in composition across stand age and soil layers. By contrast, the pool of archaeal *amoA* genes did not, indicating similar genetic potential for ammonia oxidation along the chronosequence. Mycelial decomposition was significantly affected by fungal species and stand age, with an average mass loss of 54%.

**Discussion:** SOM turnover was linked to microbial community composition, whereas the ubiquity of the potential for ammonium-oxidation released during SOM degradation indicates equal risk for N loss across the consequence.

**Conclusions:** SOM turnover linked to ECM dynamics are complex and our understanding of impacts of ECM-mediated transformation is still evolving.

**Keywords:** ectomycorrhizal fungi, soil organic matter, decomposition, community composition, mycelia, forest age

O-007

## Ecosystem Functioning

### ECTOMYCORRHIZAL FUNGI THAT PRODUCE MANGANESE PEROXIDASES AND THEIR NICHE(S) IN BOREAL FORESTS

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**Introduction/Aim:** Some ectomycorrhizal fungi possess genes coding for manganese-peroxidases. Ectomycorrhizal species with oxidative enzymes may decrease soil carbon stocks while maintaining forest productivity by cycling organic nitrogen, however, these fungi are difficult to study in laboratory experiments. Here we present proof of concepts for studying traits of unculturable ectomycorrhizal fungi from three studies.

**Materials and Methods:** In our first two studies, we use correlation based analyses of field-measured properties to test the effect and response traits of potential ectomycorrhizal decomposers. Sampling on a 0.1 to 10 m scale, correlations between fungal communities and manganese-peroxidase activity were evaluated. Using a national scale inventory, the niche(s) of the ectomycorrhizal decomposers assigned in the first study were specified, according to mean stand age and soil fertility. Our third study utilizes heterologous production to produce manganese-peroxidases from an ectomycorrhizal fungus.

**Results:** Manganese-peroxidase activity was correlated with fungal community composition beyond what could be explained by abiotic soil properties alone, and we assigned six potential ectomycorrhizal taxa as significantly co-localised with manganese-peroxidase hotspots. These taxa occurred more frequently in younger forests and had varying individual responses to soil fertility.

**Discussion:** There was little support for our hypothesis that ectomycorrhizal decomposers would have their main niche in older and less fertile forests, rather, there was niche variation among the investigated taxa. We produced active heterologous enzymes from *Cortinarius aurae*, which will be used to test their capacity to degrade persistent organic matter.

**Conclusions:** These studies progress the knowledge on ectomycorrhizal fungi with decomposer capacities and put forward a toolbox for studying uncultured fungi.

**Keywords:** Ectomycorrhizal fungi, Manganese-peroxidases, boreal forests, traits, decomposition, heterologous production

O-008

## Ecosystem Functioning

## DO FERTILIZER-INDUCED CHANGES IN SOIL CARBON AND FUNGAL COMMUNITIES LAST AFTER CLEARCUTTING?

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**Introduction/Aim:** Fertilization is a common practice to improve productivity in Fennoscandian boreal forests. But apart from boosting tree growth it can also increase soil carbon stocks by altering fungal communities. Increased nitrogen availability has been shown to negatively affect ectomycorrhizal fungi with extensive nitrogen-mining decomposer capabilities. Their disappearance is hypothesized to cause the increased carbon stock after fertilization. However, boreal production forests are often harvested through clearcutting, which annihilates ectomycorrhizal fungi. What happens to the fertilizer-induced increase in organic matter and to fungal communities during the immediate years after clearcutting is hitherto unknown.

**Materials and Methods:** In this study, we collected samples from clearcuts of previously fertilized and unfertilized forest stands, either within the first year or 4-13 years after clearcutting. We determined soil carbon stocks, fungal biomass (qPCR) and fungal communities (PacBio ITS sequencing) of the organic layer.

**Results:** First-year clearcuts from fertilized forests contained 30 % more soil carbon than those from unfertilized forests, while there was no difference in carbon stock among the older clearcuts. Fungal biomass did not differ in the first year after clearcutting but was significantly higher in the previously fertilized stands 4-13 years after clearcutting. Fungal communities in clearcuts from unfertilized forests tended to be less diverse and had lower evenness than in clearcuts from unfertilized forests.

**Discussion:** Overall, our results suggest that the fertilizer-induced increase in soil carbon rapidly disappears after clearcutting, which corresponds to a shift in the fungal communities.

**Conclusions:** Clearcutting results in a rapid turnover of fungal communities and reduces fertilizer-induced changes in soil carbon.

**Keywords:** Forest fertilization, Clearcutting, Ectomycorrhizal fungi, Saprotrophic fungi, Boreal, forests, Soil carbon

O-009

## Ecosystem Functioning

### ATMOSPHERIC DEPOSITION OF NUTRIENTS AND SOIL FACTORS ACT AS FILTERS ON COMMON AND RARE SPECIES OF ECTOMYCORRHIZAL FUNGI IN BRITISH OAK WOODLANDS

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**Introduction/Aim:** Nitrogen deposition is consistently reported as a significant driver of turnover in communities of ectomycorrhizal fungi (EMF), however, there has been little work to-date on deposition of a wider range of atmospheric nutrients. Furthermore, most techniques used to study EMF community structure place more weight on rare species turnover, yet factors influencing common species may have a greater impact on plant-soil feedbacks. We examined EMF communities of mature oak in British broadleaved woodlands across a wide biogeographical gradient.

**Materials and Methods:** We used multisite generalised dissimilarity modelling to explore the relative importance of spatial and environmental filters acting on rare and common species of EMF of oak across nineteen British woodlands.

**Results:** We found that atmospheric nutrients were the most important factors structuring British oak EMF communities and suggest that their EMF gamma diversity could be much higher.

**Discussion:** In agreement with other studies, oxidised and reduced nitrogen deposition were found to be consistent and important filters acting on EMF communities. Distance had little to no effect on community structure. Rare taxa were influenced by soil K, C:N ratio, and precipitation and common taxa by deposition of non-marine sulphur. Deposition of calcium and magnesium cations influenced all but the rarest taxa.

**Conclusions:** Factors associated with EMF compositional turnover differed between common and rare taxa demonstrating the need to consider these components of communities independently. Beyond nitrogen deposition a range of other atmospheric nutrients influence EMF community structure at landscape scales.

**Keywords:** ectomycorrhizal, fungi, MSGDM, zeta diversity.

O-010

## Ecosystem Functioning

### COLD TEMPERATURE GROWTH AND FREEZING RESISTANCE OF ECTOMYCORRHIZAL AND SAPROTROPHIC FUNGI

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**Introduction/Aim:** Ectomycorrhizal fungi (EM) and saprotrophic fungi in soils are subject to low or freezing temperatures over winter. Depending on the snow depth winter soil temperatures can be around 0°C or below -10°C.

**Materials and Methods:** Using 40 ectomycorrhizal and wood degrading saprotroph isolates obtained from mountain regions either in Austria, Mongolia or Slovenia, the effect temperature on radial growth was assessed at 22, 15 and 4 °C, and the effect of freezing at -4 and -18 °C. In addition, root ectomycorrhizas were collected from high elevation stands and subjected to freezing. Metabolic activity in both in vitro cultures and root tips was estimated using the reduction of triphenyl tetrazolium chloride (TTC).

**Results:** Saprotrophic fungi had higher growth rates than ectomycorrhizal fungi, although within both habits there were considerable differences between taxa. Taxa with the highest growth rates showed the greatest sensitivity to temperature, and had the highest TTC reduction. Low temperatures and freezing resulted in an increase in the specific dry weight of the fungal colonies (mg cm<sup>-2</sup>). This was due to both a thickening hyphal mat also due to an increase in dry mass of the hyphae. This occurred even during the 3-month period the fungi were frozen. Freezing decreased the TTC reduction in root tip ectomycorrhizas.

**Discussion:** In in vitro cultures, both ectomycorrhizal and saprotrophic fungi appear to be metabolic active even during freezing.

**Conclusions:** The cold sensitivity of fungi is not clearly related to the expected soil temperatures at the source of origin.

**Keywords:** Ectomycorrhizal fungi, Saprotrophic fungi, Freezing, Growth, Triphenyl tetrazolium chloride reduction,



O-011

## Ecosystem Functioning

### EXPLORATION SPACE OF ECTOMYCORRHIZAL MYCELIA INCREASES BELOWGROUND CARBON ALLOCATION BY EUROPEAN BEECH TREES

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**Introduction/Aim:** Ectomycorrhizal fungi develop extensive underground hyphal networks to forage for soil nutrients and compete with other fungi, yet the effects of these activities on plant carbon assimilation and allocation to fungal partners remain unclear.

**Materials and Methods:** We investigated how a tree's mycorrhizal mycelium expanding into the area of neighboring trees impacts plant carbon assimilation and belowground allocation. Young Beech trees were planted in soil-filled pots, along with mesh-bags containing a  $^{15}\text{N}$ -labeled organic nitrogen source accessible to mycorrhizal fungi. Pairs of pots, separated by sand, were placed a few centimeters apart in a box. Some boxes allowed ectomycorrhizal hyphae to grow from one tree's pot into the neighboring pot. After six months, one tree from each pair was exposed to  $^{13}\text{C}$ - $\text{CO}_2$ , and the distribution of  $^{13}\text{C}$  and  $^{15}\text{N}$  was analyzed using isotope-ratio mass-spectrometry and  $^{13}\text{C}$  phospholipid-fatty acid analysis.

**Results:** A significant portion of the tree biomass nitrogen came from the mycorrhizal-exclusive mesh-bags, which were highly enriched in  $^{13}\text{C}$  compared to the surrounding soil. The nutrient bags of neighboring (unlabeled) trees also showed high  $^{13}\text{C}$ -enrichment, indicating that ectomycorrhizal fungi bridged the sand to access the neighbor's nitrogen source.

**Discussion:** Trees with mycorrhizal partners expanding into their neighbor's area nearly doubled their  $\text{CO}_2$  assimilation and belowground carbon allocation, without receiving additional nitrogen in return, as their own nitrogen source was exploited by fungal invaders from the adjacent tree.

**Conclusions:** This study suggests that exploration space of mycorrhizal mycelia and fungal competition may play a significant role in determining the sink strength for plant photosynthates in temperate forest soils.

**Keywords:** Ectomycorrhiza, Plant C assimilation, C allocation to mycorrhizal fungi, temperate forests, Fungal interactions

## O-012

### Ecosystem Functioning

#### INFLUENCE OF DIVERSE AGRICULTURAL PRACTICES ON THE ARBUSCULAR MYCORRHIZAL STATUS OF ARABLE CROPS IN HUNGARY

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**Introduction/Aim:**The appreciation of the significance of soil biology is on the rise today, especially due to the soaring input costs for agriculture and the advance of regenerative agricultural practices. Yet, stakeholders and decision-makers often overlook the importance of soil conservation and regeneration, and the integration of soil biological services into agricultural practices. It is particularly true for the Central-Eastern European region, including Hungary, shaped by agricultural land structures and farming traditions developed during the former socialist industrialised agriculture. Since most arable crops form arbuscular mycorrhiza, it is a major task to understand the mycorrhizal status of agricultural soils and the impact of different agricultural practices. Conventional methods, such as tilling and the application of chemicals significantly diminish the richness and variety of AM fungi. Nonetheless, the degree to which organic or regenerative farming practices can amplify the mycorrhizal capacity of soils remains unclear.

**Materials and Methods:**In Hungary, numerous field studies, primarily on maize, examined the mycorrhizal colonization and diversity of crop plants.

**Results:**Collaborations with universities and companies engaged in active agricultural practices revealed apparent variations in root colonization patterns, primarily attributable to agricultural setups and soil nutrient levels.

**Discussion:**These findings offer valuable insights into the variability of mycorrhizal potential in lowland agricultural fields.

**Conclusions:**They aid in the selection of appropriate soil biological assessment methodologies to facilitate and monitor the impact of regenerative practices and mycorrhizal inoculation practices on soil health.

**Keywords:** arbuscular mycorrhiza, mycorrhizal colonisation, molecular diversity, regenerative agriculture

## LT-011

### Ecosystem Functioning

## MYCORRHIZAL ASSOCIATIONS MODIFY TREE DIVERSITY–PRODUCTIVITY RELATIONSHIPS ACROSS EXPERIMENTAL TREE PLANTATIONS

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**Introduction/Aim:** Decades of studies have demonstrated links between biodiversity and ecosystem functioning (BEF), yet the generality of the relationships and the underlying mechanisms remain unclear, especially for forest ecosystems.

**Materials and Methods:** By using eleven tree-diversity experiments, we tested tree species richness–community productivity relationships and the role of arbuscular (AM) or ectomycorrhizal (ECM) fungal-associated tree species in these relationships.

**Results:** Tree species richness had a positive effect on community productivity across experiments, modified by the diversity of tree mycorrhizal associations. In communities with both AM and ECM trees, species richness showed positive effects on community productivity, which could have resulted from complementarity between AM and ECM trees. Moreover, both AM and ECM trees were more productive in mixed communities with both AM and ECM trees than in communities assembled by their own mycorrhizal type of trees. In communities containing only ECM trees, species richness had a significant positive effect on productivity, whereas species richness did not show any significant effects on productivity in communities containing only AM trees.

**Discussion:** AM and ECM tree species may have complementary resource niches that can enhance the nutrient uptake efficiency of mixed AM-ECM tree communities. Other processes could also contribute to the complementarity between AM and ECM trees, such as biotic feedbacks mediated by mycorrhizal fungi.

**Conclusions:** Our study provides novel insights into mechanisms underlying the context dependency in BEF relationships by suggesting that tree-mycorrhiza interactions can modify BEF relationships.

**Keywords:** Arbuscular mycorrhizal fungi, biodiversity–ecosystem functioning, complementarity effects, ectomycorrhizal fungi, plant-mycorrhiza interactions, tree species diversity

**LT-012**

**Ecosystem Functioning**

**PROTEOMIC PROFILE ALTERATIONS OF THE ECTOMYCORRHIZAL FUNGUS LACCARIA BICOLOR IN RESPONSE TO CADMIUM STRESS**

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**Introduction/Aim:** Ectomycorrhizal fungi play a potential role in the bioremediation of heavy metal-polluted areas. The present study focuses on the proteomic responses of the ectomycorrhizal fungus *Laccaria bicolor* to cadmium stress.

**Materials and Methods:** Proteins were isolated from Cd treated and nontreated mycelium of *L. bicolor*. The proteomic analysis of isolated proteins was carried through label-free LCMS-QTOF. The Differentially expressed proteins were annotated through Gene Ontology and their metabolic pathways using KEGG classification.

**Results:** Comparative proteomic analysis of *L. bicolor* reported 997 differentially expressed proteins under Cd stress. The KEGG annotation of these differentially expressed proteins revealed that Cd-induced toxicity in ECM fungus *L. bicolor* by altering various metabolisms like carbohydrate metabolism, nucleotide metabolism and energy metabolism. Cd suppressed the carbohydrate metabolism, thus inducing energy stress inside the cell. It also altered the cell's genetic information processing like DNA replication, DNA repair, transcription, translation, protein folding, and chromosome metabolism, thus causing genetic instability in *L. bicolor*. Cd also interrupted the cellular homeostasis by altering the cell's transport system and vesicular trafficking. In defence, the ECM fungi confront the Cd-induced toxicity by upregulating the enzymes involved in mitigating oxidative response, including enzymes involved in glutathione metabolism, signalling pathway, and increasing the amino acid and protein biosynthesis.

**Discussion:** These observations provide a deep understanding of the Cd toxicity mechanisms and highlight biomarkers for Cd toxicity in *L. bicolor*. The functional and expression verification of the differentially expressed proteins were validated through complementation assays.

**Conclusions:** This study also provides the reference dataset on fungal proteome changes under Cd stress.

**Keywords:** Ectomycorrhizal fungi, *Laccaria bicolor*; qTOF-LC-MSMS, cadmium toxicity, oxidative stress

## LT-013

### Ecosystem Functioning

#### PERVASIVE SHIFTS IN SOIL FUNGAL COMMUNITIES IN TRANSFORMING MOUNTAIN FORESTS

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**Introduction/Aim:** Forests are experiencing significant shifts due to intensified disturbances. Ungulate herbivory further threatens tree regeneration, potentially leading to shifts from forests to non-forests. This study investigates the impact of such ecological regime shifts on soil fungal communities and their functions within a temperate mountain ecosystem.

**Materials and Methods:** Soil samples were collected from *Picea abies* stands and 18 storm and insect damaged forest sites. Disturbed sites varied in time since disturbance and vegetation type, with half showing successful tree regeneration and half showing complete regeneration failure dominated by grasses. High-throughput sequencing, qPCR and enzyme assays were used.

**Results:** Disturbances significantly altered soil fungal communities, increasing species richness and diversity while shifting community composition. Ectomycorrhizal fungi were reduced post-disturbance due to tree loss but gradually returned as trees regrew. In grass-dominated areas, ectomycorrhizal fungi were absent and saprotrophic basidiomycetes, particularly *Clavaria* and *Hygrocybe*, increased. Despite community shifts, total fungal DNA remained stable. Enzyme activity patterns varied with dominant vegetation, with decreases in phenol oxidase and phosphatase activity in grass-dominated areas, indicating changes in organic matter decomposition.

**Discussion:** The observed dynamics in fungal communities and enzyme activities highlight the critical role of vegetation type in determining soil ecosystem functions. This interplay suggests that changes in forest composition, whether through natural regeneration or failure due to disturbances, have profound implications for nutrient cycling and organic matter decomposition.

**Conclusions:** The resilience of fungal communities in regenerating forests underscores the potential for ecosystem recovery post-disturbance, while the shift towards saprotrophic fungi in grass-dominated areas signals a fundamental change in ecosystem functioning.

**Keywords:** Forest disturbance, Ecosystem functioning, Ectomycorrhizal fungi, Organic matter decomposition

## LT-014

### Ecosystem Functioning

## INVESTIGATING THE CONTRIBUTION OF COMMON MYCORRHIZAL NETWORKS TO THE DROUGHT TOLERANCE OF UK FORESTS

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**Introduction/Aim:** Ectomycorrhizal fungi (EMF) contribute to the health of tree species employed in UK forestry by facilitating resource acquisition and modifying soil quality. EMF frequently link multiple trees to form common mycorrhizal networks (CMNs). Currently, due to the use of varied and limited methodologies, there is no consensus on the contribution of CMNs to forest ecosystem health and stability among researchers. In particular, a paucity of studies on water translocation through CMNs hinders understanding of their contribution to the drought tolerance of UK forests.

**Materials and Methods:** To address this, in a microcosm experiment a donor *Pinus sylvestris* seedling will be separated from two conspecific, neighbouring receivers by mesh barriers which prevent the passage of roots and water, but not hyphae or rhizomorphs. Subsequently, in each microcosm the connections to one of the receivers will be severed and the receivers in half of the microcosms will undergo a drought treatment.

**Results:** The receivers which have maintained hyphal connections to the donor are expected to be more drought tolerant than those with severed connections.

**Discussion:** Radioisotopes will be applied towards the end of the experiment to investigate resource exchange dynamics. The experiment will then be replicated with *Betula pendula* as the donor, to determine the influence of donor species identity on results.

**Conclusions:** eDNA sequencing of soil samples from forests with the same component tree species as the experiments will dictate the wider relevance of the experimental findings and inform if and how the design and management of drought tolerant forests in the UK should consider CMNs.

**Keywords:** Ectomycorrhizal, Network, Drought, Microcosms, eDNA

LT-015

## Ecosystem Functioning

### GROWTH, BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES OF THREE TREE SPECIES TO ECTOMYCORRHIZAL FUNGI UNDER DIFFERENT MOISTURE CONDITIONS

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**Introduction/Aim:** Ectomycorrhizal fungi play important roles in seedling survival, establishment, nutrient supply, protection against pathogens, drought tolerance and growth. This study aims to investigate growth, physiological and biochemical responses in seedlings of *Parkia biglobosa*, *Terminalia superba* and *Gmelina arborea* grown under four different moisture conditions.

**Materials and Methods:** The screenhouse experiment carried out at College of Forestry, Ibadan, Nigeria, was a factorial 3 x 4 x 2, laid out in complete randomized design with split-split plot arrangement. The factors were 3 tree species, 4 moisture conditions and two levels of ectomycorrhizal fungi.

**Results:** Results revealed that *Gmelina arborea* had significantly higher contents of soluble sugar, proline, ascorbic acid, total chlorophyll, ethylene and glutathione reductase than other trees. Plants grown in ectomycorrhizal fungi soil had insignificantly higher contents of soluble sugar, proline, ascorbic acid, chlorophyll and ethylene. At 25 % evapotranspiration potential (ETP), ectomycorrhizal fungi significantly increased proline content in *Terminalia superba*. At 50 % ETP, proline significantly increased in seedlings of *Terminalia superba* and in *Parkia biglobosa* inoculated with ectomycorrhizal fungi.

**Discussion:** Consequently, all the growth parameters (plant height, stem girth and leaf area), root parameters (root length and number of lateral roots) and shoot weight were significantly higher in *Gmelina arborea* than in other tree species. However, ectomycorrhizal fungi inoculation significantly improved the growth parameters in *Parkia biglobosa* and *Terminalia superba* especially at 25 % and 50 % ETP.

**Conclusions:** It was concluded that *Parkia biglobosa* and *Terminalia superba* benefited more significantly from ectomycorrhizal fungi inoculation under water stress condition while *Gmelina arborea* did not.

**Keywords:** *Gmelina arborea*, *Terminalia superba*, *Parkia biglobosa*, Ectomycorrhizal fungi, Evapotranspiration potential.

LT-016

## Ecosystem Functioning

### THE RESTRICTION OF PLANT DERIVED C FLOW TO SOIL AFFECTS ROOT LITTER DECOMPOSITION WITH DELAY BUT MODIFIES SOIL FUNGAL COMMUNITY AS IN GADGIL THEORY

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**Introduction/Aim:**The climate warming and increased CO<sub>2</sub> levels may accelerate photosynthesis and increase the flow of C to soil. Plant-derived C inputs may enhance the microbial activity and degradation of root litter and soil organic matter (SOM). Our aim was to study the plant-dependency of root and SOM decomposition, with a particular focus on microbial dynamics and the potential role of Gadgil effect.

**Materials and Methods:**In a four-year experiment conducted in a boreal pine forest in Finland, we restricted plant-derived C input to soil by implementing various plots with combination of aboveground vegetation and belowground trenching treatments. We quantified 1) the root litter decomposition rate, the structure of root litter associated fungal and bacterial communities, as well as microbial activities, and 2) the fungal biomass and community structures associated with SOM. The data is compared to published soil CO<sub>2</sub> flux data.

**Results:**Our data showed that the manipulation of C fluxes altered the structure of fungal and bacterial communities associated with the root litter, but significant effects were observed only after 3-4 years, characterized e.g. as an increasing proportion of symbiotrophs. In SOM, treatments that restricted plant-derived C led to a decrease in the relative abundance of ectomycorrhizal fungi and an increase in saprophytic fungi.

**Discussion:**Our results indicated that the initial decomposition of root litter is not dependent on plant-derived C. Regarding SOM decomposition, the changes in the soil fungal communities support the 'Gadgil effect' theory.

**Conclusions:**Root litter and SOM decomposition vary in their dynamics with respect to plant-derived C.

**Keywords:** Trenching, plant-derived C, Gadgil, plant-associated fungi, decomposition



**LT-017**

## **Ecosystem Functioning**

### **FROM ASHES TO INSIGHTS: MYCORRHIZAL FUNGI ROLE IN POST-FIRE LANDSCAPES**

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**Introduction/Aim:**Communities in fire-affected ecosystems possess unique traits that aid survival and ecosystem recovery post-fire. As fires increase in frequency and intensity due to climate change, understanding the functions of these communities is essential. This work aimed to identify key functional traits of mycorrhizal fungi that correlate with fire regime and vegetative composition.

**Materials and Methods:**Thirty sclerophyll forest sites were selected within the Sydney Basin, Australia. These sites burned in the 2019-2020 Black Summer fires and varied in historical fire severity and interval. Vegetative composition, fungal communities, soil carbon and nutrient availability were analysed from each site. Mycorrhizal fungal biomass was harvested using mesh bags allowing us to measure functional traits associated with hyphal chemistry (carbon, nitrogen, and phosphorus concentrations) and morphology (hyphal diameter and branching).

**Results:**Fungal composition was significantly associated with fire interval and nutrient availability. Fungal biomass was harvested from twelve sites and is currently being analysed to assess how compositional shifts impact function directly (via fire interval) and indirectly (via nutrient availability).

**Discussion:**While previous work has been done on the presence or absence of mycorrhizal fungi post-fire, generally using DNA-based approaches, there is limited knowledge about the functions they serve. Here we show a significant effect of fire regime and nutrient availability on mycorrhizal fungi and will use trait data to elucidate the functional consequences of these communities.

**Conclusions:**By integrating responses associated with stoichiometric and morphological traits, fungal community composition, and environmental factors, the research seeks to provide a holistic understanding of the functional implications arising from these variations.

**Keywords:** Fire, Mycorrhizae, Fungal Traits, Stoichiometry, Recovery, Community Ecology

**LT-018**

**Ecosystem Functioning**

**MICROCOSM EXPERIMENTS SUGGEST POSITIVE INTERACTIONS BETWEEN ERICOID AND ECTOMYCORRHIZAL SYMBIOTIC SYSTEMS ON RECALCITRANT SUBSTRATES**

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**Introduction/Aim:**In nature, ericoid mycorrhizae (ErM) are found in complex environments characterized by acidic soils, low nutrient availability and high organic matter content. They have retained a vast reservoir of enzymes that enable them to degrade organic compounds and mobilize nutrients in these habitats. ERM therefore play an important ecological role in the forest understories, influencing soil carbon and nitrogen dynamics as well as interaction between microbial communities. Given that research on ERM association and community interaction remains limited and underdeveloped, we aim to clarify what kind of relationship - facilitation or competition - exists between ERM and ectomycorrhizal (ECM) fungi in hostile environments.

**Materials and Methods:**In this context, a microcosm system was set up to investigate the co-cultivation effects of ERM/ECM fungi (belonging to the species *Oidiodendron maius*, *Hyaloscypha hepaticicola* and *Paxillus involutus*) and plants (*Vaccinium myrtillus*, *V. corymbosum* and *Pinus sylvestris*) under controlled conditions.

**Results:**Measurement of mycorrhization rate, assessment of plants ecophysiological parameters and molecular analyses were performed.

**Discussion:**Preliminary results indicated that ERM fungi are highly resistant to prohibitive conditions, compared with ECM fungi, and that they strongly promote the growth and development of both host and non-host plants.

**Conclusions:**These results may have potential applications in reforestation (after dieback or fires) or in the restoration of degraded soils.

**Keywords:** ericoid mycorrhizal fungi, *Vaccinium*, fungal interactions, multiple plant-fungal associations

**LT-019**

**Ecosystem Functioning**

**ISOTOPIC INVESTIGATION OF THE DECOMPOSITION OF MYCORRHIZAL ROOTS INTO DISTINCT CARBON POOLS IN MANAGED GRASSLANDS**

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**Introduction/Aim:**Arbuscular mycorrhizal (AM) fungi contribute not only to plant health but also to soil organic matter (SOM) formation and carbon (C) accumulation through nutrient exchange and the buildup of their own biomass and necromass. However, this pathway to SOM-C storage and the impact of land management on AM contributions to SOM-C are not fully understood.

**Materials and Methods:**To address this knowledge gap, we used multiple pulses of  $^{13}\text{C}$ -CO<sub>2</sub> to isotopically label *Lolium perenne* grown with four treatments: (1) an inoculation of AM spore-rich sandy soil, (2) a single application of nitrogen-phosphorus-potassium (NPK) fertilizer, (3) a combination of spore-rich soil and NPK, and (4) a control. We are measuring  $^{13}\text{C}$  respiration and distribution into plant, SOM, and microbial pools at multiple timepoints over a subsequent 6 months of decomposition.

**Results:**Initial results show lower  $^{12}\text{C}$  and  $^{13}\text{C}$ -CO<sub>2</sub> respiration in fertilizer treatments in the first month of incubation, suggesting that NPK addition early in the season may affect the decomposition of root and AM tissues later on.

**Discussion:**Altered plant carbon to nitrogen ratios or inhibited AM hyphae growth may explain these differences, which will be investigated further.

**Conclusions:**Tracing  $^{13}\text{C}$  into various biomarkers and SOM pools during decomposition will shed light on plant-AM C storage and the effects of fertilization on this important process.

**Keywords:** carbon, decomposition, arbuscular mycorrhizal fungi, isotopes, grasslands, land management

LT-020

## Ecosystem Functioning

### ECTOMYCORRHIZAL DECOMPOSITION RESPONSES TO ELEVATED ATMOSPHERIC CARBON DIOXIDE ENRICHMENT OF A MATURE TEMPERATE DECIDUOUS FOREST

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**Introduction/Aim:**Addressing climate change concerns, this study investigates the impacts of elevated atmospheric carbon dioxide (eCO<sub>2</sub>) in a mature temperate forest on plant-soil feedbacks, focusing on the decomposition of organic matter in soil as influenced by ectomycorrhiza (ECM). While ECM aids nutrient absorption, concerns arise regarding potential resource competition and its implications for carbon and nutrient cycling in mature, nutrient-limited forests.

**Materials and Methods:**Decomposition studies in the absence and presence of ECM colonization trials were conducted at the University of Birmingham Free-Air Carbon Dioxide Enrichment Facility (BIFoR-FACE). We deployed two mesh sizes of ECM in-growth bags (1- $\mu$ m and 41- $\mu$ m) containing sand and 1mm-mesh inner litter bags with soil organic matter or oak leaf litter for decomposition measurements over time. Here we present initial results after 4-months incubation.

**Results:**We observed that the 1- $\mu$ m mesh effectively reduced ECM colonization without significantly affecting moisture inside. Bags with ECM colonization exhibited higher microbial respiration rates (mean increases 31.2% in control arrays,  $p < 0.05$ , and 40.1% in eCO<sub>2</sub> arrays,  $p < 0.001$ ).

**Discussion:**Our preliminary findings suggest that ECM influences organic matter decomposition and responds to eCO<sub>2</sub>. Ongoing research will assess the broader implications on decomposition, analysing the relationship between ECM colonization and mass loss. Future investigations using gene sequencing and extended incubation periods are planned.

**Conclusions:**Overall, our ECM-exclusion experiments are expected to help understand the influence of ECM on organic matter decomposition and how it responds to eCO<sub>2</sub>. This study could have significant implications for understanding the role of mycorrhizal associations in ecosystem functions and their responses to global changes.

**Keywords:** Ectomycorrhiza, Plant-soil feedbacks, Carbon cycling, Organic matter decomposition, Elevated atmospheric carbon dioxide.

LT-021

## Ecosystem Functioning

### FINE DINING WITH AN ENDANGERED MARSUPIAL: 23 YEARS OF TRUFFLE-LIKE FUNGI CONSUMPTION BY THE LONG-FOOTED POTOROO

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**Introduction/Aim:** Hypogeous fungi form truffle-like sporing bodies under or at the surface of the soil. Australia hosts a high diversity of endemic hypogeous fungi, accounting for one third of species globally. Most hypogeous fungi form ectomycorrhiza with dominant forest trees like Eucalyptus, where they facilitate nutrient and water uptake and promote host resistance to pathogens. These fungi rely on excavation and consumption by animals for spore dispersal, and in turn, provide a valuable food source for many native mammals. The endangered long-footed potoroo (*Potorous longipes*), an endemic Australian marsupial, may be the most fungi-dependent mammal worldwide, consuming over 85.8% of its diet as fungi. Despite their crucial role in consuming and dispersing hypogeous ectomycorrhizal fungal spores, therefore promoting forest health, the spatial and temporal patterns of fungal consumption by the long-footed potoroo remain poorly understood.

**Materials and Methods:** To address these knowledge gaps, this study utilises eDNA metabarcoding of LFP scats collected over a 23-year period in south-eastern Australia. Multivariate analyses will be used to characterise the fungal diet of the long-footed potoroo over time and across the landscape, in correlation with climatic fluctuations and disturbances.

**Results:** (Data is currently being analysed, results will be produced prior to ICOM12.)

**Discussion:** (Discussion to be developed after data is analysed.)

**Conclusions:** Exploring the fungal species consumed by the long-footed potoroo will contribute to our understanding of hypogeous species diversity, distribution, and ecological relationships with this endangered marsupial. This research will also inform conservation efforts to support the long-footed potoroo, hypogeous fungi, and their habitat.

**Keywords:** hypogeous, ectomycorrhizal, dispersal, ecology, mycophagy

LT-022

## Ecosystem Functioning

### MACROLEPIOTA PROCERA AS A SOURCE FOR AG-MYCOGENIC NANOPARTICLES SYNTHESIS AND THEIR CHARACTERIZATION

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**Introduction/Aim:**Fungi have the remarkable ability to secrete/produce proteins, polysaccharides, vitamins, and amino acids which can be incorporated as stabilizing, reductant, and capping mediators in the production of biogenic metallic nanomaterials.

**Materials and Methods:**The prime objective of the research work was the mycosynthesis of biogenic metallic nanomaterials. The prime objective of the current study was *Macrolepiota procera* (Scop.) Singer extract based production of silver-mycogenic nanoparticles (AgmyNPs) by solution combustion method.

**Results:**The AgmyNPs were characterized by various analytical techniques. They produce peculiar spectral peaks of UV–Vis spectroscopy at 380 nm, X-ray diffraction (XRD) at  $2\theta$  values of  $38.1^\circ$ ,  $44.1^\circ$ ,  $67.4^\circ$  and  $77.7^\circ$ . The XRD study also corroborates the presence of alkali metal that is Ag and face-centered cubic (FCC) structure of AgmyNPs, and Fourier-transform infrared (FTIR) spectroscopy from 800 to  $3726\text{cm}^{-1}$  which confirmed the formation of the AgmyNPs. AgmyNPs were further investigated for their antibacterial potential against gram-positive bacteria (*Bacillus licheniformis*, *B. meurilis*) and gram-negative bacteria (*Azotobacter pasteurensis*, *A. rhizospherensis*).

**Discussion:**The antibacterial testing was executed at four different concentrations of the dilutions of the prepared by AgmyNPs employing well diffusion method.

**Conclusions:**As compared to Penicillin, a standard antibiotic, AgmyNPs showed maximum zones of inhibitions of 8 mm, 2.25 mm against *A. parteurensis*, *A. rhizospherensis* while no inhibitory zone was observed against *B. licheniformis*. The excellent zone of inhibition was noticed against *B. meurilis*.

**Keywords:** Ag-mycogenic nanoparticles (AgmyNPs), *M. procera*, Mushroom extract, Antibacterial activity, Inhibitory zone

**LT-023**

## **Ecosystem Functioning**

### **INTENSIVE GRASSLAND MANAGEMENT ACCELERATES SOIL PHOSPHORUS CYCLING**

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**Introduction/Aim:** Understanding the effects of grassland management types on soil phosphorus (P) cycling is vital to develop sustainable farming strategies. Grassland management practices, including biomass removal for silage or hay, grazing with manure return, and nitrogen fertilizer application, may interact to affect soil P cycling, often involving a combination of these activities at varying intensities. Additionally, the function and diversity of arbuscular mycorrhizal (AM) fungi may vary with different grassland management types, suggesting a need for further elucidation of their roles in soil P cycling.

**Materials and Methods:** Here we assessed different soil P fractions and their correlation with biotic and abiotic factors under three typical grassland management types across the UK.

**Results:** Our results showed that intensive grassland management significantly increased the content of all P fractions except organic P, while the percentage of organic P in the P pool significantly decreased.

**Discussion:** The contents of soil residual P and primary mineral P were significantly correlated with the increased abundance of specific families of AM fungi (Archaeosporaceae and Claroideoglomeraceae) under intensive grassland management.

**Conclusions:** These results indicate that more intensive grassland management may accelerate soil P cycling and improve the utilization of soil recalcitrant P.

**Keywords:** Grassland management, arbuscular mycorrhizal fungi, soil phosphorus cycling, organic phosphorus

**LT-024**

**Ecosystem Functioning**

**THE POTENTIAL OF AMF IN CONTROLLING GHG EMISSION FROM TROPICAL FOREST SOILS AND ITS MECHANISM STUDY**

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**Introduction/Aim:**Tropical forests represent a major GHG source in the natural ecosystems. Previous studies have reported the important roles of AMF in soil C&N dynamics. However, how AMF controlling GHG from tropical forest soils are still unclear, since AMF is widely distributed in the tropical forest.

**Materials and Methods:**This research used a combination of field in-situ experiments and controlled pot experiments to establish soil environments infected with different AM fungal abundances, where we monitored the changing trends of N<sub>2</sub>O and CO<sub>2</sub> emission flux, and key functional genes of carbon and nitrogen metabolism.

**Results:**The study found that setting up in-growth soil core in tropical forest soil can effectively reduce the abundance of AM fungi in the forest soil. The decrease in the abundance of AM fungi in the soil has a more significant impact on CO<sub>2</sub> emissions in tropical rainforest soil, as the abundance of AM fungi increases, the amount of AM fungi in the soil increases at the same time. The main source of N absorption by AM fungi in tropical rainforest soil is nitrate.

**Discussion:**The results have shown that changes in the abundance of AM fungi can mediate changes in the diversity of the soil bacterial community, and also reduce the abundance of functional genes related to N metabolism in the soil. Changes in these functional genes may be related to changes in GHG from the forest soil.

**Conclusions:**These research results can provide new scientific theoretical basis for the reduction of greenhouse gas emissions in forest ecosystems and the protection of forest fungal resources.

**Keywords:** AMF, Tropical forests, GHG, microorganism, soil microbial communities, qPCR



LT-025

## Ecosystem Functioning

### MYCORRHIZAL PRODUCTION AND COMMUNITY PHENOLOGY PATTERNS ARE SEASONAL AND MAY BE COUPLED WITH FINE-ROOT PHENOLOGY

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**Introduction/Aim:** Mycorrhizal fungi account for 15–45% of annual forest carbon allocation, leaving substantial uncertainty in mycorrhizal contributions to whole-ecosystem functions. It remains unclear what conditions favor these belowground investments and how they vary over time and by host tree type. Furthermore, diverse interactions among forest mycorrhizal networks add complexity to predictions about the phenology of mycorrhizal fungi, their traits, and their community compositions.

**Materials and Methods:** We estimate the timing of production and community shifts of mycorrhizal fungi based on minirhizotron images and high-throughput amplicon sequencing data from soil across subsets of monodominant stands of 10 temperate tree species, which vary in plant functional traits including leaf habit, mycorrhizal type, and evolutionary group at the Morton Arboretum in northern Illinois, USA.

**Results:** Current data show that ectomycorrhizal fungal production varies seasonally and inter-annually. Ectomycorrhizal mycelial production tended to occur during transitional seasons, primarily in the Fall with higher soil moisture and cooler temperatures, either in synchrony with or shortly after fine-root production, depending on host tree species. Compared to root production, ectomycorrhizal rhizomorphs and mycelia appeared more consistent across plots, despite substantial variation across years.

**Discussion:** These findings reveal that ectomycorrhizal mycelial production is at least partly related to the timing of fine-root production in some tree hosts, but that it is also more phenologically consistent than root growth. Ongoing and future work will analyze complementary data on the phenology of whole-community shifts in arbuscular and ectomycorrhizal fungi.

**Conclusions:** Our study resolves key sources of uncertainty in the timing of forest tree investment in mycorrhizal symbioses.

**Keywords:** phenology, trait, productivity, forest, temporal patterns

P-042

## Ecosystem Functioning

### ECM COMMUNITY SHIFTS IN POST-WILDFIRE SCENARIOS: WHO ARE THE FIRE FUNGI?

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**Introduction/Aim:** Following large-scale severe wildfires, the recovery of forests is linked to the survival and viability of ectomycorrhizal (ECM) fungal propagules in soil, which act as symbiotic partners for most forest tree species. ECM fungi play a crucial role in nutrient supply, stress resilience, and plant-soil interactions. This study aims to unravel how fire severity affects soil fungal communities and the extent to which this effect is modulated by potential post-fire erosion.

**Materials and Methods:** Three months after the fourth most destructive wildfire of Spain in 2021 (23,000 ha), topsoil (0-5 cm) and subsoil (5-10 cm) samples were collected encompassing unburnt zones, and zones burnt at low severity (tree canopies unaffected) and at high severity (total scorched tree canopies). The latter were further stratified by slope (moderate/high). Soil analyses covered physical-chemical properties, soil activity (i.e.: microbial biomass carbon), and fungal indicators such as ergosterol. Additionally, high-throughput Illumina sequencing of the ITS region was conducted.

**Results:** Results will reveal (1) whether unburnt and burnt soils share the same ECM propagules reservoir, (2) if dominant fungal species/genera emerge in burnt soils, indicating specific fire-adapted fungi, and (3) to what extent the shift in community composition is amplified along a gradient of fire severity and post-fire erosion.

**Discussion:** Anticipated outcomes based on existing literature suggest distinct fungal community assembling in unburnt versus burnt soils, higher species richness in unburnt soils, and an increased presence of single dominant fire-resistant species in burnt soils.

**Conclusions:** Our findings underscore the importance of considering ectomycorrhizal communities in wildfire management and restoration strategies.

**Keywords:** Ectomycorrhizal fungi, fire ecology, resistant propagules, community assembling, metabarcoding

P-043

### Ecosystem Functioning

#### DECOMPOSITION OF MYCORRHIZAL FUNGAL NECROMASS IN THE ARCTIC TUNDRA: INSIGHTS FROM A THREE-YEAR CASE STUDY

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**Introduction/Aim:**Fungal necromass decomposition plays a key role in nutrient cycling and carbon storage in terrestrial ecosystems, but its dynamic in the Arctic tundra is still poorly understood.

**Materials and Methods:**In a three-year field experiment conducted in Svalbard, we investigated the decomposition of two different types of fungal necromasses, using the ectomycorrhizal fungus *Laccaria laccata* as a high-quality substrate and the endophytic fungus *Phialocephala fortinii* as a low-quality substrate.

**Results:**Our findings suggest that the Arctic tundra follows similar principles of fungal necromass decomposition observed in other ecosystems. This process involves a rapid initiation phase replaced by a slower decomposition phase that persists after three years, highlighting the presence of poorly decomposable material. This persistence suggests an important role for fungal necromass in soil organic matter accumulation and carbon storage that warrants further investigation. The composition of fungal communities associated with decomposing fungal necromass remains relatively stable throughout decomposition, with no changes in species richness or turnover. Notable changes are limited to shifts in the relative abundance of taxonomic and ecological groups on the substrate. Interestingly, ectomycorrhizal fungi appear later in the decomposition process, suggesting their potential contribution to carbon stability in Arctic tundra soils.

**Discussion:**In this study, we identified fungi that are part of the so-called "core necrobiome", but we also recorded ecosystem-specific observations.

**Conclusions:**Our study provides deeper insight into the complex dynamics of fungal necromass decomposition.

**Keywords:** mycorrhizal fungi, fungal necromass decomposition, fungal community, necrobiome, Arctic tundra

P-044

## Ecosystem Functioning

### INTENSIVE AGRICULTURAL MANAGEMENT REDUCES THE TRANSFER OF PHOSPHOR BY ARBUSCULAR MYCORRHIZAL FUNGI

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) are known to trade phosphorus for carbon with their host plant, making them of crucial importance for crop nutrition. While the impact of agricultural practices on AMF community composition is well described, it is far less investigated to which extent agricultural practices affect the ability of AMF to supply phosphorus to plants.

**Materials and Methods:** Field soils were collected from 60 sites in Switzerland, including organic arable fields, conventional arable fields, conventional vegetable fields and extensive grasslands. We developed a compartment system with a hyphal compartment labeled with <sup>33</sup>P and inaccessible to plant roots. We tracked the mycorrhizal transfer of <sup>33</sup>P from the root-free compartment to *Plantago lanceolata* shoots.

**Results:** We observed that <sup>33</sup>P recovery in *P. lanceolata* shoots was significantly higher in soils from extensive grasslands. Soils from organic and conventional systems resulted in similar <sup>33</sup>P recovery rates. The transfer of <sup>33</sup>P to *P. lanceolata* was positively correlated with the abundance of AMF in roots, while it was negatively affected by the plant-available phosphorus in soils.

**Discussion:** We showed that mycorrhizal communities originating from grassland soils transfer more phosphorus to their host plant than AMF communities from arable lands. We observed that increased phosphorus availability in soils reduces the transfer of <sup>33</sup>P to plants by arbuscular mycorrhizal fungi. The identification of key taxa for <sup>33</sup>P transfer will be performed by analyzing the AMF community composition.

**Conclusions:** Our findings highlight that communities of arbuscular mycorrhizal fungi from undisturbed environments with low phosphorus availability are more efficient to supply phosphorus to plants.

**Keywords:** Agriculture, Arbuscular mycorrhizal fungi, Management intensity, Plant nutrition,

P-045

## Ecosystem Functioning

### HOW TREE DIVERSITY AND MYCORRHIZAL TYPE AFFECT SOIL CARBON STABILIZATION

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**Introduction/Aim:** Forests play a key role in global carbon (C) storage. Important for long-term C storage is the formation of mineral-associated organic matter (MAOM) in forest soils, which is protected through chemical bonds and occlusion from decomposition. Recent research revealed variations in forest C cycles depending on tree diversity and mycorrhiza, but we still lack mechanistic knowledge about the role of the mycorrhizal symbiosis in soil C stabilization.

**Materials and Methods:** Our study aims to quantify soil organic matter fractions in relation to tree diversity and mycorrhizal association type in the MyDiv tree diversity experiment. We estimate fungal and plant residue contributions to MAOM formation by Bayesian Inference Isotopic Mixing Models, in which we compare <sup>13</sup>C and <sup>15</sup>N (nitrogen) natural abundances of soil organic matter fractions with those of leaf litter, fine roots, saprotrophic and ectomycorrhizal sporocarps and arbuscular mycorrhizal hyphae. In addition, we measure the timing and quality of fine root and hyphal turnover and rhizodeposition to determine their impact on MAOM formation.

**Results:** First results reveal a tendency towards increased C and N stocks in MAOM at higher tree diversity (independent from the mycorrhizal association type).

**Discussion:** This may indicate an importance of molecular diversity of organic C for soil C persistence.

**Conclusions:** Combining soil organic matter fractionation by natural stable isotope analysis with measurements of root and hyphal turnover and rhizodeposition may allow to elucidate the dynamics of plant-soil C transfer in arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) trees and enhance our understanding of soil C stabilization in diverse temperate forests.

**Keywords:** temperate forests, mineral-associated organic matter, carbon isotopes, nitrogen isotopes, arbuscular mycorrhizal fungi, ectomycorrhizal fungi

P-046

## Ecosystem Functioning

### SOIL COMPACTION REVERSED THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON SOIL HYDRAULIC PROPERTIES

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) typically provide a wide range of nutritional benefits to their host plants, and their role in plant water uptake, although still controversial, is often cited as one of the hallmarks of this symbiosis. Less attention has been paid to other water dynamics related effects that the presence of AMF in soils may have. Evidence that AMF can affect soil hydraulic properties is only beginning to emerge.

**Materials and Methods:** In two greenhouse pot experiments with dwarf tomato plants (*Solanum lycopersicum*, cv. 'Micro Tom'), we tested whether the presence of AMF (*Rhizophagus irregularis* 'PH5') could affect the water holding capacity (WHC) of the substrate (sand-zeolite soil mixture).

**Results:** While in the first experiment AMF slightly but significantly decreased WHC, in the second experiment we found the opposite effect - the mycorrhizal substrate retained more water than the non-mycorrhizal substrate.

**Discussion:** Since the same substrate was used and the other conditions were mostly comparable in both experiments, we explain the contrasting results by different substrate compaction, most likely caused by different pot shapes. It seems that in compacted substrates, AMF may have no effect or even decrease their WHC. On the other hand, the AMF hyphae interweaving the pores of less compacted substrates may increase the capillary movement of water throughout such substrates and cause slightly more water to remain in the pores after the free water has drained.

**Conclusions:** We believe that this phenomenon is worthy of attention by mycorrhizologists and merits further investigation of the role of AMF in soil hydraulic properties.

**Keywords:** arbuscular mycorrhizal fungi, water holding capacity, sand-zeolite-soil mixture, pot shape, dwarf tomato

P-047

### Ecosystem Functioning

#### ECTOMYCORRHIZAL COMMUNITIES OF ADULT AND YOUNG SWISS STONE PINE AND EUROPEAN LARCH AT HIGH ALTITUDINAL SITES OF THE ALPS

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**Introduction/Aim:** *Pinus cembra* and *Larix decidua* are typical and important tree species in European subalpine habitats. However, their ectomycorrhizal (EcM) fungal communities have not been thoroughly investigated in natural conditions. Here, we aim to identify these EcM communities in high-altitudinal forests in the Alps. We explore the mycobiont diversity between naturally rejuvenated and adult trees, compare EcM colonization patterns in geographically disjunct areas, and in sites with contrasting climatic conditions.

**Materials and Methods:** Our approach combines morphotyping of mycorrhized root tips combined molecular analysis.

**Results:** EcM fungal communities of *P. cembra* and *L. decidua* are diverse, with a total of 25 and 68 ECM species, respectively. Both, adult and young trees show a 100% mycorrhization rate. We observed distinct species diversity between seedlings and adult trees, regardless of the host. The two hosts exhibit contrasting patterns in species diversity. For example, *L. decidua* harbours higher EcM species richness in dry sites compared to humid ones. Additionally, the EcM community composition is strongly influenced by geographic region for *P. cembra*, while it is less predicted by our studied factors for *L. decidua*.

**Discussion:** The importance of host-specialist EcM fungi is emphasized. Additionally, our results underscore the importance of generalist fungi in enhancing the adaptability of the plant host to changing climatic conditions. Differences in EcM community responses between (sub-)alpine plant species are highlighted in accordance with the distinct ecological niche of their hosts.

**Conclusions:** The EcM fungal communities associated with (sub-)alpine *P. cembra* and *L. decidua* is rich and diverse at high altitudinal sites in the Alps.

**Keywords:** *Larix decidua*, Alpine larch, Suillus, Alps, rare species turnover, seedlings

P-048

## Ecosystem Functioning

### MYCORRHIZAL TYPES MODULATE TREE DIVERSITY EFFECTS ON FOLIAR ELEMENTAL POOLS

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**Introduction/Aim:**Species-specific differences in nutrient acquisition strategies allow for complementary use of resources among plants in mixtures, which may be further shaped by mycorrhizal associations. Empirical evidence of this potential role of mycorrhiza is scarce, particularly for tree communities. Tree species predominantly associate with either arbuscular mycorrhizal fungi (AM) or ectomycorrhizal fungi (EM) which are characterized by contrasting nutrient economies which may allow for better resource partitioning in mixed tree stands.

**Materials and Methods:**In the tree diversity experiment MyDiv, three levels of tree species richness (monocultures, two- and four-species mixtures) were crossed with either AM, EM, or a mixture of both mycorrhizal types. Here, we investigated the effects of tree species richness and mycorrhizal type on elemental concentrations, ratios and pools above- and belowground. For this, soil, soil microbial biomass, and tree foliage were analyzed for carbon (C), nitrogen (N), and phosphorous (P) dynamics.

**Results:**We demonstrate that higher tree diversity results in larger foliar C and P pools for AM, EM, and AM+EM tree stands, and greater N pools in EM and AM+EM communities. The results for tree foliage pools also show that AM+EM communities exhibit additive effects, rather than an overperformance. However, analyses of the biodiversity effects (complementarity, selection, and net effect) showed that complementarity effects in AM+EM stands were significantly larger compared to selection effects in EM tree stands.

**Discussion:**Our results indicate that positive effects of tree diversity on aboveground nutrient storage are mediated by complementary mycorrhizal strategies.

**Conclusions:**This highlights the importance of using tree mixtures to achieve more multifunctional afforestation.



**Keywords:** biodiversity effects, mycorrhizal fungi, MyDiv, nutrient dynamics, plant-soil interaction, tree species richness

P-049

## Ecosystem Functioning

### DO ECTOMYCORRHIZAL FUNGI DRIVE DIFFERENCES IN SOIL ORGANIC MATTER BETWEEN PINE AND OAK FORESTS?

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**Introduction/Aim:** Mycorrhizal strategies can affect the composition of soil organic matter (SOM). However, the relationship of SOM and mycorrhizal strategy differs across systems, likely due to functional diversity in ectomycorrhizal trees and fungi. Ectomycorrhizal oaks and pines co-dominate fire-adapted woodlands across the southeastern United States. They produce litter of different quality and associate with distinct communities of ectomycorrhizal fungi.

**Materials and Methods:** We sampled soils from nineteen pairs of adjacent oak- or pine-dominated forest plots in the Appalachian Piedmont and Blue Ridge. We separated particulate and mineral-associated fractions and quantified carbon and nitrogen in each fraction and in bulk soil.

**Results:** We found a higher proportion and stock of mineral-associated soil carbon in oak versus pine plots, while the proportion and stock of mineral-associated nitrogen did not differ. The effect of tree identity on particulate carbon stocks and carbon to nitrogen ratio depended on prescribed fire. Pine soils held more particulate carbon than oaks only in the absence of recent fire.

**Discussion:** Differences in SOM between oak and pine forest soils partially depend on the presence or absence of fire, likely due to differences in fuel chemistry and flammability. Next, we will use metabarcoding to compare the influence of ectomycorrhizal fungal community composition versus tree litter quality on differences in SOM.

**Conclusions:** Mineral association is a key driver of soil carbon accumulation and stability. Understanding the influence of tree and fungal identity on SOM stocks and chemistry will help inform land management to maximize ecosystem services and allow more accurate modeling of soil carbon in ectomycorrhizal-dominated landscapes.

**Keywords:** soil organic matter, functional diversity, ectomycorrhizae, biogeochemistry, mineral-associated organic matter

P-050

### Ecosystem Functioning

#### **A REFLECTION OF THEIR ROOTS: HOW SOIL CONDITIONING AND NEIGHBOR EFFECTS SHIFT SEEDLING OUTCOMES AND DROUGHT RESPONSE**

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**Introduction/Aim:**Environmental factors such as nutrient and water conditions, access to symbionts, or the accumulation of enemies can drive changes in seedling growth and morphology. To better understand the multiple interacting drivers existing in natural contexts, this project investigates how soil conditioning and neighboring plants interact to change seedling outcomes and responsiveness to drought.

**Materials and Methods:**In a greenhouse, bigcone Douglas fir and canyon live oak seedlings were conditioned for one year to soils with home, away, or no microbial inoculum. After one year, seedling growth and mycorrhization were measured, and seedlings were co-planted in a fully factorial design to test for the interacting effects of prior soil conditioning, neighboring seedlings, and drought on seedling performance.

**Results:**Initial soil conditioning had significant and lasting effects on physiology and growth. In shared pot communities, neighbor species and neighbor soil conditions were found to have weaker but significant impacts on the aboveground growth of seedlings. When subjected to drought, firs were more likely to decline in performance after home soil conditioning (negative plant soil feedback), particularly with oak neighbors. Oaks did not show productivity responses to drought but did decline with home soil conditioning and conspecific competition.

**Discussion:**Seedling outcomes cannot be understood without clarity on their growing context. Additionally, species interactions may drive systemic outcomes through both direct and indirect pathways, complicated even more by stress.

**Conclusions:**By outlining these stark context-specific differences in seedling outcomes, we highlight the nuances of plant-soil feedbacks and the difficulty of predicting forest community trajectories in a dynamic climate.

**Keywords:** ectomycorrhizae, forests, plant-soil feedbacks, community ecology

P-051

## Ecosystem Functioning

### PARTITIONING OF RHIZOSPHERIC, HETEROTROPHIC AND MYCORRHIZAL SOIL CO<sub>2</sub> FLUX COMPONENTS AND THEIR RESPONSE TO DROUGHT IN THREE DIFFERENT ECOSYSTEMS

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**Introduction/Aim:**The amount of carbon stored in the biomass, soil and flux vary depending the land cover types. By being a source of CO<sub>2</sub> and a pathway of carbon to the soil organic matter, the role of arbuscular mycorrhizal fungi (AMF) in carbon balance is unquestionable. Our ongoing experiment focusing on the contribution of AMF respiration to soil respiration (Rs) in three different ecosystems – grassland, cropland, forest – has been set up in 2022. Our aim is to determine the proportion of Rs components and to monitor diurnal and seasonal changes in carbon fluxes in different vegetation types. The Central-Hungarian sandy grassland is a drought prone area where the seasonal variation of plants' demand for water results in diverging ecosystem responses depending on the seasonal timing of these events.

**Materials and Methods:**Continuous measurements of Rs were conducted with an automated Rs system in four different treatments: i) undisturbed, root and AMF included (Rs), ii) disturbed, root and AMF included (Rsdist), iii) root excluded (Rhet+myc) and iv) root and AMF excluded (Rhet) plots. Net ecosystem exchange of CO<sub>2</sub> and meteorological variables were monitored by an eddy covariance system. Soil temperature and moisture were also measured.

**Results:**The mean fluxes of Rs, Rsdist, Rhet+myc and Rhet in grassland were 1.62, 1.53, 1.60 and 1.35 μmol CO<sub>2</sub> m<sup>-2</sup> h<sup>-1</sup>, respectively during the studied period.

**Discussion:**The CO<sub>2</sub> emission rates show a seasonal pattern with changes corresponding to drought events.

**Conclusions:**Our conclusion is that CO<sub>2</sub> emission rates are greatly affected by drought events and their seasonal timing.

**Keywords:** arbuscular mycorrhizal fungi, soil respiration, carbon dioxide, grassland, drought

P-052

## Ecosystem Functioning

### LIMITED PHOSPHORUS SUPPLY AND AMF COLONIZATION ENHANCE UPTAKE OF IRON BOUND PHOSPHORUS IN ARABLE SOILS

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**Introduction/Aim:**We tested the capacity of farmed crops to mobilize P bound to goethite (a common iron oxide in soils) in three distinct agroecosystems: 1) Kernza (*Thinopyrum intermedium*) grown in monoculture or intercropped with Alfalfa (*Medicago sativa*), in a perennial system with limited nutrient supply. This system was compared with winter wheat grown with a conventional fertilizer regime. 2) Sugar beet grown in a long-term fertilization experiment under sufficient or deficient P availability. 3) Rotational soybean grown in an acid P-fixing soil from Kenya with or without fertilizer and/or biochar addition.

**Materials and Methods:**Desorption of P from goethite was tested in root ingrowth cores buried in the soils.

**Results:**We found a strong capacity to desorb P from goethite in the soybean experiment, especially when biochar, but no fertilizer, was added. This capacity was positively related to the amount of AMF biomass (PLFA/NLFA 16.1ω5) colonizing the goethite substrate. Sugar beet was also able to mobilize goethite bound P, but only in the rhizosphere soil under limited P supply. In contrast, neither Kernza nor winter wheat were able to desorb P from goethite.

**Discussion:**Enhanced utilization of poorly available P sources that are common in many arable soils may be achieved by reducing P application rates and adding biochar through a positive feedback on mycelial symbiosis and growth in AMF crops.

**Conclusions:**The optimal rate of P fertilizer and biochar amendment, and other management practices, needed to realize high yield with efficient use of bound P must be identified for making appropriate recommendations.

**Keywords:** arbuscular mycorrhiza, legacy P, goethite, soybean, kernza, sugar beet

P-053

## Ecosystem Functioning

### **DISTURBANCE ADAPTED? MUCOROMYCOTINA FINE ROOT ENDOPHYTES INCREASE WHILE ARBUSCULAR MYCORRHIZAL FUNGI DECREASE AFTER FIRE AND GRAZING IN A GRASSLAND**

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**Introduction/Aim:** Natural disturbances (fire and wild herbivory) can reduce the abundance of mycorrhizal fungi in grasslands and savannas. However, the recently defined Mucoromycotina Fine Root Endophytes (MFRE) are phylogenetically distinct from arbuscular mycorrhizal fungi (AMF) and may respond differently to these disturbances.

**Materials and Methods:** Plant biomass, soil physico-chemical characteristics, soil fungal diversity (ribosomal 18S ITS), and root colonization were sampled across a range of disturbances in experimental plots within a South African mesic grassland (30.40°S; 29.01°E). Moderate, heavy or no grazing was simulated by defoliating the native grass sward (predominantly *Themeda triandra*) to varying degrees, combined with varying recovery periods. Also, a fire event was compared to herbivore impact simulated through trampling and dung inputs.

**Results:** Colonization of grass roots by AMF was largely unaffected by grazing but decreased from ~50% to ~35% with fire and/or trampling ( $P < 0.05$ ). By contrast, MFRE colonization increased under heavy grazing especially when combined with moderate recovery periods ( $P < 0.05$ ). Most markedly, colonization by AMF decreased with fire and/or trampling while MFRE colonization increased by ~25% ( $P < 0.05$ ).

**Discussion:** These trends may be linked to observed increases in soil cations and altered plant biomass with disturbance and will be further explored along with fungal amplicon sequence variants using ordination techniques. We speculate that MFRE play an ecological role after acute disturbance events (fire and trampling), while AMF are more important following chronic moderate disturbances (grazing).

**Conclusions:** MFRE likely play an important ecological role in naturally disturbed open and grassy ecosystems but their functional role in plant nutrition remains uncertain.

**Keywords:** disturbance, grassland, nutrient cycling, rangeland

**P-054**

## **Ecosystem Functioning**

### **VERTICAL ASSESSMENT OF MYCORRHIZAL FUNGAL COLONIZATION AND SOIL MICROBIAL COMMUNITIES IN TWO DIFFERENT SOIL CLASSES**

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**Introduction/Aim:**The correlation between soil properties, depth, and arbuscular mycorrhizal fungal (AMF) communities has received insufficient attention in academic research. Our study investigates vertical changes in AMF colonization capacity and the soil microbiome.

**Materials and Methods:**Conducted in an alkaline grassland under regenerative agricultural management in the Hungarian Great Plain, the research involved a soil survey in August 2023, utilizing a pre-existing digital soil map for the study area and adopting the World Reference Base (WRB) system for soil classification. Genetic horizons were sampled and analyzed in the laboratory to determine soil chemical and physical characteristics.

**Results:**Soil colonization potential assessment was performed on a Solonetz and a Gleysol using trap plant cultures, with genetic horizons sampled in August and November 2023 at depths exceeding 100 cm. By evaluating colonization of roots grown in samples collected from different soil horizons in two seasons we aimed to identify soil properties significantly influencing AMF presence and monitor temporal changes in colonization capacity of soils. For microbiome mapping, amplicons produced by TS1-2, 16S rDNA, and AMF-specific primers from soil and root samples were sequenced by the Illumina MiSeq platform.

**Discussion:**Findings indicate that soil properties significantly affect microbial communities across various soil horizons, with AMF root colonization observed at depths exceeding 100 cm, representing a non-linear decrease in colonization capacity with depth.

**Conclusions:**Soil properties have a significant effect to the AMF root colonization and the microbiome of the soils. AMF communities are present in the deeper horizons of the soil as well.

**Keywords:** Digital soil mapping, AMF root colonization, sequencing, soil microbiome

P-055

## Ecosystem Functioning

### ARBUSCULAR MYCORRHIZAL FUNGI SUPPRESS SOIL NITRIFICATION

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**Introduction/Aim:** Arbuscular mycorrhizal (AM) fungi represent an important component of soil microbiomes, and are implicated in multiple plant and ecosystem functions. Whether and how the fungi interact with ammonia oxidizing prokaryotes in soils, which are responsible for conducting the rate-limiting step of nitrification, has long been unclear.

**Materials and Methods:** We conducted several pot experiments with mock microbial communities or with unsterile soils containing indigenous ammonia oxidizers placed in root-free compartments of mycorrhizal and nonmycorrhizal microcosms.

**Results:** Consistently, the abundance of ammonia oxidizing bacteria (AOB) was strongly suppressed by the actively growing hyphal networks of AM fungi, whereas the abundance of ammonia oxidizing archaea (AOA) was mostly unaffected. The size of the suppression effect on the AOB was comparable with the effect of some of the commercially used synthetic nitrification inhibitors.

**Discussion:** In spite of this unusually strong suppression, it seems that the underlying mechanism of the interaction is most likely the competition for free ammonium ions between the AM fungi and AOB, rather than production of elusive biological nitrification inhibitor(s) by the fungi. This new knowledge invites further questions about co-existence and co-functioning of the AM fungi and ammonia oxidizing prokaryotes in field settings and also demands further experimentation *in vitro*. The latter is particularly challenging due to difficulties to isolate and culture not only the AM fungi, but also the ammonia oxidizing microorganisms.

**Conclusions:** Understanding the interactions between ammonia oxidizers and AM fungi appears important for efficiency of nitrogen (re)cycling in the soil-plant systems, and for limiting mineral nitrogen losses from the (agro)ecosystems, above all.

**Keywords:** ammonia oxidizing bacteria and archaea, competitive interactions, hyphosphere, inhibitors, nitrogen cycling, quantitative real-time PCR



P-056

## Ecosystem Functioning

### ROOT-ASSOCIATED FUNGAL COMMUNITIES DIFFER BETWEEN PHOSPHORUS-RICH AND POOR SOILS IN PANAMA BUT DO NOT ACCOUNT FOR DIFFERENCES IN SEEDLING GROWTH

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**Introduction/Aim:** Panama Canal watershed soils vary almost as much in phosphorus (P) as those of the entire Amazon Basin. This variation is associated with high compositional turnover, with >50% of tree species showing preferences for high or low P soils. The traits underlying P specialization, however, remain unclear. We tested whether low-phosphorus specialist species obtain greater benefits from mycorrhizal communities from low-phosphorus soils.

**Materials and Methods:** We grew seedlings of 9 tree species differing in P requirements in sterile soil collected from either a low (283 ppm total P) or high (794 ppm) P site and then added either a live or sterile inoculum consisting of soil and roots collected from each site. We measured seedling growth, nutrition, mycorrhizal infection and sequenced root fungal communities.

**Results:** Seedlings always grew faster in the high-P soil, where the impact of the inoculum type was generally small. In contrast, in the low-P soil, seedlings grew faster with live vs dead inoculum, but contrary to expectation, had 2-3 fold faster growth rates when grown with high-P versus low-P soil inoculum. While arbuscular mycorrhizal (AM) colonization rates were generally similar between inoculum types, AM communities were distinct. Furthermore, seedlings were also infected with mucoromycotina, but only in the combination of low-P soil and low-P inoculum source.

**Discussion:** The capacity to grow in low P soils does not appear to be attributable to beneficial associations with AM fungi.

**Conclusions:** Root-associated fungal communities vary with inoculum source and soil type, but cannot account for observed species performance and distribution patterns.

**Keywords:** arbuscular mycorrhizal fungi, habitat specialization, mucormycotina, seedling performance

P-057

## Ecosystem Functioning

### GLOMALIN-RELATED SOIL PROTEIN BENEFITS SOIL CARBON SEQUESTRATION WITH TROPICAL COASTAL FOREST RESTORATION

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**Introduction/Aim:** Glomalin-related soil protein (GRSP), produced by arbuscular mycorrhizal fungi (AMF), plays a crucial role in enhancing soil structure and promoting the sequestration of soil organic carbon (SOC) during vegetation restoration. It is important to assess the impact of GRSP on soil properties in tropical coastal areas.

**Materials and Methods:** A study involving four distinct restoration practices: barren land (BL, serving as an unrestored control), a *Eucalyptus exserta* (EF) forest, a mixed broadleaved forest (MF), and a secondary natural forest (SF) was selected.

**Results:** The implementation of vegetation restoration measures led to significant increases in both easily-extractable GRSP (EE-GRSP) and total GRSP (T-GRSP) compared to BL. The proportions of EE-GRSP/SOC and T-GRSP/SOC ranged from 1.6%-2.0% and 6.5%-15.8%, respectively. <sup>13</sup>C NMR demonstrated that the O-alkyl-C fraction of SOC, which is relatively easily degradable, was notably higher under MF compared to EF and SF, while the recalcitrant aromatic-C or alkyl-C fractions were most abundant under SF or EF, respectively. Furthermore, a significant positive correlation was observed between the GRSP/SOC ratio and aromatic-C, as well as between GRSP and soil aggregate stability.

**Discussion:** Our study underscores the substantial contribution of GRSP to SOC and highlights its role in enhancing SOC sequestration through the promotion of soil aggregate stability and the accumulation of recalcitrant SOC.

**Conclusions:** Among these artificial or natural growing forest restorations, the mixed forest restoration practice with native tree species provides a promisingly preferable restoration strategy in heavily eroded land restoration from the aspect of soil aggregation and SOC sequestration.

**Keywords:** mixed broadleaved forest, *Eucalyptus exserta* forest, secondary natural forest, soil aggregate, soil carbon chemical composition

P-058

## Ecosystem Functioning

### TRACKING DISPERSAL OF ECTOMYCORRHIZAL FUNGI TO INFORM FOREST RESTORATION ON EX-ARABLE LAND

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**Introduction/Aim:** To meet UK Net Zero goals, 21% of low-grade farmland will be targeted for woodland creation through planting and natural colonisation. Compared to woodland soils, ex-arable soils typically have low organic carbon content, high fertility and compaction, dominance of bacteria and arbuscular mycorrhizal fungi, and few ectomycorrhizal fungi (EMF). Lack of EMF may restrict the growth and survival of key tree species, e.g., pedunculate oak and silver birch. As part of “Fungi4Restor”, a wider multi-partner research project led by Forest Research, this study aims to elucidate EMF dispersal and colonisation pathways in ex-arable land.

**Materials and Methods:** Airborne dispersal was assessed using a total of 75 rainwater traps along 25 transects from mature woodlands into ex-arable land, across four sites in south-east England. Samples were stained with Melzer’s reagent and EMF spores were counted with a haemocytometer and x1000 light microscope. Soil colonisation was assessed by collecting host fine roots and sampling foraging hyphae with mesh bags. DNA was extracted for ITS2 amplification and amplicon-sequencing using Illumina Novaseq.

**Results:** Spore counts revealed a decrease in a total number of fungal spores along the increasing distance gradient. Metabarcoding analysis will identify the species composition of the EMF community.

**Discussion:** Our experiment will help to unravel the relative contributions of airborne and soil colonisation pathways and the dispersal capabilities of EMF into ex-arable settings.

**Conclusions:** Our experiment contributes evidence to inform whether artificial inoculation of seedlings might be needed to help improve their survival and growth in ex-arable settings.

**Keywords:** ectomycorrhiza, dispersal, colonisation, forest restoration

P-059

## Ecosystem Functioning

### FUNGAL COMMUNITY STRUCTURE CHARACTERISATION OF THREE DIFFERENT TREE SPECIES GROWING AT METAL-ENRICHED SITE

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**Introduction/Aim:** Long-term lead mining and smelting in Žerjav (Slovenia) has resulted in metal pollution and environmental degradation. Our previous research showed that associations of plants with fungal endophytes might alleviate stress and enhance the survival of plants at metal-enriched sites. This work aimed to identify the fungal colonisers of three different tree species at the metal-enriched site.

**Materials and Methods:** The root samples of *Salix caprea*, *Betula pendula*, and *Pinus sylvestris*, with their rhizosphere soils, were collected at two sampling sites with different metal pollution levels. The frequencies of arbuscular mycorrhiza (AM) and dark septate endophytes (DSE) were estimated. Ectomycorrhizal (EM) roots were assigned to 10 distinctive morphotypes, and two of each EM morphotype detected on each tree species were selected for molecular identification. Parameters such as pH, organic content, and total metal content were measured in soil.

**Results:** EM dominated all tree species, while colonisation with DSE was the highest in *S. caprea*. A positive correlation between the DSE and increased soil metals was confirmed. Fungi from Pezizales and Thelephorales were the most abundant in *B. pendula*, while Helotiales and Sordariales dominated in *S. caprea*. Most of the fungi colonising *P. sylvestris* remained unidentified, while Pyronemataceae prevailed among the rest.

**Discussion:** These results are in line with our previous research on *S. caprea*. This study reported for the first time the mycorrhizal status of *B. pendula* and *P. sylvestris* in the Žerjav area.

**Conclusions:** Our results showed that the tree species is a more important factor determining mycorrhizal colonisation rather than the sampling site (pollution level).

**Keywords:** dark septate endophytes, ectomycorrhiza, polluted area, Helotiales, Pezizales

P-061

## Ecosystem Functioning

### SHIFTS IN PLANT MYCORRHIZAL TYPE FOLLOWING EXTENSIVE TREE MORTALITY COINCIDE WITH RESILIENCE OF SOIL CARBON STORES IN BOREAL FORESTS

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**Introduction/Aim:**The mountain pine beetle outbreak caused extensive tree mortality in Canadian boreal forests. Many ectomycorrhizal (EM) pine stands have been replaced by arbuscular (AM) mycorrhizal shrubs. Given the distinct effects EM and AM fungi have on carbon cycling, shifts from EM- to AM-dominance may affect soil carbon stores.

**Materials and Methods:**We sampled soil and surveyed vegetation in 80 lodgepole pine-dominated sites, split by disturbance severity (forests with >70% pine basal area killed by beetle versus intact forest) and soil texture (coarse versus fine). Trees in disturbed forests were killed about 15 years ago. We separated soils into mineral-associated organic matter (MAOM) and particulate organic matter (POM) pools and measured their carbon and nitrogen concentrations.

**Results:**Disturbed forests were dominated by AM vegetation. Carbon concentrations of bulk mineral soil remained largely unaffected by tree mortality. Disturbed forests contained proportionally more soil carbon in the MAOM fraction compared to intact forests. Soil C/N was lower in both POM and MAOM in disturbed than in intact forests.

**Discussion:**Lowered C/N in POM and MAOM suggests that decomposition increased in disturbed forests. Carbon inputs from AM-dominated vegetation may have compensated carbon losses from soils, resulting in no soil C concentration changes. These results are consistent with emerging hypotheses on the effect of AM vegetation on soil organic matter, however, a change in abiotic conditions with extensive tree mortality independent of changes in mycorrhizal type could also explain the results.

**Conclusions:**Carbon storage in these forest soils was resilient to extensive tree mortality. Multiple explanations exist for the underlying mechanisms.

**Keywords:** boreal forest, *Dendroctonus ponderosae*, lodgepole pine, mountain pine beetle, *Pinus contorta*, soil carbon

P-062

## Ecosystem Functioning

### HOW DO DIFFERENT MYCORRHIZAL TYPES AFFECT THE DYNAMICS OF PLANT C INPUT TO SOIL? INSIGHTS FROM A MINI FOREST EXPERIMENT

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**Introduction/Aim:** Mycorrhizal associations play a crucial role in shaping soil organic carbon (C) dynamics and stocks within forest ecosystems. However, understanding the specific effects of different tree mycorrhizal types, such as arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) associations, on organic C allocation in the plant-soil system and especially soil respiration remains limited.

**Materials and Methods:** This study reports on a lysimeter experiment to assess the transfer of plant organic C via mycorrhizal hyphae to the soil and its subsequent effect on soil respiration for different AM and ECM tree species. Ten tree species were planted in lysimeters, with five trees associated with each mycorrhizal type. After one year of establishment, trees are labeled periodically (i.e., every month during the growing season) with  $^{13}\text{C}$  by exposure to  $^{13}\text{C}$ -enriched  $\text{CO}_2$ , and soil respiration (including its  $\delta^{13}\text{C}$  signature) is measured in situ using a  $\text{CO}_2$  isotope laser analyzer (G2132-i, Picarro, Inc.). The  $^{13}\text{C}$ -labeling is also used to trace the C input via hyphae to various soil fractions.

**Results:** Data on soil C budgets and fluxes in the plant-soil system depending on the mycorrhizal type as well as the short-term effects of mycorrhizal types on organic C allocation and soil respiration will be presented.

**Discussion:** Patterns of soil C fluxes influenced by mycorrhizal type and tree species will be discussed.

**Conclusions:** These results will highlight the consequences of mycorrhizal type for forest C cycling and potential strategies for enhancing soil C stocks in temperate forest ecosystems

**Keywords:** Mycorrhizal associations, Soil carbon dynamics, Soil respiration, Forest ecosystems

**P-064**

### **Ecosystem Functioning**

#### **ENHANCING PEPPER SEEDLING GROWTH WITH INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGI FROM MONO AND MIXED CROPPING PEPPER PLANTATIONS IN LAMPUNG INDONESIA**

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**Introduction/Aim:**Arbuscular Mycorrhizal Fungi (AMF), naturally present in the soil, form symbiotic relationships with various plants, including peppers. These fungi provide numerous benefits to plants, although their presence and effectiveness can be influenced by the specific fungal species and host plant types. In Lampung, Indonesia, peppers are cultivated in both monoculture and mixed cropping systems.

**Materials and Methods:**Soil samples from mono and mixed cropping pepper plantations in Lampung, were subjected to a 3-month culture trapping process using maize as a host plant to propagate AMF propagule present in the soil. The spore from the culture were molecularly identified and then evaluate for their effectiveness by inoculating them onto 7-week-old pepper seedlings.

**Results:**Nine AMF types were identified. Among them, five AMF types were present in both types of pepper plantations, while two types were exclusive to monoculture, and another two types were exclusive to mixed cropping. These AMF significantly improved pepper seedling growth, as indicated by increases in the number of leaves, total leaf area, shoot fresh and dry weights, and AMF root colonization

**Discussion:**Differences in certain types of AMF between monoculture and mixed cropping plantations are influenced by plant types and farm conditions. Mixed cropping includes more main crops and various types of shade trees, resulting in higher vegetation density and a more humid environment compared to monoculture.

**Conclusions:**The indigenous AMF are believed to have adapted and are compatible with pepper plants, allowing them to colonize the pepper seedling roots in the nursery and subsequently enhance seedling growth.

**Keywords:** Pepper cropping system, diversity, molecular identification, and pepper nursery

P-065

## Ecosystem Functioning

### INFLUENCE OF PLANT COMMUNITY ON ARBUSCULAR MYCORRHIZAL FUNGI: AN APPROACH TO DRY HABITATS RESTAURATION IN OCEANIC ISLANDS

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**Introduction/Aim:**Arbuscular mycorrhizal fungi (AMF) play a crucial role in ecological restoration, particularly in arid environments, by facilitating plant establishment. In addition, prioritizing locally adapted AMF enhances ecosystem restoration. This research focuses on assessing how the plant community affects AMF abundance in arid environments and how different plant consortia can influence the propagation of mycorrhizal inoculum.

**Materials and Methods:**Seven microenvironments were studied in an arid ecosystem at risk of desertification in Canary Islands, each with different vegetation cover and richness. A floristic inventory was conducted and spores of AMF present in the soil were quantified. AMF propagation was assessed using different trap plant consortia. The spores were counted after 3 months of cultivation. Identification of mycorrhizae was performed by extraction of DNA from the roots of the trap plants and amplification and sequencing of an 18S rRNA fragment of the mRNA.

**Results:**Spore density varied significantly between microenvironments, with an average of 5 spores/g soil. Vegetation cover showed the greatest influence on AMF density. Finally, spore propagation was higher (increase of 9.16 spores/gr.), in the native and diverse plant consortium, comprising various species of AMF.

**Discussion:**Contrary to the hypothesis, spore quantification is only modulated by plant cover, however multiplication was influenced by plant diversity.

**Conclusions:**In this heterogeneous environment, spore density is controlled by vegetation cover rather than host identity. Considering the assembly mechanisms of these symbionts, this work demonstrates that the use of trap plants native to the study area and belonging to different phylogenetic groups is beneficial for a greater spread of the inoculum.

**Keywords:** Arbuscular mycorrhizal symbiosis, spore density, mycorrhizal inoculum, vegetation cover, trap culture, ecological restoration.



P-066

## Ecosystem Functioning

### **SURVIVING TREES ARE KEY ELEMENTS IN THE FATE OF ECTOMYCORRHIZAL COMMUNITY AFTER SEVERE BARK-BEETLE FOREST DISTURBANCE**

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**Introduction/Aim:** Bark-beetle outbreaks are a critical event in the Norway spruce forest life cycle. However, gaps still remain in our knowledge of disturbance effects on ectomycorrhizal fungi (EMF), key players in forest productivity and nutrient cycling. While majority of available studies focused on the EMF communities in managed forests, context of natural disturbances remains understudied.

**Materials and Methods:** We investigated an unmanaged natural spruce forest in the Bohemian Forest (Czech Republic) affected by a severe forest dieback. EMF communities were described using soil DNA sequencing for 41 plots within this area.

**Results:** After decade since the disturbance, the forest structure in the study area (~60 ha) ranged from open canopy and sparse tree cover to areas with dense spruce regeneration to patches of closed-canopy forest. We found that relative abundance of EMF in soils was positively related to amount of surviving trees and regeneration density. Additionally, the number of surviving trees, but not the regeneration density, had positive effect on EMF species richness and tended to support preservation of late-successional EMF species.

**Discussion:** The stands with sparse surviving trees tended to be dominated by early stage fungi, and conversely, plots with the highest densities of survived trees tended to harbour more of late-stage EMF species. However, our data didn't imply a tight interlacing of the stand regeneration stage and EMF community composition.

**Conclusions:** Our results suggest that trees that survive bark beetle disturbance are key for the fate of the EMF community in natural forests.

**Keywords:** ectomycorrhizal fungi, forest disturbance, natural regeneration, DNA metabarcoding

P-067

### Ecosystem Functioning

#### **ASSESSING THE DEGRADED AND ENHANCED MUTUALISM HYPOTHESES BY QUANTIFYING A NATIVE AND INVASIVE HOSTS' DEPENDENCE UPON AND RESPONSIVENESS TO AM FUNGI**

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**Introduction/Aim:** Invasive plants are a wide-scale and costly issue around the globe, but their interactions with ubiquitous arbuscular mycorrhizal fungi (AMF) in exotic ranges remain poorly defined. The enhanced mutualism hypothesis suggests that invasive plants receive benefits from AMF outside of their native range because invaders can form novel symbioses and/or associate with cosmopolitan fungi. In contrast, the degraded mutualism hypothesis proposes that invasive plants receive little to no benefit and are poorly colonized by AMF.

**Materials and Methods:** We set out to investigate both hypotheses by quantifying the dependence upon and responsiveness to a suite of native range fungi by two congeners: native Pasture Thistle (*Cirsium discolor*) and invasive Canada Thistle (*Cirsium arvense*). We grew both species with and without AMF along a soil P fertility gradient.

**Results:** We found that both Thistles were facultatively dependent upon AMF, or capable of growing without AMF. Canada Thistle exhibited a small negative maximum responsiveness to AMF, while Pasture Thistle had a maximum responsiveness of 0.123g, or 35%, at 7.8 ppm soil P. Canada Thistle was more colonized by AMF than Pasture Thistle ( $26.6 \pm 3.2\%$  (SE) and  $19.3 \pm 3.4\%$ , respectively).

**Discussion:** The negative responsiveness of Canada Thistle corresponds with the degraded mutualism hypothesis; however, its high colonization concurs with the enhanced mutualism hypothesis.

**Conclusions:** Our findings suggest that neither the degraded nor enhanced mutualism hypotheses apply to Canada Thistle, at least in terms of P benefit. Pasture thistle, however, most likely benefits from a shared evolutionary history with native AMF.

**Keywords:** Degraded mutualism hypothesis, Enhanced mutualism hypothesis, Invasive Plant, Responsiveness, Dependence, Phosphorus

P-068

## Ecosystem Functioning

### THE EFFECT OF TILLAGE ON AM FUNGAL COMMUNITIES ACROSS A 3000 KM LATITUDINAL GRADIENT IN EUROPE

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**Introduction/Aim:** This study assesses the impact of different tillage practices on arbuscular mycorrhizal fungi (AMF) across various climates. By comparing fields with standard, reduced, and no-tillage, we aim to uncover the effects of tillage intensity on AMF diversity and community structure. Understanding these impacts is vital for developing agricultural practices that support soil ecosystem services. This study contributes to the broader knowledge of how agricultural interventions influence soil biology, guiding strategies for maintaining soil fertility and ecosystem functions and enhancing the sustainability of agricultural systems.

**Materials and Methods:** 84 soil samples were collected from seven conventional fields subjected to standard, reduced, and no-tillage practices, spanning a 3000 km latitudinal gradient across Europe within the EJP Soils EU project. Each soil sample underwent DNA extraction, followed by AMF-specific amplicon generation using the primers AMV4.5NF/AMDGR. The resultant amplicons were aligned with the MaarJAM database for AMF identification, allowing an assessment of tillage impacts on AMF diversity across varied soil types.

**Results:** Reduced tillage enhanced AMF richness and diversity. While geographical location strongly affected AMF community structure, tillage intensity did not show significant effects. Standard tillage resulted in a lower abundance of the genus *Claroideoglomus*.

**Discussion:** none at all

**Conclusions:** These findings highlight the beneficial impact of reduced tillage on AMF biodiversity and demonstrates that location, rather than tillage has the biggest effect in determining AMF community composition within European agricultural systems.

**Keywords:** Tillage, Arbuscular mycorrhiza, DNA, Diversity

P-069

## Ecosystem Functioning

### LINKS BETWEEN SOIL PHYSICAL AND ARBUSCULAR MYCORRHIZAL SIGNATURES IN RESPONSE TO TILLAGE AND CROP RESIDUE MANAGEMENT

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**Introduction/Aim:** Improving soil health is a key sustainability goal, garnering significant attention for the development of agricultural management practices supporting this objective. Arbuscular mycorrhizal fungi (AMF) serve as soil health indicators and are linked to various soil benefits. Conservation agriculture (CA) emerges as a system promoting agricultural sustainability and enhancing soil quality through the combination of minimal soil disturbance and residue retention, influencing microbial biomass. The study explores the long-term effects of CA on AMF biomass. The hypothesis posits that implementing conservation practices will positively influence AMF biomass, subsequently enhancing SSS, porosity, and organic carbon content, thereby contributing to comprehensive soil health.

**Materials and Methods:** The study exploited 2 experimental sites following CA practices for 20 years. Soil samples were collected from two depths and analyzed for SSS and porosity indices. AMF biomass was estimated via phospho- and neutral-lipid fatty acids (PLFA/NLFA).

**Results:** CA practices positively affected AMF biomarkers. PLFA exhibited a significant correlation with wet aggregate stability and organic carbon. A milder response was observed for NLFA. Both biomarkers were positively correlated with soil pore indices, but this correlation was not consistent across sites. Correlations were stronger with decreasing tillage intensity.

**Discussion:** The study showcased CA's beneficial effects on AMF. It also highlighted AMF's role in supporting soil wet stability and improving soil pore characteristics. Finally, it noted that CA practices may potentially improve AMF's contribution to soil health.

**Conclusions:** Findings suggest that applying essential conservation practices, fostering the growth of AMF has the potential to enhance topsoil structural properties.

**Keywords:** Soil health, Arbuscular mycorrhizal fungi, Conservation agriculture, Long-term field experiment

P-070

## Ecosystem Functioning

### USING NATIVE SOIL INOCULUM IN ECOLOGICAL RESTORATION: DOES IT HELP?

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**Introduction/Aim:**Riparian forests have a variety of interlinked habitats and are resource-rich with high biodiversity, but often converted to agricultural land uses. Associated changes to mycorrhizal communities may limit the reestablishment of native plants. We asked if the origin of soil inoculum influenced plant growth traits, foliar nutrients, and root-associated fungal community composition.

**Materials and Methods:**We grew three Myrtaceous species in soil from three different soil inoculums (sterilised, pasture and riparian forest). Plants were harvested after 28, 84 and 140 days, to measure plant traits, foliar nutrients, and root-associated fungal communities.

**Results:**We found substantial mycorrhizal colonisation on roots from plants grown in inoculum from pasture and riparian soil, but few differences in plant growth traits and foliar nutrients with inoculum type, time of harvest or species. Root fungal community showed marked differences between plant species, inoculum, and harvest time. Pathogenic fungi are influential in predicting and reducing the number of leaves and root volume in young trees at 28 days, but after 140 days of growth, this switches to beneficial fungi which also increase carbon in leaves.

**Discussion:**Fungal associations with roots differed between plant species and over time, not inoculum type and had little influence on plant traits. These effects might increase over time.

**Conclusions:**Here we shed light on the different capacities of soil inoculums, finding that soil inoculums do not influence revegetation outcomes. Instead, plants have different capacities to cultivate a specialist root-associated fungal community that is similar in different soil inoculums.

**Keywords:** mycorrhizal colonisation, plant traits, ectomycorrhizae, plant roots, Pezizomycetes, restoration

P-071

## Ecosystem Functioning

### RESPONSE OF THE FUNCTIONAL TRAITS OF AMF AND DSE IN EUGENIA WINZERLINGII, OWING TO THE SEASONAL VARIATION IN THE FLOODABLE LOWLAND FOREST IN MEXICO

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**Introduction/Aim:**The objective of this study was to assess the impact of seasonality on arbuscular mycorrhizal fungi (AMF) and dark septate endophytic fungi (DSE), colonization in relation to *E. winzerlingii* development, while determining how the functional traits of the AMF and DSE community colonizing these species' seedlings are altered by seasonal flooding or drought.

**Materials and Methods:**To observe the differences between, a group of 120 individuals was marked and sampled to evaluate their functional growth traits. Additionally, 30 individuals per season were collected for evaluating AMF and DSE functional traits. Traits such as growth rate, survival rate, stem height, leaf count, root length, as well as total nitrogen and phosphorus concentration in seedlings, exhibited no statistically significant inter-seasonal disparities.

**Results:**During the rainy season, seedlings demonstrated important divergence in AMF colonization (63%) in comparison to the dry season (46%). Likewise, during the rainy season, DSE colonization was documented at 75%, while during the dry season, it amounted to 65%. Evidence of intraradical fungal structure formation were universal among all seedlings, although extraradical mycelium length remained invariant across seasons.

**Discussion:**Seedlings of *E. winzerlingii* within the floodable lowland forest appear inherently adapted and thus evince resilience, as seasonal variation appears to have no discernible impact on fungal activity or the length of extraradical mycelium.

**Conclusions:**This influence manifests in the alteration of fungal structure formation and the correlation between variables of AMF and DSE, particularly during the dry season.

**Keywords:** AM Fungi, DSE Fungi, extraradical mycelium, seasonal flooding, symbiotic fungi, symbiotic seedling colonization

## Ecosystem Functioning

### EFFECT OF MYCORRHIZA, ZEOLITE, AND SUPERABSORBENT ON GROWTH AND PRIMARY ESTABLISHMENT OF SOME RANGELAND PLANTS IN ABANDONED CEMENT MINE SOILS

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**Introduction/Aim:**The purpose of this study was to investigate the possibility of increasing the establishment and growth of *Achillea millefolium* L., *Agropyron elongatum* (Host). P. beauv, *Agropyron desertorum* (Fisch. Ex Link) Schult. and *Artemisia sieberi* Besser plant through their cultivation along with zeolite, superabsorbent and mycorrhiza in greenhouse and minefield of Mashhad Shargh cement factory.

**Materials and Methods:**First, the seeds were planted in the greenhouse conditions and then when the seedlings were transferred to the pots in separate treatments, the *Glomus intraradices* (1:10), zeolite (2%) and superabsorbent (0.4%) based on the randomized complete block design with four replications were grown at the greenhouse. In a field experiment, the seeds of species were first cultivated in greenhouse conditions (seedling trays) and then they were transferred to paper pots filled with soil from the mining area and after a month, the pots were moved to the field (about 760 square meters). Seedlings were irrigated only once at the time of cultivation.

**Results:**According to the results, in greenhouse conditions, the colonization percentage of *G. intraradices* with plant roots was higher than the percentage of plant root colonization in two years of field experiments.

**Discussion:**In greenhouse conditions, zeolite, superabsorbent, and mycorrhiza treatments improved growth characteristics and early establishment of seedlings compared to control treatment.

**Conclusions:**Among the studied plants, respectively, *Agropyron elongatum* and *Agropyron desertorum* (grasses), *Artemisia sieberi* (shrub), and *Achillea millefolium* (Forbe) had the most successful establishment percentage in the field and could be used for vegetation restoration.

**Keywords:** Heavy metals, rangeland, mycorrhiza, Mashhad

# **Omics and Ecology**



**O-013**

**Omics and Ecology**

**SPATIAL AND TEMPORAL DYNAMICS OF AM SYMBIOSIS AT SINGLE-CELL RESOLUTION**

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**Introduction/Aim:**The intimate and ancient symbiosis between plant root systems and beneficial arbuscular mycorrhizal (AM) fungi can improve plant growth and resilience to water deficit. AM symbiosis is both spatially and temporally dynamic. Specialized fungal structures, known as arbuscules, are central for the exchange of nutrients between the interacting partners, though they develop and collapse over the course of a few days. In this project, we aim to identify key molecular pathways underpinning AM symbiosis at single-cell resolution in rice.

**Materials and Methods:**We leveraged translating ribosome affinity purification (TRAP) to profile the rice transcriptome in response to AM colonization by *Rhizophagus irregularis*. To determine the spatial expression of key genes, we applied single-cell spatial transcriptomics using a panel of 100 gene targets from both rice and *R. irregularis* (Molecular Cartography, Resolve Biosciences, Agrigenomics grant).

**Results:**We generated transgenic TRAP lines driven by three different AM-specific promoters which are expressed across distinct stages of arbuscule development. Here, we report the discovery of key molecular pathways which are specifically activated during early, mid or late arbuscule development. Furthermore, we reveal the spatial expression patterns of important plant and fungal genes at single-cell resolution.

**Discussion:**By integrating transcriptome and spatial expression data, we can better resolve the spatial and temporal dynamics of AM symbiosis.

**Conclusions:**In summary, our study provides new insights into gene activity across the arbuscule lifespan, changing our understanding of this intimate association.

**Keywords:** transcriptome, spatial expression, single-cell, multiomics, rice

O-014

## Omics and Ecology

### MOLECULAR CROSS-TALK IN ECTOMYCORRHIZAL SYMBIOSIS: FROM FUNGAL EFFECTORS TO POPLAR MYC2 TRANSCRIPTION FACTORS

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**Introduction/Aim:** Ectomycorrhizal (ECM) symbiosis plays a pivotal role in temperate and boreal forest carbon cycling, nutrient mobilization, plant development, and fitness. The molecular events leading to ECM formation have been partially elucidated, revealing the influence of fungal effectors on the plant immune system. Previous studies have shown that the *Laccaria bicolor* MiSSP7 effector is capable of interacting with the jasmonic acid (JA) co-repressor JAZ6, interfering with the JA signalling pathway and inhibiting the activity of MYC2 transcription factors. In this study, we investigated the molecular events triggered by LbMiSSP7 to elucidate the contribution of poplar MYC2s to the establishment of ECM symbiosis.

**Materials and Methods:** Transgenic poplars overexpressing or silencing the two paralogs of MYC2 were produced, and their ability to establish ectomycorrhizal symbiosis was assessed. We coupled these analyses with transcriptomics and DNA affinity purification sequencing.

**Results:** MYC2s overexpression led to a decrease in fungal colonization, whereas its silencing increased it, revealing their crucial role in ECM symbiosis. We identified poplar genes which direct or indirect control by MYC2 is required for ECM establishment.

**Discussion:** The enrichment of terpene synthase genes in the MYC2-regulated gene set, together with the identification of certain root monoterpenes as inhibitors of fungal growth and ECM formation, suggests a complex interplay between host monoterpenes and ECM fungi.

**Conclusions:** These results highlight the significance of poplar MYC2s and terpenes in mutualistic symbiosis. These findings deepen our understanding of the molecular mechanisms underlying ECM symbiosis and underscore the need of further exploration of the plant balance between defence and symbiotic relationships.

**Keywords:** Ectomycorrhiza, jasmonic acid, plant-microbe interactions, symbiosis

O-015

## Omics and Ecology

### THE MYCORRHIZAL LIFESTYLE OF MUCOROMYCOTINA FINE ROOT ENDOPHYTES THROUGH A GENOMIC LENS

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**Introduction/Aim:** Fine root endophytes belonging to the Mucoromycotina clade (MFRE) potentially play an important role in terrestrial nutrient cycling through their dual saprotrophic and mycorrhizal traits. Over the past decade, microscopic and physiological studies have yielded valuable insights into the mycorrhizal relationship between MFRE and plants, demonstrating intracellular colonisation and nutrient-for-carbon exchange. However, our understanding of the molecular mechanisms underpinning the saprotrophic and mycorrhizal lifestyles of MFRE remains limited.

**Materials and Methods:** In this study, we apply genomic, cytological and molecular genetic approaches to two MFRE isolates to investigate symbiotic and saprotrophic strategies in this mycorrhizal lineage. We generated the first genome assemblies corresponding to MFRE by combining complementary short-read (Illumina) and long-read (Nanopore) sequencing technologies.

**Results:** Leveraging comparative genomic and transcriptomic analyses, we have identified distinct signatures for transporters and secreted proteins (hydrolases, effector candidates) during in vitro and in planta conditions.

**Discussion:** Collectively, our findings underscore a unique molecular blueprint characterising the saprotrophic and mycorrhizal lifestyles of MFRE, setting them apart from other mycorrhizal fungi.

**Conclusions:** This study provides exciting new insights into the common and contrasting mechanisms underlying their plant-associated lifestyles and reveals the presence of saprotrophic traits in MFRE. It highlights the importance of isolating, sequencing and studying species from this group of mycorrhizal fungi to fully understand the ecosystem services provided by soil fungi.

**Keywords:** Mucoromycotina Fine Root Endophytes, comparative genomics, transcriptomics, confocal microscopy, transporters, effectors

O-016

## Omics and Ecology

### EXTENSIVE DIFFERENTIATION OF TRANSCRIPTOMIC RESPONSES TO SOIL ACIDITY AMONG GEOGRAPHIC ISOLATES OF *RHIZOPHAGUS CLARUS*: ARE VIRUSES ALSO INVOLVED?

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**Introduction/Aim:** Acid tolerance of arbuscular mycorrhizal (AM) fungi critically affects survival/growth of the host plants in acidic soil, but the mechanism underlying remains unknown. Fungi harbor diverse viruses, and some viruses enhance stress tolerance of the hosts. We approached the mechanism via dissecting transcriptomic and viromic responses to soil acidity, comparing geographic isolates.

**Materials and Methods:** Three strains of *R. clarus*, RF1 from acid sulfate soil in Northern Japan, HR1 from acid sulfate soil in Central Japan, and CK001 from tropical peat soil in Indonesia, were grown with *Lotus japonicus* at pH 3.8 – 5.5, and the extraradical mycelia were subjected to RNA-Seq, followed by read mapping to the CDS and viral sequences found in the three.

**Results:** Although many genes were upregulated in response to soil acidity in each strain, only 41 out of the 35,000 genes in the genome were commonly upregulated among the three. Certain similarity in the expression profiles were observed between those from acid sulfate soils (RF1 and HR1), but the one from peat soil (CK001) showed a very different profile. Interestingly, RF1 and HR1 upregulated two argonaute genes, AGO5 and AGO6, key components of antiviral RNAi machinery, and downregulated AGO9, which accompanied by a decrease in a partitivirus (RF1) and increases in totiviruses and mitoviruses (RF1 and HR1) in acidic pH.

**Discussion:** The extensive differentiation in transcriptomic response suggests that local soils are a strong driver for ecotypic differentiation.

**Conclusions:** The individual ecotypes evolved specific strategies to cope with soil acidity, in which viruses are likely to play a role.

**Keywords:** acid tolerance, arbuscular mycorrhizal fungi, ecotypes, transcriptomics, viruses

O-017

## Omics and Ecology

### GENETIC AND FUNCTIONAL TRAITS LIMIT THE SUCCESS OF COLONISATION BY ARBUSCULAR MYCORRHIZAL FUNGI IN A TOMATO WILD RELATIVE

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**Introduction/Aim:** To understand whether domestication had an impact on susceptibility and responsiveness to arbuscular mycorrhizal fungi (AMF) in tomato (*Solanum lycopersicum*), we investigated two tomato cultivars ('M82' and 'Moneymaker') and a panel of wild relatives including *S. neorickii*, *S. habrochaites* and *S. pennellii* encompassing the whole *Lycopersicon* clade.

**Materials and Methods:** Plants were inoculated with the AMF *Funneliformis mosseae* and biometric and molecular analyses were carried out.

**Results:** Most genotypes revealed good AM colonisation levels while, by contrast, both *S. pennellii* accessions showed a very low mycorrhization, but normal arbuscule morphology, and a negative response in terms of root and shoot biomass. This behaviour was independent of fungal identity and environmental conditions. Genomic and transcriptomic analyses revealed in *S. pennellii* the lack of genes identified within QTLs for AM colonisation, a limited transcriptional reprogramming upon mycorrhization and a differential regulation of strigolactones and AM-related genes compared to tomato. Donor plants experiments suggested that the AMF is perceived as a cost for *S. pennellii*: *F. mosseae* could further proliferate in the roots only when it was part of a mycorrhizal network.

**Discussion:** Overall these results suggest that genetics and functional traits of *S. pennellii* are responsible for the limited extent of AMF colonisation.

**Conclusions:** These unique features make *S. pennellii* an unprecedented model to study the molecular mechanisms which regulate the extent of AM colonisation in plant roots. We suggest that the use of tomato wild relatives as a source of new alleles requires deeper analysis which also considers the plant-beneficial microbe interaction.

**Keywords:** *Solanum lycopersicum*, *Solanum pennellii*, strigolactones, transcriptomics, wild relatives

**O-018**

**Omics and Ecology**

**TOWARD 1000 MYCORRHIZAL GENOMES**

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**Introduction/Aim:**Fifteen years after sequencing the first mycorrhizal genome of *Laccaria bicolor* the number of sequenced mycorrhizal fungi has grown dramatically along with the need for additional reference genomes and quickly evolving genomic technologies to meet the growing demands.

**Materials and Methods:**Through large-scale projects like Mycorrhizal Genomics Initiative, 1000 Fungal Genomes project and genus-centric projects (e.g. *Suillus*), over 200 annotated genomes are currently available from the US Department of Energy Joint Genome Institute's MycoCosm (<https://mycocosm.jgi.doe.gov>) equipped with interactive analytical tools.

**Results:**Sequenced and analyzed in partnership with fungal research community, these data not only provide insights into biology and evolution of different types of mycorrhizal associations but also set foundation for deeper understanding of plant-fungal interactions, ecosystem functions, and functional genomics.

**Discussion:**Now, with over 2,500 fungal genomes in MycoCosm, JGI aims to expand their fungal genomics efforts by targeting 10,000 fungal genomes as a grand challenge. Drawing upon integrated genomics and multi-omics data in MycoCosm and in collaboration with research labs and culture collections, this ambitious goal seeks to build the most comprehensive collection of fungal genomes in the next 10 years.

**Conclusions:**This presents new opportunities to further scale up mycorrhizal genomics and explore the role of mycorrhizal associations at molecular level using multi-omics data and computational techniques to review at the meeting.

**Keywords:** genomics, transcriptomics, metabolomics, multi-omics, JGI

O-019

## Omics and Ecology

### METABARCODING AND REPEATED SANGER SEQUENCING OVER 27 YEARS REVEAL DISPERSAL STRATEGIES OF ECTOMYCORRHIZAL FUNGI, AND LIMITS ON POST-LOGGING SUCCESSION

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**Introduction/Aim:**We have studied succession of ectomycorrhizal fungi (EMF) within a subalpine forest since logging in 1994-1995. Initially, living ectomycorrhizas on peripheral forest trees were an important source of EMF for seedling colonization in the clearcuts. We hypothesized that more EMF taxa would spread from forest edges deeper into openings over time.

**Materials and Methods:**In 2021-22, samples were collected from 90 m-long transects centered on forest edges and extending to the centers of 1-ha openings. Mycorrhizal fungi were identified from field-collected spruce, fir and *Vaccinium membranaceum* mycorrhizas (PacBio); sporocarps (Sanger); and ectomycorrhizas of conifer seedlings grown in dried soil samples in a greenhouse (Sanger).

**Results:**At 27 years postharvest, the threshold decline in EMF richness on regenerating conifers appeared to remain at approximately 15 m into the openings. Sporocarp richness declined adjacent to, and within, openings. Some EMF colonized from desiccation-resistant propagules but were not detected on roots in the field. Of EMF taxa on conifer roots in the openings, 50% were also present on hair roots of *V. membranaceum*.

**Discussion:**Our data provide evidence that (i) EMF taxa that define the forest community colonize more effectively from hyphae than desiccation-resistant propagules; (ii) a propagule bank exists independent of living ectomycorrhizas; (iii) the ericaceous understory could act as a significant repository of EMF; and (iv) forest fragmentation is affecting reproduction of EMF.

**Conclusions:**Overall, it appears that environment filtering, not dispersal limitation, is the current constraint on succession, and that canopy closure will be required for major change in the EMF communities in the openings.

**Keywords:** ectomycorrhizal fungi, succession, propagules, community ecology, inoculum, *Vaccinium*

O-020

## Omics and Ecology

### USING META-OMICS TO STUDY CROSS-KINGDOM INTERACTIONS BETWEEN PLANTS, MYCORRHIZAL FUNGI, AND OTHER SOIL MICROBES ALONG ALTITUDINAL GRADIENTS

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**Introduction/Aim:** Understanding the intricate network of interactions between mycorrhizal fungi and other soil microorganisms is crucial for elucidating ecosystem functioning and resilience. Previous studies have shown that other soil microbes often associate with mycorrhizal fungi influencing their symbiotic relationship with plants. However, we still poorly understand the mechanistic processes behind these associations and how these interactions shape overall mycorrhizal community assembly.

**Materials and Methods:** Here we use soil shotgun metagenomics and metatranscriptomics to unravel how cross-Kingdom interactions can shape mycorrhizal communities across 137 sites in the Swiss Alps. We specifically test how taxonomic and functional diversity of soil bacteria and protists shape taxonomic and functional composition of mycorrhizal communities across altitudinal gradients (425 to 2947m a.s.l.). Metatranscriptomic short reads were used to profile the metabolically active soil microbial communities with unprecedented taxonomic resolution using the reconstructed full ITS, 16s and 18s ribosomal genes. In addition, the non-ribosomal reads were used to characterize the transcriptomic response at the community level and mapped to the metagenome assembled genomes obtained from the metagenomic dataset.

**Results:** Our results revealed an intricate network of cross-Kingdom interactions between plants, mycorrhizal fungi, and other soil microbes, elucidating their cooperative and competitive dynamics across these gradients.

**Discussion:** Overall, this study underscores the importance of incorporating both DNA and RNA based approaches to better elucidate the complex interplay of cross-Kingdom interactions driving mycorrhizal communities.

**Conclusions:** Such insights are crucial for advancing our understanding about the role of mycorrhizal fungi mediating ecosystem processes and for informing conservation and management strategies in the face of ongoing environmental changes.

**Keywords:** Metagenomics, Metatranscriptomics, Mycorrhiza, Cross-Kingdom interactions, Altitudinal gradients, Ecosystem functions.



**O-022**

**Omics and Ecology**

**EXPLORING THE DIVERSITY, FUNCTION, AND SPATIAL ORGANIZATION OF AM HYPHAE-ASSOCIATED BACTERIA**

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**Introduction/Aim:**The extra-radical hyphae (ERH) of arbuscular mycorrhizal fungi (AMF) interact with bacteria from the surrounding soil, in a zone called the hyphosphere. While certain key players have well-understood functional impacts on the AMF symbiosis (e.g. P-solubilizing bacteria), there is much still to learn about the overall hyphosphere ecosystem. In particular, the spatial organization of bacteria across hyphal networks remains largely unknown.

**Materials and Methods:**To visualize hyphae-adherent bacteria, we grow *Rhizophagus irregularis* and *Diversispora Epigaea* in co-culture with bacteria originally isolated from the hyphosphere. We have developed several hyphal-bacterial co-culture methods, including in vitro with transformed carrot roots, in sand substrates in the growth chamber with *Brachypodium distachyon*, and on cellophane and polycarbonate membranes. Hyphae are then transferred to slides and the locations of different bacterial species imaged using a fluorescence in situ hybridization approach with high taxonomic sensitivity (HiPR-FISH).

**Results:**Using a universal bacterial FISH probe, we have confirmed that our co-culturing methods result in sufficient levels of hyphal occupancy by bacteria. We are now using HiPR-FISH to further illuminate occurrence patterns of bacterial taxa with different ecological functions (e.g. phytase and chitinase activity, biofilm formation) on key features of hyphal architecture including fine absorptive hyphae, runner hyphae, and branching junctions.

**Discussion:**HiPR-FISH data will contribute to our understanding of both fine-scale ERH-bacterial interactions and interactions between different hyphosphere bacteria, and expand our toolbox for studying AMF.

**Conclusions:**Such insights will point us to new mechanistic hypotheses on how microbial activities in the hyphosphere influence the ability of AMF to take up and transport nutrients.

**Keywords:** hyphosphere, hyphal-associated microbial communities, microbial ecology, fungal-bacterial interactions

**O-023**

**Omics and Ecology**

**ARBUSCULAR MYCORRHIZAL FUNGAL NICHE DIFFERENTIATION AND COMMUNITY ASSEMBLY**

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**Introduction/Aim:**The relationship between niche differentiation and coexistence is theoretically subject to opposing forces: species persistence should be more likely when species resource requirements and tolerances match local conditions; but also when they diverge from those of potential competitors. Empirical evidence weighing these forces is inconsistent, but it is unclear whether this reflects genuine variability or inconsistency in the way that niches are defined.

**Materials and Methods:**Earlier authors have distinguished abiotic and biotic niche axes, and resource and condition axes: resources may be consumed and competed for, while conditions may be tolerated and not competed for. We used global arbuscular mycorrhizal fungal distribution data and a range of metadata to estimate different niche hypervolumes for individual taxa, and niche differentiation among co-occurring taxa.

**Results:**There was low-to-moderate correlation between estimates of differentiation derived from different niche axes, indicating that interacting arbuscular mycorrhizal fungi may intimately share niche space along some axes but be relatively independent along others. Niches of co-occurring taxa were less differentiated along condition than resource axes.

**Discussion:**In general, AM fungal communities comprise taxa with overlapping niches. They are thus predominantly influenced by the availability of taxa able to tolerate local conditions. Yet, clearer differentiation along resource than condition niche axes suggests that resource competition moderates community composition.

**Conclusions:**Simultaneously considering different niche axes can improve our understanding of community assembly. The distinction between condition and resource niches may be fundamental for teasing apart the effects of regional and local processes.

**Keywords:** Fungal niches, Realised niche, Species coexistence, Hypervolume

O-024

## Omics and Ecology

### GLOBAL PATTERNS AND DRIVERS OF THE SPECIES-AREA RELATIONSHIP FOR ECTOMYCORRHIZAL FUNGI IN FOREST FRAGMENTS

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**Introduction/Aim:**The species-area relationship (SAR) is a cornerstone of ecology, predicting that species richness amplifies with increasing habitat area. This principle is well documented for macroorganisms from local to global scales, but its applicability to ectomycorrhizal (EcM) fungi, key soil microbes that form mutualistic relationships with more than 60% of trees on Earth, in fragmented forest habitats remains unexplored on the global scale.

**Materials and Methods:**This study leverages the GlobalFungi dataset of 13,475 soil samples from 1,274 forest patches worldwide, spanning diverse climatic gradients and biome types, to examine the relationship between EcM fungal richness and each forest patch area, habitat quality and diversity.

**Results:**Our analysis revealed a global-scale positive relationship between forest patch size and EcM fungal diversity, indirectly mediated by habitat quality (including climatic, edaphic, vegetation and spatial factors) and habitat heterogeneity in forest fragments.

**Discussion:**The habitat heterogeneity and community dissimilarity primarily drive the positive SAR for EcM fungi, especially in larger, intact forest habitats. The positive area effect is most pronounced in temperate broadleaf and coniferous forests but persists across different biomes. Furthermore, patch-level human impacts, distance to roads, and landscape integrity are also significant mediators of EcM fungal diversity.

**Conclusions:**The larger forests host a higher EcM fungal diversity and a higher proportion of unique EcM species, underscoring the significance of forest size and habitat diversity for fostering biodiverse and patch-level endemic fungal communities at the landscape scale. These findings inform the interpretation and application of SAR in the contexts of EcM fungal biogeography, ecosystem functioning, and biodiversity conservation efforts.

**Keywords:** Ectomycorrhizal Fungi, Species-Area Relationship, Forest Fragmentation, Biodiversity Conservation, Habitat Loss, Ecosystem Functioning

LT-026

## Omics and Ecology

### COMMUNITY FUNCTION OF ARBUSCULAR MYCORRHIZAL FUNGI REVEALED FROM METATRANSCRIPTOMES OF COLONIZED HOST PLANT ROOTS IN AN AGRICULTURAL FIELD

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**Introduction/Aim:** Arbuscular mycorrhizal (AM), mutualistic symbioses, require reciprocal regulation between fungi and their plant hosts, yet the dynamics of this regulation remain undocumented in agricultural fields.

**Materials and Methods:** Here, we use meta-transcriptomes of mycorrhizal sorghum roots sampled weekly throughout the growing season to analyze this regulation.

**Results:** We discover that coordinated transcription of AM fungal and plant host genes early in the season features cytoskeletal genes related to formation of arbuscules, the fungal-plant organs of nutrient and mineral exchange. Late in the season, plants downregulate cytoskeletal genes, but AM fungi continue their upregulation along with genes associated with fungal sporulation and mating, suggesting the time and place to search for the undiscovered mechanisms of sexual reproduction in AM fungi. Functional complexity of AM fungi is evidenced by an increase of more than 100-fold in richness of transcribed genes, which is coordinated with upregulation of plant genes facilitating transport of fatty acids and sucrose.

**Discussion:** The co-existence of multiple, highly niche-overlapped taxa increased the functional diversity of AM fungal gene expression. The early upregulation in both AM fungus and host involves arbuscule formation and that the later upregulation seen only in AM fungi is associated AM fungal clonal sporulation, and possibly with sexual mating in mature roots or adjacent soil.

**Conclusions:** Our analysis of plant and AM fungal transcripts found in RNA-seq analysis of RNA extracted from field samples helps disentangle the complex system where several, coexisting AM fungi must coordinate development with their host plant.

**Keywords:** community function, arbuscular mycorrhizal fungi, metatranscriptomes, dual-RNA

**LT-027**

**Omics and Ecology**

**SPECIALIZED ECTOMYCORRHIZAL METABOLITES REGULATE SYNTHETIC FUNGAL COMMUNITIES AND THEIR INTERACTIONS**

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**Introduction/Aim:** Ectomycorrhizal fungi (EMF) are beneficial fungi colonizing multiple host plants' root tissues. The interaction between EMF and their host is likely governed by metabolites, which act as direct lines of communication among organisms in the rhizosphere. However, the metabolites or signals that are produced when a fungus is alone or with other microbes are understudied. Our goal is to identify and characterize the repertoire of metabolites produced when EMFs are in co-culture.

**Materials and Methods:** Firstly, to develop a functioning EMF synthetic community, we conducted co-culture assays between five EMFs isolated from *Populus* roots or within a *Populus* plantation. The aim was to find a combination of EMF that produces a repertoire of metabolites that promote the growth of other fungal members. Since it is known that nutrient availability influences metabolite production, we utilized four different substrate media, two nutrient-rich and two nutrient-deprived.

**Results:** Our preliminary results determined that *Hebeloma brunneifolium* promoted the growth of nearly all the co-occurring fungi in a nutrient-poor environment; however, this was not reciprocal, with no growth benefit of *H. brunneifolium*. Regardless of the media, *Laccaria bicolor* and *Cenococcum geophilum* had the most beneficial interactions with co-occurring fungi.

**Discussion:** Based on the above results, we determined that *C. geophilum*, *H. brunneifolium*, and *L. bicolor* would benefit each other for fungal growth.

**Conclusions:** Altogether, our results show that we can construct a synthetic EMF community that will be symbiotic with each other and likely have a positive phenotypic effect on the host plant.

**Keywords:** Mycorrhization, beneficial fungi, metabolomics, proteomics

LT-028

## Omics and Ecology

### GENOME-WIDE ASSOCIATION ANALYSIS OF ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES IN *SORGHUM BICOLOR*

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**Introduction/Aim:** Plant-microbial interactions are difficult to untangle, as they are governed by plant genetics, microbial genetics, the environment, and their interactions. To understand the complex interactions between AMF and their plant hosts, we examined *in situ* responses of the diverse *Sorghum bicolor* Bioenergy Association Panel to factorially manipulated nitrogen and phosphorus treatments.

**Materials and Methods:** 337 sorghum genotypes were grown across 12 blocks (4 fertilizer treatments, 3 replicates per block) in Watkinsville, GA, USA. Roots were harvested after 8 weeks of in-field growth and aboveground biomass traits were recorded after 5 months. AMF communities were assessed through amplicon and whole genome shotgun sequencing, and used as input phenotypes in subsequent genome-wide association analysis.

**Results:** Preliminary analysis indicates that while environmental context expectedly plays the largest role in governing plant-microbial interactions and crop yields, both host genetics and the environment-genetic interaction have consequential effects. There is substantial variation between genotypes in their response to nitrogen fertilizer in aboveground yield traits, though comparable effects have not yet been detected in the belowground community.

**Discussion:** We present one of the first large-scale genome-wide association studies incorporating both AMF abundance and taxonomic identity as input phenotypes. This should allow identification of key genes associated both with the mycorrhizal symbiosis and governing plant-AMF associations at the level of individual taxa.

**Conclusions:** Dissecting the genetic-environment interactions of the microbial symbiosis may contribute both to sorghum breeding efforts and to the identification of the most appropriate genotypes to plant in a given location thereby allowing reduced external inputs to generate sufficient agricultural outputs.

**Keywords:** Microbiome, Genetics, AMF, Genotype x Environmental Interactions

LT-029

## Omics and Ecology

### DRIVERS OF ARBUSCULAR MYCORRHIZAL FUNGI BIOGEOGRAPHY DIFFER AT LOCAL SCALES

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) are significant root biotrophs that promote carbon and nutrient transfer across the plant-soil interface. Few reports have disentangled the drivers of AMF biogeography from those of their plant hosts.

**Materials and Methods:** We minimized host influence by studying AMF associated with closely related plants across a large geographical area, and evaluated the contributions of geographical distance, topography, soil edaphic conditions and soil microclimate on AMF community assembly at local and regional scales. We collected 156 soil samples (10 cm to >548 km apart) from areas dominated by *Avena* spp. (Wild Oat) from three California Mediterranean grasslands. *Avena*-associated AMF communities were surveyed using 18S primers (WANDA/AML2).

**Results:** AMF communities differed significantly among grasslands; only 10% of the 1263 AMF OTUs were present at all three sites. AMF distance decay rates showed large differences between the three grasslands ( $z$  value 0.02-0.05), where high spatial turnover of environmental drivers (pH, texture, mineral nutrients, microclimate) was associated with higher distance decay. Null-model analysis revealed that selection (a relative importance of 23-43%), dispersal limitation (18-34%) and other stochastic phenomena (32-40%) are all important assembly processes shaping AMF communities in these grasslands. AMF taxa that likely produce larger spores had lower occupancy, supporting the hypothesis that spore size may be an important trait for AMF dispersal

**Discussion:** No single environmental driver was a predictor of AMF community assembly, and instead assembly was controlled by multiple ecological processes at the local scale.

**Conclusions:** This work advances our understanding of what controls AMF community assembly over local and regional scales.

**Keywords:** AMF, biogeography, arbuscular mycorrhizal fungi, community assembly, grasslands

**LT-030**

**Omics and Ecology**

**PATTERNS OF LOCAL ENDEMISM AND NICHE LIMITS OF AUSTRALIAN FOREST-TREE SYMBIOSES**

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**Introduction/Aim:**Australia is one of the most biologically unique countries in the world. Recent research has advanced our understanding of global patterns of mycorrhizal and nitrogen-fixing tree and root-associated microbe diversity, but an Australian perspective is lacking. We are mapping patterns of local endemism and assessing niche limits of mycorrhizal and nitrogen-fixing trees alongside their root symbiotic partners to highlight conservation priorities and identify key threats to tree symbiotic guilds in the context of global change.

**Materials and Methods:**Our research uses publicly available soil metabarcoding and tree distribution data, representing over 700 forest plots for soil microbes and 80,000 forest tree surveys covering 2,600 tree species. We focus on niche limit proxies for global change factors, namely nitrogen deposition (soil nitrogen), heat (mean temperature of the hottest period), drought (mean moisture index for the lowest moisture period), and fungal pathogen load (proportions of soil pathogens).

**Results:**We hypothesise that (1) distinct patterns of endemism will emerge between symbiotic microbes and their hosts; (2) the niche limits of arbuscular mycorrhizal trees will be narrower compared to nitrogen-fixing and ectomycorrhizal trees; and (3) dual-mycorrhizal trees will have the wider niche limits for trees due to their root plasticity potential.

**Discussion:**We expect that our result will not follow the ecological paradigms of the northern hemisphere.

**Conclusions:**By advancing our understanding of the complex interplay between Australian trees and their symbiotic partners, we will highlight conservation priorities and identify key threats to symbiotic guilds in the face of global change from a southern hemisphere perspective.

**Keywords:** Biogeography, forests, trees, fungi, endemism, niche-limits



**LT-031**

**Omics and Ecology**

**IMPORTANCE OF DROUGHT CONDITIONS IN STRUCTURING ROOT FUNGAL COMMUNITIES OF HEALTHY AND DECAYING JUVENILE BEECH TREES**

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**Introduction/Aim:** Soil fungal communities associated with trees play an essential role in forest ecosystems by providing water, nutrients, and protection against biotic and abiotic stress. However, it remains unclear how these communities respond to drought stress when associated with their host forest tree species.

**Materials and Methods:** At six sites across Switzerland severely affected by drought conditions, following a water stress gradient, and well characterized environmentally over the past 10 years, we tracked the fungal communities associated with healthy and decaying beech root systems using DNA metabarcoding.

**Results:** Fungal richness and diversity correlated positively with an increasing water stress and slightly between beech health status. Fungal communities were dominated by ectomycorrhizal (EcM) and saprotrophic fungi, and their relative abundance was altered by beech health status but not by drought. The EcM fungi *Lactarius* was significantly associated with drier sites and healthy beech root systems, while saprotrophic fungi were mainly associated with decaying beech. Fungal community assemblies were more deterministic in decaying beech than in healthy beech root systems. Specifically, the stochasticity of fungal communities' assembly increased with a rising water stress only for healthy beech, and was mainly driven by saprotrophs.

**Discussion:** Drought-related conditions rather than other environmental parameters and health status of the beech trees were the major drivers of fungal communities, even though their assembly processes differed between fungal functional traits.

**Conclusions:** This study highlights the importance of drought conditions for the assembly of fungal communities. It also points to an interplay between fungal functional traits and host health status when facing environmental constraints.

**Keywords:** Drought stress, fungal community, stochasticity, EcM, *Lactarius*

LT-032

## Omics and Ecology

### FUNGAL-AMF COMMUNITIES ARE MORE SENSITIVE TO FERTILIZATION AND PLANT GENOTYPE THAN BACTERIAL COMMUNITIES IN *SORGHUM BICOLOR*

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**Introduction/Aim:** Under climate change, environmental stressors like drought challenge crop productivity and yield stability. Sorghum forms associations with microbial partners to ameliorate the negative impacts of climate change.

**Materials and Methods:** We analyzed the effects of fertilization, plant genotype and plant age on the C utilization of rhizosphere microbial communities using Biolog plates. We also analyzed how these factors influence the composition and diversity of rhizosphere bacterial and fungal communities by sequencing the 16S and ITS2 regions using Illumina MiSeq PE300.

**Results:** We found that fertilization and plant genotype have minimal effects on the functional diversity of microbial communities in the rhizosphere. When looking at the sequencing data, we found that neither fertilization, plant genotype, plant age nor their interaction affected significantly bacterial Shannon diversity index and species richness. On the contrary, we found that the interaction between fertilization and plant genotype significantly affected fungal Shannon diversity and composition. A total of seven arbuscular mycorrhizal fungi (AMF) operational taxonomic units (OTUs) were detected in our data set comprising two families, Glomeraceae and Claroideoglomeraceae. Fertilization and plant genotype significantly affected the relative abundance of *Glomus* sp. while fertilization and plant age significantly affected the relative abundance of *Dominikia* sp.

**Discussion:** These results suggest that fungal-AMF communities are more responsive to fertilization and plant genotype compared to bacterial communities. This could be because plant-AMF interactions are dependent on the levels of nitrogen and phosphorus in the soil.

**Conclusions:** Future work will analyze sequence data using specific AMF primers to compare patterns between bacterial, total fungi and AMF communities.

**Keywords:** AMF, fertilization, plant genotype, sorghum bicolor, microbial communities

LT-033

## Omics and Ecology

### NEW INSIGHTS INTO THE TELOMERE-TO-TELOMERE REFERENCE GENOME OF THE UBIQUITOUS FOREST ECTOMYCORRHIZAL FUNGUS *CENOCOCCUM GEOPHILUM*

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**Introduction/Aim:** *Cenococcum geophilum* is one of the ectomycorrhizal fungi most frequently found on tree roots in temperate forests. Yet, the molecular mechanisms underlying its symbiosis and drought tolerance remain poorly understood.

**Materials and Methods:** We sequenced DNA of the *C. geophilum* reference strain 1.58, assembled the genome using PacBio HiFi and Hi-C technologies, and annotated it using the JGI annotation pipeline. We classified transposable element (TE) families and assessed their divergence times, and characterised TEs subject to repeat-induced point (RIPs) mutations. We searched for evidence of Starship elements with known captain-like genes.

**Results:** We recovered telomeres from each of the seven chromosomes and estimated the genome size at 178.5 Mbp. Approximately 80% of the genome was composed of repeats, a large proportion of which showed evidence of RIP mutations (~70%). We found that three TE families accounted for more than 50% of the assembly, including LTRs (46.1%), LINEs and DNA transposons (12.34%), which were intact and still active. We observed TE proliferation in two distinct periods, without assignment to specific TE families. Finally, we identified more than 500 captain-like genes that were presumably part of Starship fragments.

**Discussion:** Our outcomes demonstrate that *C. geophilum* genome expansion was driven by a few TE families over a long period. The predominance of the RIP mechanism highlights its effectiveness for low-frequency mating. The numerous Starship fragments may explain the presumed gene exchange between *C. geophilum* lineages.

**Conclusions:** This work highlights the extreme dynamics of the *C. geophilum* genome and calls for a deeper analysis of the currently being generated graph pangenome.

**Keywords:** *Cenococcum geophilum*, comparative genomics, element transposable, Starship, repeat induced point mutation

LT-034

## Omics and Ecology

### ARBUSCULAR MYCORRHIZAL-INDUCED GENE EXPRESSION IN THE LEAVES OF *SOLANUM TUBEROSUM* VARIETIES

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**Introduction/Aim:** Most potato symbiosis transcriptomics studies focus on root systems, yet systemic responses in the leaf maintain symbiosis by provision of a carbon source. The aim of this study was to elucidate potato leaf transcriptomics induced by arbuscular mycorrhizal fungal symbiosis in Sifra and Mondial varieties.

**Materials and Methods:** Using half-closed arbuscular mycorrhiza plant systems, Sifra and Mondial micro-propagated plantlets were inoculated with *Funniformis mosseae*. RNA was extracted from leaves of inoculated and un-inoculated control plantlets and used to construct cDNA sequence libraries. Sequencing was performed using Nanopore technology. Bioinformatics analyses of the sequences were performed using the nf\_core nanoseq pipeline launch version 3.1.0, followed by downstream processing using R software.

**Results:** Sifra had 24 Differentially expressed genes (DEGs), 8 upregulated and 16 downregulated. Similarly, 16 significant DEGs were identified in Mondial. 10 were upregulated and 6 downregulated. Pairwise comparisons between Sifra and Mondial were based on the top 100 genes. Sixty-nine DEGs were identified (34 upregulated and 35 downregulated). Most DEGs between Mondial and Sifra were involved in abiotic and biotic stress.

**Discussion:** Inoculation induced the expression of HSP20-like chaperones, Protein kinase superfamily protein, NIN like protein, lipoxygenase, NB-ARC domain-containing disease resistance protein and MAPK/ERK kinase kinase. This indicates that *F. mosseae* can prime Sifra and Mondial plants against biotic and abiotic stress. Upregulation of genes involved in metabolites with anti-cancer properties illustrate the potential of *F. mosseae* to improve human health.

**Conclusions:** Overall, the study highlights the benefits of arbuscular mycorrhizal fungi on plant fitness and enhanced production of beneficial secondary metabolites.

**Keywords:** Gene expression, potato varieties, disease resistance, secondary metabolites

LT-035

## Omics and Ecology

### COMPARATIVE RNA SEQUENCING-BASED TRANSCRIPTOME PROFILING OF TEN GRAPEVINE ROOTSTOCKS: SHARED AND SPECIFIC SETS OF GENES RESPOND TO MYCORRHIZAL SYMBIOSIS

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**Introduction/Aim:**Arbuscular mycorrhizal symbiosis (AMS) significantly enhances water and nutrient uptake in plants while providing various ecosystem benefits. Among the world's major crops, grapevine stands prominently. Grapevine scions are commonly grafted onto diverse rootstocks, which impart varying degrees of resistance against pests, resilience to environmental stress, and influence scion physiology. Yet, the precise regulatory mechanisms and transcriptomic adaptability of rootstocks in response to mycorrhization remain elusive.

**Materials and Methods:**In our study, we conducted RNA sequencing-based transcriptome profiling to compare the responses of ten different grapevine rootstocks to AMS facilitated by *Rhizophagus irregularis*. We further examined the expression levels of these genes, comparing them to over 2000 transcriptome profiles from diverse grapevine varieties and tissues as well as transcriptomic data from *Medicago truncatula* roots.

**Results:**While uncovering shared regulatory mechanisms, we also identified rootstock-specific responses to Ri colonization. Notably, a set of 353 genes exhibited regulation by AMS across all ten rootstocks..

**Discussion:**We compared the response of the 351 genes upregulated by mycorrhiza in grapevine to their counterparts in *Medicago truncatula*, based on existing transcriptomic studies on mycorrhizal colonization. Remarkably, over 97% of the grapevine genes homologous to *M. truncatula* were expressed in at least one mycorrhizal transcriptomic study, with 64% observed across all RNAseq datasets

**Conclusions:**At the intra-specific level, we identified both shared and unique grapevine rootstock genes responsive to Ri symbiosis for the first time. At the inter-specific level, we delineated a shared subset of mycorrhiza-responsive genes, shedding light on the intricate dynamics of plant-fungal symbiotic interactions.

**Keywords:** Grapevine rootstock, *Rhizophagus irregularis*, RNA-sequencing, Transcriptome profiling, gene expression atlas

LT-036

## Omics and Ecology

### TRANSCRIPTOMIC INSIGHTS IN ABILITIES OF ORCHID MYCORRHIZAL FUNGI TO ASSIMILATE NITRATE

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**Introduction/Aim:** Fungi belonging to the polyphyletic Rhizoctonia group (Tulasnellaceae, Ceratobasidiaceae, Serendipitaceae) are primarily soil saprotrophs, yet they also form symbiotic relationship with orchid seeds, stimulating germination and seedling growth. Orchid tissues are typically rich in nitrogen, which likely originates from fungi and serves as a key nutrient for orchid growth. While Ceratobasidiaceae have been shown to utilize both organic and inorganic nitrogen forms, including ammonium and nitrate, some Serendipitaceae and possibly all Tulasnellaceae lack a nitrate assimilation system. Nevertheless, few studies have explored nitrate metabolism in rhizoctonian isolates. Here we investigated the transcriptome of nine rhizoctonian strains obtained from orchid roots, comparing nitrate assimilating and non-assimilating strains.

**Materials and Methods:** The strains were cultivated on a medium enriched with nitrate (0.32 gL<sup>-1</sup>) and on water agar as a control. RNA from the mycelial biomass was analysed by RNA-Seq, followed by *de novo* assembly.

**Results:** Four strains showed significantly higher fungal biomass under nitrate conditions compared to the control. These four strains contained the genes related to nitrate assimilation, such as ammonium transporter (MEP), nitrite reductase (NIT6), nitrilase (NIT1), nitric oxide synthase (NOSIP), nitrate reductase (NR), and nitrate/nitrite transporter (NRT2). Conversely, nitrate non-assimilating strains showed only partial expression of these genes, as MEP, NIT1 and NOSIP.

**Discussion:** Nitrate assimilation involves the reduction of nitrate to nitrite by NR and further reduction to ammonium by NIT6. The absence of these genes in non-assimilating strains suggests a breakdown in this process.

**Conclusions:** Additionally, both the ability and incapacity to assimilate nitrate occurs across all three families, contradicting current assumptions.

**Keywords:** orchid mycorrhizal fungi, nitrate assimilation, functional genes, Tulasnellaceae, Ceratobasidiaceae, Serendipitaceae

**LT-037**

**Omics and Ecology**

**VARIATION IN ARBUSCULAR MYCORRHIZAL ASSOCIATIONS LEADS TO DIVERGENT RHIZOSPHERE CHEMISTRY AND SPECIFIC PLANT-SOIL FEEDBACK**

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**Introduction/Aim:**Species-specific feedback between plants and soil microbial communities is an important driver of vegetation dynamics and productivity. Arbuscular mycorrhizal fungi colonise the roots of most terrestrial plants but are expected to have little impact on specific feedback due to their low host specificity.

**Materials and Methods:**Here we tested if variability in mycorrhizal association strength and associated rhizosphere chemistry among co-existing temperate grassland plant species can lead to species-specific feedback.

**Results:**We detected more divergent plant-soil feedback in the presence than in the absence of mycorrhizal fungi, particularly among species with contrasting mycorrhizal association strengths.

**Discussion:**The plant species that produced the most positive, self-promoting feedback was characterized by a strong shift in rhizosphere metabolites related to stress and immune responses, while the metabolomes of species with neutral to negative feedback were unresponsive to mycorrhizal fungal presence.

**Conclusions:**Thus, mycorrhizal fungi can generate divergent plant-soil feedback, which can be predicted from species-specific changes in rhizosphere metabolome.

**Keywords:** arbuscular mycorrhizal, metabolomics, plant-soil feedback, root exudates

LT-038

## Omics and Ecology

### DIVERSITY OF FUNGAL ENDOSYMBIONTS IN NATURAL PLANT POPULATIONS IN CONTRASTING HABITATS WITHIN PEAK DISTRICT NATIONAL PARK, UK

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**Introduction/Aim:** Mucoromycotina ‘fine root endophytes’ (MFRE) are a recently characterised group of mycorrhizal fungi, known to contribute to plant nutrition. This could be important across different habitats; however, little is currently known about the natural diversity, distribution, and host and habitat range of these fungi.

We aimed to address this by determining frequency, diversity, host range, and relatedness of both arbuscular mycorrhizal fungi (AMF) and MFRE in wild plants at a landscape scale utilising natural edaphic gradients across the Peak District National Park, UK.

**Materials and Methods:** We quantified relatedness and interactions between AMF and MFRE fungal communities within and between habitats, including diverse grasslands, moorlands, and woodlands, characterising the host range and habitat preferences of these fungal groups. We sampled roots from *Trifolium repens*, *Trifolium pratense*, *Ranunculus repens*, *Plantago lanceolata*, *Holcus lanatus*, and *Juncus effusus* at four sampling time points to reveal seasonal fungal signatures, host preference, and impact of nutrient status and management on plant-fungal interactions at contrasting sites.

**Results:** We found distinct MFRE and AMF populations at each site. Further analysis will reveal how the fungal communities relate to soil chemical properties, and whether plant or fungal species are selective when choosing their symbiotic partners in complex, natural ecosystems.

**Discussion:** Characterising and understanding plant-fungal interactions on a landscape scale is an important step in understanding functional and evolutionary significance of MFRE symbiosis, and their potential resilience in a rapidly changing world.

**Conclusions:** Our findings provide new insights into the importance of MFRE communities and their relationship with a diverse range of plants.

**Keywords:** Mucoromycotina ‘fine root endophytes’, endosymbiosis, fungal diversity, amplicon sequencing



LT-039

## Omics and Ecology

### IMPROVED CHARACTERISATION OF MYCORRHIZAL FUNGAL COMMUNITIES USING NEW PRIMERS AND A COMBINATION OF ILLUMINA AND LONG-READ SEQUENCING

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**Introduction/Aim:**Characterising mycorrhizal fungal communities has always been challenging. It's often considered this cannot be done using classical fungal ITS primers as they underrepresent the diversity of certain groups, especially the arbuscular mycorrhizal fungi (AMF) and mucoromycotina fine root endophytes (MFRE) due to primer site sequence divergence.

**Materials and Methods:**We investigated the specificity of commonly used fungal 18S and ITS primers and designed a new primer set with predicted broader coverage in silico, which we tested on field-collected DNA extracts using Illumina sequencing. Furthermore, we used long-read sequencing (PacBio) to pair 18S sequences with ITS types to merge phylogenetic certainty (18S) with high resolution of subpopulations (ITS).

**Results:**We found that a large pool of Illumina amplified sequence variants (ASVs) were not identified to phylum level using standard fungal databases. Pairing ITS regions with 18S using long-read sequencing allowed assignment of a large proportion of previously unassigned reads to phylum level. Unassigned sequence numbers varied between primer sets. The new primers included a relatively high proportion of MFRE and AMF sequences not captured by existing primers.

**Discussion:**Primer choice can heavily bias fungal community characterisation, by omitting previously undescribed subgroups of understudied phyla. This can undermine efforts to understand the true importance and function of the mycobiome.

**Conclusions:**We found that primer choice affects MFRE amplification, and suggest a new, more inclusive primer set, and a method for assigning ITS types to phylum level, further improving available database annotations.

**Keywords:** Fungal diversity, amplicon sequencing, long read sequencing, Mucoromycotina fine root endophytes

**LT-040**

**Omics and Ecology**

**META-TRANSCRIPTOMICS REVEALS FUNCTIONAL DIFFERENTIATION OF MYCORRHIZAL AND ENDOPHYTIC FUNGI ON QUAKING ASPEN**

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**Introduction/Aim:** Mycorrhizal and endophytic fungi expand the metabolic capabilities of their plant hosts by expressing unique molecular functions. However, it is not known whether fungi co-occurring in a community express a core set of shared molecular functions or alternatively exhibit functional complementarity.

**Materials and Methods:** We used meta-transcriptomics to construct gene profiles of 135 root-associated fungal communities of quaking aspen (*Populus tremuloides*) across a spatiotemporal gradient in semi-arid natural forests in the Uinta Mountains, Utah, USA. High throughput meta-transcriptomic RNA sequencing was used to generate a de novo assembled gene catalog that was functionally and taxonomically annotated. Functional annotations identified genes potentially involved in symbiotic signaling as well as those involved in soil organic matter modification and nutrient acquisition.

**Results:** Results demonstrate that plant-symbiotic fungi express a core set of genes, but also bring unique functions that are strongly structured by fungal evolutionary history.

**Discussion:** Community functional diversity strongly depends on fungal taxonomic diversity, which indicates that loss of fungal symbiotic diversity could leave plants with a reduced metabolic capacity that may hinder its ability to persist under variable environmental conditions. Turnover in gene functional profiles across the dataset was primarily due to turnover in community composition and to a lesser extent by gene regulatory mechanisms. Fungal communities were generally stable across time, but showed strong habitat specificity.

**Conclusions:** Our data provide a rare window into the community molecular functioning of plant-associated fungi in their native habitat.

**Keywords:** Meta-transcriptomics, functional differentiation, mycorrhizal, endophytic fungi, quaking aspen

**P-072**

**Omics and Ecology**

**NON-DESTRUCTIVE IMAGING AM FUNGAL-PLANT INTERACTIONS IN ABOVEGROUND PLANT TISSUES**

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**Introduction/Aim:**Arbuscular mycorrhizal (AM) fungi are known to alter plant metabolite content both above and belowground. Previous studies have identified at least one metabolite (blumenol-B) known to correlate with AM fungi, and compounds like blumenols have a ring structure that should be detectable when excited by a laser by Raman spectroscopy. Our aim was to identify compounds that vary with AM fungal colonization in aboveground plant tissues using Raman spectroscopy.

**Materials and Methods:**We grew multiple plant species (tomato, little bluestem grass, lettuce, and onion) with and without AM fungi in a series of experiments and imaged the leaves using Raman spectroscopy. In some experiments we applied plant stresses to determine if this would alter plant metabolism. In addition, we synthesized blumenols and observed blumenols and other common plant metabolites (carotenoids, chlorophyll, etc.) using Raman spectroscopy.

**Results:**Plants colonized by AM fungi display different Raman spectra, and changes in spectra are related to the abundance of carotenoids. To confirm this we assessed carotenoid concentrations using traditional methods and Raman spectroscopy. Carotenoid responses due to AM fungi can be observed in multiple plant species under stress. AM fungal community composition also alters carotenoid responses.

**Discussion:**Spectra of AM fungal and non-AM fungal colonized host plants are dominated by carotenoids and chlorophyll which may obscure other less abundant molecules (e.g., blumenols). AM fungi consistently influence carotenoids, however variation in this influence occurs with stress and AM fungal colonization.

**Conclusions:**AM fungi influence the Raman spectra of colonized plants regardless of plant species.

**Keywords:** carotenoid, blumenol, Raman spectroscopy, stress, AM fungal colonization

**P-073**

**Omics and Ecology**

**SOIL PATHOGENIC FUNGI, BUT NOT ARBUSCULAR MYCORRHIZAL FUNGI, COINCIDE WITH NON-NATIVE PLANT SPECIES IN PARTIALLY RESTORED MONTANE GRASSLANDS**

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**Introduction/Aim:**Inoculation with soil containing native arbuscular mycorrhizal fungi (AMF) has successfully supported restoration of some grasslands. However, other microorganisms like pathogenic fungi are also present in soils and may counter or supersede facilitation by AMF during restoration. In Banff National Park (BNP), Canada, restoration of montane grasslands has been stalled by the presence of non-native plant species. We hypothesized that a distinct, species-poor community of AMF would coincide with the dominance of non-native plants in these partially restored sites.

**Materials and Methods:**We surveyed plant and fungal community composition of partially restored sites and those representing restoration targets, namely native grasslands and savannah-type ecosystems in the Park. In addition to AMF, we also characterized soil pathogens by sequencing the rDNA regions SSU and ITS1, respectively.

**Results:**We found higher plant species diversity in native grasslands compared to partially restored sites. AMF diversity and community composition did not differ between native grasslands and partially restored sites. Diversity of soil pathogens was higher in partially restored sites than in native grasslands and savannahs. Soil pathogen composition in partially restored sites differed from both restoration target ecosystems and coincided with the composition of the different plant communities.

**Discussion:**In the montane grasslands of Banff National Park, AMF appear less coupled than pathogenic fungi to plant community composition.

**Conclusions:**Soil pathogens, and not AMF, may underlie the persistence of non-native plants in partially restored sites.

**Keywords:** soil pathogens, grassland restoration, non-native plants, montane grasslands, inoculum

P-074

## Omics and Ecology

### LIGHT INTENSITY ALTERS SELECTION STRATEGY OF JUVENILE C<sub>4</sub> GRASS FOR ARBUSCULAR MYCORRHIZAL FUNGAL PARTNERS

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**Introduction/Aim:** Frequent disturbance selects compatible plants and arbuscular mycorrhizal (AM) fungi to facilitate the rapid establishment of vegetation in harsh environments. Cost of AM fungi may affect establishment success of juvenile plants, particularly under the canopy of adult plants that restrict their photosynthesis. Mycorrhizal cost may vary with the colonization strategies of the fungi, e.g., between those that colonize via soil propagules (SP fungi) and hyphal networks [network-competitor (NC) fungi]. We hypothesized that juvenile plants preferentially associate with NC fungi over SP fungi because their carbon cost could partially be covered by the original (adult) host.

**Materials and Methods:** Intact root-soil-core samples (NC inocula) and sieved root-zone-soil samples (SP inocula) were collected from coastal sand dunes dominated by the C<sub>4</sub> grass *Miscanthus sinensis*. *M. sinensis* seedlings were grown for 2 months either with the NC or SP inoculum to define colonization strategy of individual OTUs or with both (NC+SP) to compare competitiveness between the two strategists with or without shading. Fungal LSU rDNA was amplified from DNA extracted from the roots and sequenced.

**Results:** Juvenile plants showed no preference for NC fungi but shading increased genus richness and altered community composition at the genus level. Several SP fungi of *Dominikia*, *Archaeospora*, *Scutellospora*, and *Claroideoglossum* occurred preferentially under the shade.

**Discussion:** These observations indicate that host photosynthetic activity alters competitiveness of AM fungi in the community.

**Conclusions:** Under the canopy of adult plants, juvenile plants may have no preference for fungal colonization strategies, but rather can select low-cost AM fungi.

**Keywords:** arbuscular mycorrhizal fungi, colonization strategy, mycorrhizal cost, light intensity, network competitors, soil propagules

P-076

## Omics and Ecology

### DEPICTING THE MYCORRHIZAL COMMUNITY WITHIN THE RHIZOSPHERE OF PLANTS IN A NEOTROPICAL DRY FOREST

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**Introduction/Aim:** Arbuscular Mycorrhizal Fungi (AMF) can be used to mitigate water stress in cultivated plants, especially when this AMF are adapted to drought. Thus, our aim was to characterize AMF community within the rhizosphere of native plants as part of a bioprospecting initiative aimed at identifying microbes capable of enhancing plant resilience to water stress.

**Materials and Methods:** We utilized high-throughput sequencing of the partial 18S rRNA gene to characterize the AMF in the rhizosphere of *Neoglaziovia variegata* (commonly known as 'caroa') and *Tripogonella spicata* (referred to as the resurrection plant). Sampling was conducted within the Caatinga biome, a neotropical dry forest situated in northeastern Brazil.

**Results:** Alpha diversity analyses revealed that *T. spicata* exhibited superior richness and diversity, as indicated by the Observed ASVs and Shannon index, respectively. Conversely, *N. variegata* showcased a higher level of modularity within its mycorrhizal network compared to *T. spicata*. Among the detected genera, *Glomus* emerged as the most prevalent in both plants, followed by *Gigaspora*, *Acaulospora*, and *Scutellospora*, each exceeding a 10% abundance threshold. Remarkably, *Gigaspora*, *Diversispora*, and *Ambispora* were exclusively identified within the rhizosphere of *N. variegata*, while *Scutellospora*, *Paraglomus*, and *Archaeospora* were unique to *T. spicata*'s rhizosphere.

**Discussion:** The mycorrhizal fungal community within the rhizosphere of each plant exhibits distinct compositional, structural, and modular attributes.

**Conclusions:** In conclusion, it seems that these differentiation could differentially assist plants in the hostile environment.

**Keywords:** fungal ecology, drought, bioprospection, semi-arid

**P-077**

**Omics and Ecology**

**DIFFERENTIAL FUNGAL NICHE MODIFICATION ALTERS ECTOMYCORRHIZAL FUNGAL COMMUNITY ASSEMBLY DURING NECROMASS DECOMPOSITION**

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**Introduction/Aim:** Dead ectomycorrhizal (ECM) fungal mycelium (hereafter called necromass) harbors a unique set of fungi during its decay process. Yet, ECM fungi are more abundant at later stages of decomposition. To understand the impact of an early-arriving species (i.e., priority effects) on the ECM fungal community associated with decaying necromass, we deployed a laboratory- and field-based experiment using the ECM fungus *Hyaloscypha bicolor* necromass.

**Materials and Methods:** We used mesh bags to pack 100 mg of freeze-dried necromass and incubated them in 250 mL microcosms filled with sterilized soil. For the laboratory experiment, two fungal strains (*Trichoderma* and *Metarhizium*) were added to the microcosms in single and paired inoculations and harvested after two- and four weeks. For the field experiment, necromass was pre-colonized with a fungus for two weeks. Then all bags were buried in a *Pinus strobus* forest and harvested after four-, eight-, and 12 weeks.

**Results:** At week four, necromass pre-colonized by *Metarhizium* was more acidic, had a higher water content, and had more mass remaining relative to necromass pre-colonized by *Trichoderma*. These effects on necromass remained in the field after four and eight weeks before attenuating at week 12.

**Discussion:** *Tomentella* (ECM genus) was more abundant after eight weeks in necromass pre-colonized by *Trichoderma*. Yet, pre-colonization of necromass by either fungus significantly reduced the alpha- and beta-diversity of ECM fungi after three months post-deposition.

**Conclusions:** Collectively, our results indicate that strong priority effects during necromass decomposition are caused by niche modification and can alter the ECM fungal community in the field.

**Keywords:** priority effects, ectomycorrhizal necromass, niche, decomposition

P-078

## Omics and Ecology

### TOWARDS NEW GROUP-SPECIFIC PRIMERS FOR LONG-READ METABARCODING OF ARBUSCULAR MYCORRHIZAL FUNGI

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**Introduction/Aim:**Arbuscular mycorrhizal fungi (AMF) are key participants in terrestrial habitats through their interactions with vascular plants. While their association with plants indicates a near-omnipresent distribution, surveying these organisms on a large scale is a challenge. One approach to do so is to use metabarcoding of soil or plant host samples to assess their distribution. However, commonly applied rDNA metabarcoding region(s) for AMF include limited phylogenetic information. In this study, long-read sequencing of a longer stretch of the rDNA operon is investigated as a potential improvement to increase taxonomic resolution and detectability.

**Materials and Methods:**New group-specific primers for AMF have been developed and will be used in a larger downstream survey to investigate the distribution of AMF across major environmental gradients in Norway, including both natural and anthropogenically influenced habitats.

**Results:**Results from the preceding in-silico evaluation of the new AMF primers, as well as long-read sequence data from mock communities evaluating the usability of the primers in-vitro (PCR), will be presented. In addition, we will present initial analyses of long-read sequences from environmental samples.

**Discussion:**The performance of the new long-read AMF-specific primers will be compared against existing long- and short-amplicon primers and their usability will be discussed.

**Conclusions:**Long-read sequencing using novel group-specific primers provides an exciting opportunity to generate more phylogenetically informative data for AMF from environmental samples.

**Keywords:** AMF, metabarcoding, long-read sequencing, primer testing



P-079

## Omics and Ecology

### INVESTIGATING THE *TUBER BORCHII* AND *SUILLUS COLLINITUS* PAN-GENOMES FOR INSIGHTS INTO LOCAL-SCALE ADAPTIVE EVOLUTION

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**Introduction/Aim:** Ectomycorrhizal fungi play a crucial role in forest ecosystems, including carbon cycling and dynamics. Despite their ecological importance, the genetic and functional diversity of these fungi remains largely unexplored. This study aimed to elucidate the genomic signatures of local adaptation among ectomycorrhizal fungi with varying phenotypes across different habitats.

**Materials and Methods:** As part of a research collaboration with the DOE Joint Genome Institute (proposal: 10.46936/10.25585/60000526), our study focuses on whole genome re-sequencing of a set of isolates from two widespread ectomycorrhizal fungal species, *Tuber borchii* and *Suillus collinitus*, collected from diverse microclimatic soils. Sequences were aligned to the available genomes of *T. borchii* and *S. collinitus*, allowing us to identify gene sequence variations, including single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels). Using these data, we were able to perform population genomic analysis.

**Results:** Population genomic analysis highlighted the allelic diversity of functional genes in the two fungal species, including Carbohydrate-Active enZymes (CAZyme) genes, crucial for carbon processing in forest soils.

**Discussion:** The results from our study are providing novel insights that help decipher the pan-genomes of ectomycorrhizal fungi within the same habitat and their genomic adaptations to local environments.

**Conclusions:** Overall, understanding genomic signatures of adaptation not only advances our knowledge of ectomycorrhizal fungi functional diversity but also informs conservation strategies and forest management practices in the face of climate change.

**Keywords:** ectomycorrhizal fungi, genomics, adaptive evolution, SNPs, InDels

P-080

## Omics and Ecology

### IMPACT OF *RHIZOPHAGUS CLARUS* AND PLANT GROWTH-PROMOTING BACTERIA INOCULATION ON THE ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITY OF BEAN ROOTS IN BRAZIL

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**Introduction/Aim:** Inoculation with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPB) are increasingly being implemented in agriculture for improving crop health and productivity. However, the interactions between inoculants and the resident microbiota are not yet fully understood. Therefore, the aim of this study was to explore the impact of AMF and PGPB inoculation on the native AMF community in bean (*Phaseolus vulgaris* L.) roots.

**Materials and Methods:** Bean seeds were sown in an agricultural area in the Brazilian savanna region in 2023. Plants were inoculated with individual PGPR species (*Azospirillum brasilense*, *Burkholderia vietnamiensis*, and *Nitrospirillum amazonense*), with half of them also receiving *Rhizophagus clarus* inoculum. The experiment was set as a randomized block design. Two months later, root samples were collected and root AMF communities were evaluated following a metabarcoding approach.

**Results:** Inoculation of *R. clarus* significantly influenced the presence of other AMF taxa, notably leading to an increase in the relative abundance of *Redeckera* sp. and *Kamienskia* sp. in roots. Inoculation with PGPR also affected the relative abundance of AMF in bean roots, resulting in a general increase in *Claroideoglossum* abundance.

**Discussion:** Furthermore, *N. amazonense* inoculation enhanced the presence of *Microdominikia* in bean roots, although the combined inoculation of *R. clarus* and *N. amazonense* moderated this increase. Interestingly, in this treatment, there was an increase in the relative abundance of *Gigaspora margarita*.

**Conclusions:** Overall, these findings highlight the complexity of microbial interactions in agricultural systems and contribute to unveil the dynamics between these beneficial microorganisms and their impact on plant health and productivity.

**Keywords:** Glomeromycota, community composition, *Phaseolus vulgaris*, inoculation, savanna region

P-081

## Omics and Ecology

### BEYOND THE HOST - MULTISCALE DETERMINANTS OF ARBUSCULAR MYCORRHIZAL COMMUNITIES IN LOWLAND NEOTROPICAL FORESTS OF FRENCH GUIANA

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**Introduction/Aim:**The biogeography of arbuscular mycorrhizal fungi (AMF) is not well understood in tropical forests that are characterized by high local and global diversity of AMF-associated tree species. Some tree species may be distributed frequently at the regional scale, but not be locally dominant and could host specialized AMF communities, such as Fabaceae species in French Guiana. Considering potential local environmental variation and isolation of trees, both environmental (edaphic) and geographical distance may contribute to selection of AMF, beyond the host. The importance of these factors may differ between AMF communities in roots versus rhizosphere soil and relative to fungal and prokaryote communities.

**Materials and Methods:**Root and rhizosphere soil samples were collected from 84 individuals of *Dicorynia guianensis* (Fabaceae) across seven sites (up to 180 km distance) in French Guiana. Community composition of AMF, fungi and prokaryotes were assessed using amplicon sequencing, and soil physicochemical properties measured.

**Results:**High local and regional diversity of AMF, especially on roots, was found. AMF communities were not shaped by geographical distance at a regional scale, and only influenced by local differences in soil parameters when associated with roots. A similar pattern was observed for fungal and prokaryote communities.

**Discussion:**These results highlight the high diversity of AMF associated with a single host tree species in the Neotropics and question the drivers of local below-ground microbial diversity and dispersal abilities at a regional scales.

**Conclusions:**This first regional scale study of AMF diversity in French Guiana begs further consideration of both neutral and deterministic factors hidden beyond the host.

**Keywords:** Arbuscular mycorrhizal fungi, biogeography, distance decay, Neotropics, soil

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## Omics and Ecology

### GENE EXPRESSION CHANGES IN A DROUGHT-TOLERANT *CENOCOCCUM GEOPHILUM* STRAIN DURING THE STRESS AND RECOVERY PERIODS

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**Introduction/Aim:** Drought stress poses considerable challenges to forest ecosystems. The ectomycorrhizal fungus *Cenococcum geophilum* is commonly found in ecosystems experiencing environmental stress. Previous research has demonstrated that *C. geophilum* mycelium exhibits greater resilience to drought than other ectomycorrhizal fungi and enables host growth under stressful conditions. However, there are still gaps in our understanding of the mechanisms underlying *C. geophilum* recovery from drought stress.

**Materials and Methods:** The drought-tolerant *C. geophilum* isolate 1.093 was chosen for transcriptome analysis. We used polyethylene glycol (PEG) to mimic the effects of drought. Mycelia growing in medium with and without PEG (-1.5 MPa) were sampled at 6h, 24h, and 48h. After sampling part of the cultures, the remaining mycelia were transferred to no-PEG medium for recovery for 24h and 48h. Triplicate samples were snap-frozen, total RNA was extracted and sequenced using a 150bp pair-end Illumina platform.

**Results:** PEG treatment produced significant changes in gene expression as early as six hours after treatment. At each time point, a distinct group of genes was highly expressed, but a common set of 150 transcripts was identified at all time points, including many transporter genes or stress-related genes, such as genes encoding heat-shock proteins, oxidoreductases, and glutathione-transferases, all of which quickly returned to the control expression levels after transfer to the no-PEG medium.

**Discussion:** *C. geophilum* isolate 1.093, known for its drought tolerance, exhibited significant alterations in gene expression in response to drought conditions.

**Conclusions:** Remarkably, this isolate has demonstrated the ability to quickly regain its viability upon transitioning back to favorable conditions.

**Keywords:** *Cenococcum geophilum*, drought, ectomycorrhiza, transcriptome

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**Omics and Ecology**

**ARBUSCULAR MYCORRHIZAL FUNGI AND THE TROPICAL RAIN FOREST REGENERATION**

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**Introduction/Aim:** Tropical rain forest regeneration (TRFReg) is a very complex process defined by changes along time of plant community composition and abiotic factors that occurred after a gap canopy opening, considering that above and belowground constituents, especially arbuscular mycorrhizal fungi (AMF), are entangled we expected that after gap formation, aboveground changes will have consequences on the AMF component. Our main goal was to analyse AMF community responses along TRFReg in Los Tuxtlas, Veracruz.

**Materials and Methods:** We selected 15 gaps of consecutive ages, and three undisturbed sites (MTRF). Our response variables were: plant and AMF species richness and composition, AMF mycelia, colonization, and biomass, and soil abiotic variables.

**Results:** We recorded 51 AMF species, AMF composition was not correlated with gap age. We found that AMF species richness had the lowest values in the early stage (E). AMF spore communities together with mycelia and col% varied among regeneration stages, regardless the stage. E sites had low NH<sub>4</sub> and MTRF sites had the lowest total colonization values and the highest plant species richness.

**Discussion:** We did not find a pattern among plant community and AMF responses along TRFReg, probably because of the low canopy openness impact on soil, soil matrix acts as a buffer, and regardless the aboveground consequences, soil organisms are not strongly disturbed; however we have to be aware that our study corresponds to the first phase of the TRFReg, and may be in the long term will be different.

**Conclusions:** During first stages of TRF regeneration, above and belowground compartments function independently among each other.

**Keywords:** Successional stages, belowground component, AMF functional responses, tropical rainforest

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## Omics and Ecology

### THE INFLUENCE OF SOIL PHOSPHORUS, PLANT HOST AND SEASON ON THE DIVERSITY AND COMPOSITION OF GLOMEROMYCOTINIAN AND MUCOROMYCOTINIAN ARBUSCULAR FUNGI

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**Introduction/Aim:**Traditionally, all arbuscular mycorrhizal fungi were thought to belong to the Glomeromycotina, a diverse group of well-described plant growth-promoting symbionts. In recent years 'Fine Root Endophytes' of the Mucoromycotan subphylum Mucoromycotina have provided evidence of a morphologically distinct group of arbuscular fungal symbionts. Initial evidence has indicated that this newly identified symbiosis may act similarly to those formed by Glomeromycotinian fungi. However, while important to plant microbiome diversity, function and health they remain poorly described.

**Materials and Methods:**This project compared the drivers of Glomeromycotinian and Mucoromycotinan community composition under differing environmental conditions at the 'Cowlands Long-term Experimental Site' Wexford, Ireland. Here natural grazing conditions have been maintained since 1968, with varied annual applications of phosphorus in trial plots. Root samples were collected from *Trifolium repens* and *Lolium perenne* from low (0kg/ha/yr) and high (30kg/ha/yr) phosphorus treatment plots across 4 time points and 18S rRNA sequencing used to investigate diversity and composition of each fungal group.

**Results:**While occupying similar niches, Glomeromycotinian and Mucoromycotinan fungi exhibited distinct responses to soil nutrients. While drivers of the two communities were largely comparable, the impact of phosphorus was not. Elevated phosphorus concentration was linked to a direct reduction in Glomeromycotan richness, but had no effect on Mucoromycotinan richness. For both groups of fungi other soil nutrients (N, Ca, S, Fe etc) had direct positive effect on richness. Plant host and time had limited effects on either fungal group's composition.

**Discussion:**

**Conclusions:**We conclude that while generally occupying similar niches, Glomeromycotinan and Mucoromycotinan fungi exhibit distinct responses to soil nutrients.

**Keywords:** Mucoromycotina, Glomeromycotina, Phosphorus, Arbuscular, Mycorrhiza

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## Omics and Ecology

### SEASONS CHANGE AND SO DO FUNGI: A REVIEW OF ECTOMYCORRHIZAL FUNGAL PHENOLOGY

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**Introduction/Aim:** Ectomycorrhizal fungi (EMF) are key members of forest ecosystems where they associate with the fine roots of many dominant tree species. EMF facilitate tree establishment, resilience, and survival and impact ecosystem-scale biogeochemical cycles. Investigations into EMF phenology offer opportunities to address important questions involving fungal life cycles and how seasonal shifts in EMF community structure and function impact hosts and entire ecosystems.

**Materials and Methods:** This review synthesizes reports regarding the timing of ectomycorrhizal and mycelial production, seasonal changes in diversity and function, and how EMF phenology is responding to global change. We then provide a framework for predicting EMF phenology that includes soil abiotic conditions, host effects, and key fungal traits as drivers of EMF phenological patterns.

**Results:** The timing in EM root tip, extramatrical mycelium, and fruitbody production appear to be largely decoupled, but are generally highest during the warm growing season. The timing of each of these structures can vary depending on EMF species and biome. EMF community richness and activity are also highest during the summer and early fall seasons, but some EMF species persist and remain active during the winter and spring months.

**Discussion:** Moving forward, high resolution sampling collections followed by community sequencing and measurements of EMF traits should be recorded throughout the year including periods that fall outside of the growing season typically defined by aboveground activity.

**Conclusions:** Because EMF phenology exhibits distinct seasonal peaks, disentangling the effects of different abiotic and biotic factors causing this seasonality will help in better predicting EMF impacts on hosts and ecosystem processes.

**Keywords:** mycorrhizal, phenology, trait, productivity, forest

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## Omics and Ecology

### ASSESSING THE INFLUENCE OF FORESTRY PRACTICES AND INTERANNUAL VARIATION ON ECTOMYCORRHIZAL FUNGAL COMMUNITY COMPOSITION IN AN OAK-HORNBEAM FOREST

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**Introduction/Aim:**The purpose of this study was to better understand the effects of forestry treatments (clear-cutting, gap-cutting, preparation-cutting, tree retention in clear-cut areas, and control) on the diversity and community composition of ectomycorrhizal fungi in an oak-hornbeam forest in northern Hungary. We sampled soil in all 30 plots of the Pilis Forestry Systems Experiment, operated by the Ecological Research Centre and the Pilis Park Forest Ltd., in the autumn of 2020 and 2021, 6 and 7 years after the treatments were implemented.

**Materials and Methods:**We performed ITS2 rDNA metabarcoding of the soil-borne fungal communities, completed bioinformatic analyses, and assigned the resulting high-quality fungal sequences to taxonomic and functional groups. For this presentation, we compared the richness and community composition of ECM fungi among forest treatment types and between years using analysis of variance for alpha diversity and nonmetrical multidimensional scaling and permutational analysis of variance for beta diversity analyses.

**Results:**We performed ITS2 rDNA metabarcoding of the soil-borne fungal communities, completed bioinformatic analyses, and assigned the resulting high-quality fungal sequences to taxonomic and functional groups.

**Discussion:**For this presentation, we compared the richness and community composition of ECM fungi among forest treatment types and between years using analysis of variance for alpha diversity and nonmetrical multidimensional scaling and permutational analysis of variance for beta diversity analyses.

**Conclusions:**Treatment was partly explained by altered environmental variables, such as soil water content, microclimate (predominantly relative humidity and upper soil temperature), understory vegetation, and distance from the plot to surrounding ECM host trees, while interannual variation may partly be caused by differing weather conditions during growing season, as the year 2021 was noticeably drier than 2020.

**Keywords:** ectomycorrhizal, fungal, oak-hornbeam, interannual variation



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## Omics and Ecology

### DIFFERENCES IN MYCORRHIZAL FUNGAL COMMUNITY BETWEEN TERRESTRIAL AND EPIPHYTIC ORCHIDS IN YAKUSHIMA-ISLAND, JAPAN

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**Introduction/Aim:** Epiphytic orchids account for 69% of all orchid species and 68% of all vascular epiphytes, making epiphytism particularly important for the diversification of the Orchidaceae. Mycorrhizal association may have played a crucial role in the adaptation and diversification of orchids in the canopy. The canopy environment is different from the terrestrial one, and thus mycorrhizal association in epiphytic habitats may have unique characteristics. In this study, we conducted comparisons of fungal communities between different life forms and between different substrates in Yakushima, Japan.

**Materials and Methods:** Samples included 16 terrestrial and 13 epiphytic orchid species and their associated substrates (8 soil samples and 8 bark or moss samples). Fungal communities were identified by Sanger sequencing and high-throughput sequencing. Detected fungi were classified into operational taxonomic units (OTUs) with 97% sequence similarity.

**Results:** OTU diversity of Rhizoctonia, the major orchid mycorrhizal fungal taxa, was similar between terrestrial and epiphytic orchids, but their composition differed between the two life forms. In substrate samples, soil had significantly higher OTU diversity compared to bark and moss, but the OTU diversity of Rhizoctonia was as high as that of soil.

**Discussion:** This study showed that mycorrhizal fungal communities differ between terrestrial and epiphytic orchids. This difference may be caused by the difference in fungal communities between ground and canopy habitats.

**Conclusions:** The comparable level of Rhizoctonia diversity observed in the two different habitats suggests that the presence of diverse Rhizoctonia fungi in the canopy may have contributed to the adaptation and diversification of orchids in the canopy environment.

**Keywords:** Orchidaceae, Rhizoctonia, Epiphytism, Species diversification

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## Omics and Ecology

### DIFFERENCES IN MANAGEMENT RATHER THAN SHADE HAVE STRONG EFFECTS ON ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES IN COFFEE

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**Introduction/Aim:**We used a 23-year-old experiment at the Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica to examine how AMF communities in coffee roots are shaped by shade and management. Because plant productivity is strongly tied to light availability and AM fungi differ in their carbon costs, we expected to observe differences in communities across shade treatments. We also anticipated that conventional management would reduce the abundance and diversity of AM fungi relative to organic management, due to fungicide use and increased mineral nutrient availability.

**Materials and Methods:**We used metabarcoding of the fungal ITS2 to examine AMF community composition in 10 coffee plants in each of 30 plots under four types of shade conditions (full sun; or under shade tree species *Terminalia amazonia*; *Erythrina poeppigiana*; or *E. poeppigiana* with *Chloroleucon eurycyclum*, both N-fixers) and conventional and organic management.

**Results:**AMF richness and abundances were similar across the four different shade treatments, but were much higher in conventionally managed than in organic plots. Members of Acaulosporaceae, Archaeosporaceae, and Gigasporaceae were consistently more abundant in conventionally managed plots, while Glomeraceae and Pervetustaceae were generally more abundant in organic plots, with some members showing the opposite.

**Discussion:**Contrary to expectations, shade did not shape coffee AMF communities, nor did conventional management reduce their abundance or diversity. Three families exhibited phylogenetic conservatism, while two exhibited divergence.

**Conclusions:**Type of shade had very little effect, while management had a large effect, on coffee AMF communities. Responses to management were consistent for taxa in some families but not in others.

**Keywords:** resource availability, plant productivity, community composition, species diversity

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## Omics and Ecology

### HOST IDENTITY, SOIL P AVAILABILITY AND SOIL MOISTURE EXPLAIN THE COMPOSITION AND DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN LOWLAND TROPICAL FORESTS

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**Introduction/Aim:** Arbuscular mycorrhizal (AM) fungi are considered a pervasive strategy for nutrient acquisition in lowland tropical forests. Recent work in Panama showed that tree species distributions are related to soil phosphorus (P) availability, but the species-specific composition of AM fungi remains unexplored. We evaluated the influence of host identity and soil properties on the composition and diversity of AM fungi across a gradient of soil fertility.

**Materials and Methods:** We used a long term plot network in central Panama with mapped soil properties and tree species. We sampled fine roots from 170 trees representing 24 species with varying soil P affinities for Illumina sequencing and microscopy of fungal structures.

**Results:** AM community composition strongly differed between sites that have high and low P availability, with resin P and dry season moisture as the strongest drivers of composition. Together, tree species identity and soil edaphic factors explained 13% of the variation in composition. AM fungi were less diverse in low P soils relative to high P soils.

**Discussion:** Resin P and dry season moisture are also the strongest drivers of plant species distributions, suggesting that tree species and AM fungal communities are structured via the same abiotic filters in these tropical forests. Lower AM diversity in low P soils may suggest P-limitation of certain fungal taxa, and an ability of trees to modify recruitment of AM fungi in response to soil P availability.

**Conclusions:** Overall, we found a strong effect of both abiotic and biotic filters on AM fungal community assembly in lowland tropical forests.

**Keywords:** tropical forests, arbuscular mycorrhizal fungi, community composition, plant species distribution, soil phosphorus

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## Omics and Ecology

### SEQUENCING ROOT FUNGAL COMMUNITY OF "*NOCCAEA PRAECOX*" AND "*N. CAERULESCENS*" CONFIRMS FUNGAL TAXONS BELONGING TO DSE AND AMF

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**Introduction/Aim:**Species belonging to Brassicaceae are readily denoted as non-mycorrhizal. However, they are recognised to form associations with dark septate endophytes (DSE). The same applies to metal hyperaccumulating species *Noccaea praecox* and *N. caerulescens*. The main aim was to investigate and compare fungal communities in the roots and seeds of *N. praecox* and *N. caerulescens*.

**Materials and Methods:**Next-generation sequencing was used to capture root fungal communities associated with *N. praecox* from polluted and non-polluted sites and compare them to *N. caerulescens*. Seeds were removed from the silique, and fungal endophytes of seeds and silique were isolated and identified by sequencing.

**Results:**Over 10,000 fungal sequences from the roots of both species were annotated, one-half of them belonging to the saprotroph guild and one-quarter to taxa with either pathotroph or symbiotroph lifestyles. Over 4,000 sequences belonged to DSE fungal taxa, and over 900 belonged to arbuscular mycorrhizal species (AMF). Pleosporales largely dominated the DSE root fungal community, while Archaesporales were the dominant AMF taxa. Within seed isolates, Pleiosporales were the dominant taxon

**Discussion:**Roots of *N. praecox* and *N. caerulescens* are repeatedly associated with fungal taxa belonging to DSE and AMF. Silique are structures effectively protecting the seed from fungal colonizers. Interestingly, 50% of seed isolates belonged to taxa that are in use as biological control agents.

**Conclusions:**Roots and seeds of *Noccaea* species are colonized by overlapping communities of DSE. Silique has a filtering effect on the structure of seed endophyte communities.

**Keywords:** *Noccaea*, sequencing, endophyte, root, seed

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## Omics and Ecology

### ECTOMYCORRHIZAL FUNGI MODULATE DROUGHT TOLERANCE IN PEDUNCULATE OAK VIA FINE-TUNING OF PLANT HORMONES, POLYAMINES, AND OSMOLYTES

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**Introduction/Aim:** Due to its drought sensitivity, the pedunculate oak (*Quercus robur* L.) faces a survival threat in light of climate change. Mycorrhizal fungi are among the microbes that significantly contribute to reducing the effects of climate change on trees by orchestrating biogeochemical cycles and boosting the plant's defense and immunity systems. The main aim of the study was to investigate the priming abilities of ectomycorrhizal (ECM) fungi and to assess whether their presence ameliorates drought stress in pedunculate oak. In this study, the effects of two levels of drought (mild and severe, corresponding to 60% and 30% of field capacity, respectively) on the biochemical response of pedunculate oak were examined in the presence and absence of ectomycorrhizal fungi.

**Materials and Methods:** To examine whether the ectomycorrhizal fungi modulate the drought tolerance of pedunculate oak, levels of plant hormones, and polyamines were quantified using UPLC-TQS and HPLC-FD techniques while main osmolytes (proline and glycine betaine) were determined spectrophotometrically.

**Results:** Drought increased the accumulation of osmolytes like proline and glycine betaine as well as higher polyamines (spermidine and spermine) levels and decreased putrescine levels in both, mycorrhized and non-mycorrhized oak seedlings.

**Discussion:** Furthermore, this study discovered that unstressed ECM-inoculated oak seedlings had higher levels of salicylic (SA) and abscisic acid (ABA) but not jasmonic acid (JA) than non-mycorrhized oak seedlings.

**Conclusions:** These findings highlight the beneficial function of the ectomycorrhizal fungi, in particular *Scleroderma citrinum*, in reducing the effects of drought stress in pedunculate oak.

**Keywords:** ectomycorrhizal fungi, oak, polyamines, plant hormones

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## Omics and Ecology

### THE EFFECT OF DROUGHT ON ERICOID MYCORRHIZAL FUNGAL COMMUNITIES

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**Introduction/Aim:**The boreal, alpine, and arctic heaths and forests are dominated by dwarf-shrubs along with their symbionts, ericoid mycorrhizal fungi. Dwarf-shrubs are sensitive to climate change and are already affected in large-scale diebacks. It remains to explore how their mycorrhizal symbionts mitigate damages, since little is known about these plant-fungal interactions. Ericoid mycorrhizal fungi are known to aid the host plant with nutrient uptake, but little research exists on their dealings with drought, suggesting a major knowledge gap. A better overview of belowground ericoid fungi at different drought levels might help predict future climate-change induced damage. In our study, we aim to find out if and how drought affects ericoid mycorrhizal fungal communities, and pinpoint key fungal species related to drought mitigation.

**Materials and Methods:**From an ongoing precipitation exclusion experiment in heathlands in Western Norway, we collected soil and ericoid root samples from 27 plots with no (controls), 60% and 90% rain coverage, respectively. The fungal communities are analyzed using DNA-metabarcoding of the ITS2 region.

**Results:**The results will include data from ITS2 short-read sequencing of roots and soil samples, and will be tested using community ecological analyses, comparing the three drought treatments.

**Discussion:**We expect the ericoid mycorrhizal fungal communities to differ between drought treatments. However, other factors such as soil pH, host specificity, and nutrient availability might also affect species compositions.

**Conclusions:**Our study will contribute new data on the effects of drought on ericoid mycorrhizal fungal communities in heathlands.

**Keywords:** Ericoid mycorrhizal fungi, DNA-metabarcoding, drought, climate change

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## Omics and Ecology

### DNA-BASED SPECIES IDENTIFICATION OF GREEK MACROMYCETES

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**Introduction/Aim:**Fungi comprise one of the largest and diverse groups of eukaryotes. Macromycetes, which are commonly known as mushrooms, include species in Basidiomycota and Ascomycota. Macromycetes are essential for ecosystem functioning and have high commercial value owing to their nutritional and medicinal properties. Despite the importance of macrofungi for the ecosystem and human welfare, macromycete diversity and phylogeny are poorly characterized, owing to the lack of molecular-based biodiversity descriptors supporting phenotypic classifications, especially for biodiversity rich countries such as Greece.

**Materials and Methods:**A multi-marker DNA barcoding approach was used, utilizing the Internal Transcribed Spacer 1 (ITS1) and part of the 28S nuclear ribosomal Large Subunit (nrLSU) rDNA regions, for the molecular identification of representative Greek macromycetes. Our analysis involved 103 Greek macromycetes covering seven genera of Basidiomycota (*Agaricus*, *Amanita*, *Boletus*, *Cantharellus*, *Lactarius*, *Pleurotus*, and *Russula*) and one genus of Ascomycota (*Morchella*).

**Results:**Phylogenetic inference based on the generated rDNA sequences, revealed high DNA divergence among most of the examined macromycete genera, which formed discrete monophyletic groups.

**Discussion:**Our phylogenetic analysis, in accordance with previous studies in the field, further supports the early divergence of the *Cantharellus* clade, followed by the subsequent split of the *Russulaceae* from a sister clade formed by the *Agaricus*, *Amanita*, *Boletus* and *Pleurotus* genera.

**Conclusions:**DNA barcoding using ITS in combination with 28S region is suitable to discriminate macromycetes and reveal their relations

**Keywords:** DNA barcoding, ITS, 28S, discriminate

P-094

## Omics and Ecology

### WHAT IS THE IMPACT OF FIRE ON MYCORRHIZAL SYMBIOSIS IN FIRE-NON ADAPTED ECOSYSTEMS?

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**Introduction/Aim:** Fire influences terrestrial ecosystems, affecting both fire-adapted and fire-non adapted communities. In ecosystems with infrequent fire occurrences, such as the Chilean Matorral, fire can induce substantial degradation due to species' limited adaptation to this disturbance. Despite the acknowledged importance of mycorrhizal symbioses in terrestrial ecosystems, the impact of fire on mycorrhizal symbiosis in fire-non adapted ecosystems remains poorly understood.

**Materials and Methods:** To address this gap, we conducted an experiment in the Chilean Matorral, a Mediterranean-type ecosystem non adapted to fire. Since Chilean Matorral forms mostly arbuscular mycorrhizal symbiosis, we focus on this mycorrhizal type and we assessed its colonization, spore species abundances, and diversity across fire-affected and unaffected sites, with and without woody plant species presence.

**Results:** Our findings revealed varying responses of mycorrhizal symbiosis to fire. While three out of four study sites showed a non-significant decrease in mycorrhizal colonization post-fire, one site exhibited a significant increase explained by a high vesicle formation. Additionally, arbuscule content notably declined in two fire-affected sites. Spore abundance decreased in fire-affected sites with woody plant species, while the opposite trend was observed in sites lacking woody plants and affected by fire.

**Discussion:** Furthermore, our results highlight a species-specific response of fungi to forest fires, with some species significantly more abundant in fire-affected sites, in accordance with previous findings in fire-adapted ecosystems for ectomycorrhizal fungi.

**Conclusions:** In conclusion, these observations suggest that fire has significant implications for mycorrhizal symbiosis in ecosystems with infrequent fire occurrences, contributing to our understanding of the complex interactions between fire and terrestrial communities.

**Keywords:** fire-non adapted ecosystems, Chilean Matorral, mycorrhizal colonization, mycorrhizal fungal species



P-095

## Omics and Ecology

### ADVANCEMENTS IN UNTARGETED METABOLOMICS TO CHARACTERIZE ECTOMYCORRHIZAL FUNGAL EXUDATES

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**Introduction/Aim:** Poplar species exude a tremendous diversity of compounds into the rhizosphere, transforming root-associated microbial communities, soil physiochemical properties, and ecological processes such as carbon and nitrogen cycling. Ectomycorrhizal fungi (EMF) colonizing poplar roots are expected to change the composition of this local environment. This can benefit hosts by facilitating the absorption of mineral nutrients or affecting the rhizosphere microbiome. Our goal is to develop a systematic understanding of these changes by leveraging recent advancements in untargeted metabolomics.

**Materials and Methods:** We have previously demonstrated that poplar tissue culture-agar systems are well-suited for achieving spatially resolved measurements of the rhizosphere at molecular (metaproteomics) or metabolic levels (metabolomics). We aim to extend these technical advancements to systematically assess spatial-temporal changes in root exudation with or without mycorrhizae. As a proof-of-concept, we are using tissue culture *Populus* roots colonized with the EMF, *Laccaria bicolor*. We employ innovative metabolomic approaches capable of aggregating and systematically comparing data across diverse studies and experimental conditions.

**Results:** As a result, we report identified compounds and meticulously document those that do not match commercially available molecules—a critical consideration given that modern untargeted metabolomics efforts typically identify less than 10% of the compounds measured.

**Discussion:** Here, we summarize the resulting global molecular networks, the compounds and chemistry observed, the relatedness between the observed metabolic profiles, and their importance in shaping local environments.

**Conclusions:** Overall, these capabilities will enable new measurements to better understand ectomycorrhizal fungi and their local environments

**Keywords:** Mycorrhization, beneficial fungi, metabolomics, rhizosphere, *Populus*

P-096

## Omics and Ecology

### CONTINENT SCALE SURVEY OF AERIAL ARBUSCULAR MYCORRHIZAL FUNGI OVER TIME

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**Introduction/Aim:** Aerial dispersal of arbuscular mycorrhizal (AM) fungi has been documented in certain environments. However, the widespread occurrence of AM fungal aerial dispersal and its variation across different ecosystems remains unstudied at large spatial and temporal scales. A comprehensive macroecological approach is necessary to reveal the generalizability of aerial dispersal of AM fungi across all taxa.

**Materials and Methods:** We conducted multi-year aerial sampling of AM fungi across 20 sites representing diverse ecoclimatic zones ranging from arctic tundra to tropical forest in the U.S. We used high throughput Illumina sequencing of dust samples, amplifying the small subunit rDNA region and assigned AM fungal amplicon sequence variants (ASVs) to virtual taxa using the MaarjAM database.

**Results:** We identified 1340 ASVs that align to 30 AM fungal virtual taxa and detected a range of 0 to 36 ASVs per sample across all sites. We detected AM fungi in the air of all 20 sites, regardless of the mycorrhizal type (AM/EcM/Erm) of the dominant vegetation. On average, greatest aerial AM fungal richness was detected in high elevation alpine tundra with the lowest richness in Alaskan boreal forests. Some sites exhibited more interannual variation in aerial AM fungal richness than others.

**Discussion:** Aerial AM fungal taxa present in the air will be compared to those present in the soil at each site to examine the relative roles of aerial dispersal and environmental filtering on AM fungal community structure.

**Conclusions:** These data suggest that, despite their hypogeous lifestyle, aerial dispersal in AM fungi is widespread and common.

**Keywords:** arbuscular mycorrhizas, dispersal, biogeography, environmental DNA, macroecology

P-097

## Omics and Ecology

### CORE AM FUNGAL HYPHOSPHERE MICROBIOME – ARE THERE ANY STABLE ASSOCIATIONS IN THE UNSTABLE WORLD?

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**Introduction/Aim:** There is an increasing awareness of microbes which form an integral part of eukaryotic holobionts. Some microorganisms associated with arbuscular mycorrhizal (AM) fungal hyphae have previously been shown to functionally complement the fungus. It has also been proposed that abundance of certain microbial taxa is higher within than outside of the hyphosphere, regardless of the soil properties or AM fungal species.

**Materials and Methods:** To study specificity of association of certain microbes with AM fungal hyphae across soil patches with different chemical properties, we conducted a pot experiment where root growth of mycorrhizal and nonmycorrhizal plants (*Andropogon gerardii*) was restricted to a central rhizosphere cylinder and hyphae-only patches were amended with mineral or organic nitrogen (chitin).

**Results:** Abundance of several prokaryotic taxa was promoted upon presence of AM fungal hyphae (e.g., *Ramlibacter*, *Bdellovibrio*, or *Acidobacteriota* Gp17), whereas others (e.g., *Aquicella*, *Brevundimonas*, or *Nitrospira*) were suppressed. Much fewer protistan and fungal taxa showed such clear-cut effects.

**Discussion:** In spite of their statistical significance, the above effects were dwarfed by the influence of nitrogen form on the soil microbiomes, but also by the stable isotopic labelling of the chitin (none vs.  $^{13}\text{C}+^{15}\text{N}$ ), inviting question about stability and dynamics of the microbiomes in varying environment.

**Conclusions:** We currently know close to nothing about recruitment, spatiotemporal stability and dispensability of the AM hyphal microbiomes, therefore more research into these questions is warranted. An important point in this regard is that at least some AM fungi are capable of completing their lifecycle upon absence of any other microbes.

**Keywords:** Hyphosphere, microbial community, stable isotopic labelling, chitin, mineral and organic nitrogen

P-098

## Omics and Ecology

### MICROBIAL COMMUNITY COMPOSITION AND CARBON CYCLING MEDIATED BY MYCORRHIZAL FUNGI AND SOIL MOISTURE AVAILABILITY

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**Introduction/Aim:** Mycorrhizal fungi distribute plant photosynthates below ground. A quantitative and taxonomically-resolved understanding of microbial photosynthate use in mycorrhizal soils would elucidate mycorrhizal contributions to terrestrial biogeochemistry and ecological function.

**Materials and Methods:** We used <sup>13</sup>CO<sub>2</sub> labeling, stable isotope tracing, and a multi-omics approach to investigate carbon cycling and microbial community dynamics in the rhizosphere of the bioenergy model grass *Panicum hallii*. Plants that were uninoculated or inoculated with either the arbuscular mycorrhizal fungus *Rhizophagus irregularis* or the Sebaciniales fungus *Serendipita bescii* were transplanted into field soil and maintained under water-replete or water-limited conditions for up to three months. With quantitative stable isotope probing and next-generation sequencing, we identified bacteria and fungi that incorporated <sup>13</sup>C into newly-synthesized DNA.

**Results:** Microbial taxa with distinct genomic repertoires assimilated <sup>13</sup>C under each of the fungal inoculation and soil moisture conditions. Microbial <sup>13</sup>C assimilation was greater in fungal-inoculated rhizosphere soil. Differences in soil carbon pools and fluxes suggest that inoculation with *R. irregularis* and *S. bescii* facilitated faster turnover of both native soil organic matter and fresh plant photosynthates, although this effect was dependent on soil moisture and plant phenological stage. Many of the microbial taxa that assimilated greater quantities of <sup>13</sup>C under water-limited conditions also sustained greater growth rates once soil moisture was restored.

**Discussion:** These findings demonstrate how *R. irregularis* and *S. bescii* influence rhizosphere microbial community composition and carbon cycling.

**Conclusions:** Mycorrhizal contributions to microbial photosynthate use may support ecological resilience to water limitation.

**Keywords:** Carbon cycling, rhizosphere microbial communities, genomics, stable isotope probing, drought, bioenergy grass

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## Omics and Ecology

### UNRAVELLING THE FACILITATION-COMPETITION CONTINUUM AMONG ECTOMYCORRHIZAL AND SAPROTROPHIC FUNGI

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**Introduction/Aim:** Soil fungal inter-guild interactions may impact ecosystem processes significantly. In particular, the competitive interactions between ectomycorrhizal and saprotrophic fungi could reduce organic matter decomposition through the 'Gadgil effect'. Whether fungal facilitative and competitive reciprocal interactions predictably shift with increasing environmental stress, as hypothesised by the stress-gradient hypothesis (SGH), is unclear, particularly with regard to multiple environmental resource gradients.

**Materials and Methods:** We quantified the reciprocal interactions among fungal guilds in the root tips and soil mycelia in 84 temperate forests comprising a natural gradient of soil fertility and root carbon resources. The two resource gradients were negatively related.

**Results:** In keeping with SGH, we found that the typical interactions between fungal guilds were symmetrically positive under low soil fertility or root resource gradients. The interactions were negative in fertile soils or when the root resources were more available. The inter-guild interactions varied with the spatial niche (i.e., root tips or soil mycelium) and the type of resource gradients. When both gradients were integrated, SGH held for the dominant gradient in the system.

**Discussion:** The premise of the "Gadgil effect" became apparent with increasing soil fertility. The soil fungal guilds revealed symmetrical facilitative interactions under conditions of lower soil pH and a drier and warmer climate resembling global change scenarios. These interactions potentially retain soil biodiversity and underpin forest ecosystem function.

**Conclusions:** Our findings validate the SGH in soil fungal dynamics, demonstrating that environmental resources significantly influence fungal interactions and, by extension, ecosystem processes.

**Keywords:** Gadgil effect, Stress Gradient Hypothesis (SGH), Asymmetric interactions, Temperate forests, Soil mycelium.

**P-100**

**Omics and Ecology**

**SMALL MAMMAL-MEDIATED DISPERSAL OF MYCORRHIZAL FUNGI AT A CONTINENTAL SCALE**

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**Introduction/Aim:**Increasing evidence suggests that small mammals play an important role in the dispersal of ecto- and arbuscular mycorrhizal (EcM and AM) fungi by liberating, transporting, and depositing fungal propagules. However, the relative importance of small mammals in mycorrhizal fungal dispersal may differ across ecoclimatic regions. This study examines continent-scale small mammal-mediated dispersal of EcM and AM fungi by measuring richness and community composition in scat.

**Materials and Methods:**Using next-generation amplicon sequencing of the internal transcribed spacer and small subunit regions, we identified EcM and AM fungi present in 71 small mammal scat samples from 18 sites across the U.S. Scat was collected from 14 small mammal species by the U.S.-based National Ecological Observatory Network which spans arctic to subtropical regions.

**Results:**Small mammal scat across all sites contained a total of 550 amplicon sequence variants (ASVs) matched to EcM or AM fungal taxa. We found greater total ASV richness at lower latitudes and higher AM fungi richness in female scat. Additionally, vegetation canopy type, dominant mycorrhizal type, and small mammal species influenced community composition.**Discussion:**Small mammals have been known to play a role in the dispersal of aboveground EcM fungi, but our results show that hypogeous and AM fungi are also widely dispersed by small mammals. This macroecological approach also highlights the relationship between small mammal identity, site vegetation qualities, and mycorrhizal fungi biogeography patterns.

**Conclusions:**These results emphasize the importance of examining biotic dispersal networks, particularly small mammal-AM fungi interactions, to better understand mycorrhizal fungal biodiversity across spatial scales.

**Keywords:** Dispersal, small mammal mycophagy, macroecology, next-generation sequencing, environmental DNA

**P-101**

**Omics and Ecology**

**UNVEILING BASIDIOMYCETE BIOSYNTHETIC POTENTIAL FOR SUSTAINABLE CHEMICAL PRODUCTION**

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**Introduction/Aim:**The heavy dependence on fossil chemicals as a finite and unsustainable resource calls for the exploration of alternative sources. The specialized metabolism of fungi holds promise for advancing sustainable bio-based chemical production. Cataloguing the fungal chemical and biosynthetic repertoire is crucial to leverage their full potential. Encompassing saprotrophs, mycorrhizal symbionts, and plant parasites, basidiomycetes are a major component of soil and plant microbiomes. However, this diverse fungal division remains surprisingly unexplored in terms of their specialized metabolism. A major challenge for genome-based efforts for specialized metabolite discovery is the lack of efficient bioinformatic tools for basidiomycete genomes.

**Materials and Methods:**In this study, we present the genome-guided investigation of basidiomycete specialized metabolism. We developed a bioinformatic tool that facilitates gene-calling, improving prediction of biosynthetic genes encoded in basidiomycete genomes.

**Results:**This tool enabled a thorough investigation of biosynthetic potential across a wide range of basidiomycetes.

**Discussion:**This work paves the way for heterologous expression-based discovery of previously unknown chemical architectures and enzymatic functions.

**Conclusions:**We believe this will help develop new processes for sustainable production of chemicals.

**Keywords:** Fungi, Genome Mining, Specialized Metabolism, Biomaterials, Bioenergy

**Omics and Ecology**

**IMPACTS OF MYCORRHIZAL TYPE ON TREE MYCOBIOME AND SOIL CARBON IN PLANTATION FORESTS OF SUBTROPICAL CHINA**

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**Introduction/Aim:** Trees mainly belong to arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) types. We know little about tree mycorrhizal type's impacts on tree microbiome and soil (microbial) carbon storage.

**Materials and Methods:** A field experiment of AM, EM, and AM-EM plantation was established in subtropical China in 2021. Tree's leaf and root, surface and subsurface soil samples were collected in two seasons. We adopted high-throughput sequencing, biomarkers, and soil chemical methods to explore how forest microbiome and soil carbon contents respond to AM and/or EM tree plantations.

**Results:** Our preliminary results indicated that mycorrhizal type had significant effect on fungal diversity, with higher richness observing in AM than EM trees irrespective of compartments. Higher phyllospheric fungal richness were detected in August than in April. Although mycorrhizal type significantly influenced fungal community composition, it exerted minor effects on soil total carbon (TC). Interestingly, we found that soil fungal biomass (ergosterol as proxy) were higher in EM than those of AM and AM-EM plantations regardless of soil depth. Notably, positive correlations between ergosterol and TC contents in subsurface soil were observed in EM rather than AM and AM-EM plantations.

**Discussion:** Long-term monitoring of soil carbon is essential for comparing AM and EM forests, given that no clear impacts on soil TC was detected in this study. However, we initially identified that EM tree species (e.g. *Castanopsis fissa*) seemed to sequester more soil carbon than AM trees.

**Conclusions:** Our findings highlight the difference of tree mycobiontes between AM and EM plantation and fungal contribution to soil carbon sink in subtropical forests.

**Keywords:** diversity, fungal biomass, mycorrhizal type, soil carbon, subtropical forest



**Omics and Ecology**

**PHYLOGENOMICS OF INVASIVE "AMANITA" "MUSCARIA" IN SOUTH AFRICA**

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**Introduction/Aim:** Mycorrhizal fungi have been co-transported globally for centuries with their host plants, resulting in long-term establishment of introduced populations in new habitats. The origins of these introduced fungi remain largely unknown. The "Amanita muscaria" species complex are mycorrhizal members of the division Basidiomycota. These fungi have been found globally in non-native habitats. This research aimed at determining the source and evolution of members of this species from South Africa.

**Materials and Methods:** Specimens of "A. muscaria" were collected from South Africa during an NSF funded plant-fungal co-invasions workshop and from locations in Europe and the U.S. and analysed using multi-omics techniques. After DNA extraction, short read genome sequencing and genomes assembly were conducted. Single nucleotide polymorphism (SNP) sequences were extracted from the genome sequences and used to construct a phylogenomic tree. A concatenated phylogenomic tree was also constructed using sequences of multiple DNA barcodes extracted from publicly available genome sequences and from the assembled genomes. Specialized metabolite gene clusters (SMGCs) were evaluated using comparative genomics.

**Results:** We hypothesized a European origin for the introduced populations of "A. muscaria" in South Africa due to the country's long history of plantation forestry.

**Discussion:** Our phylogenomic studies confirmed that the South African "A. muscaria" originated from populations in Europe. We also detected various largely conserved SMGCs among the genomes of the introduced and native individuals.

Conclusions:Our study provides new insights into the history of "A. muscaria"'s nearly global distribution and opens the door for new questions about the ecology and evolution of this species in its introduced ranges.

**Keywords:** Amanita muscaria, evolution, invasive fungi, multi-omics, specialized metabolite gene clusters

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## Omics and Ecology

### UNDERSTANDING POPLAR-MICROBE INTERFACES: FROM MODEL SYSTEMS TO MODEL SYNTHETIC COMMUNITIES

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**Introduction/Aim:**The current knowledge on the molecular signaling in ectomycorrhizal (ECM) interactions was obtained with a few in vitro model systems, including our model *Laccaria bicolor*-Poplar. While significant progress was made in the elucidation of signals that are essential for ECM development, very few is known on the molecular level for endophytic interactions and the possible inhibitory or synergistic effects that these fungi may have on ECM fungi and the host tree.

**Materials and Methods:**We analysed the colonisation of poplar roots by two ectomycorrhizal fungi (*L.bicolor* and *Lactarius controversus*) and two endophytes (*Hyaloscypha finlandica* and *Coniochaeta* sp) either by single inoculation or by different combinations of dual and multiple inoculations. In sterile pots, poplar plants were grown in gamma irradiated soil plus fungal inoculum. Fresh biomass was determined, colonised roots harvested for RNA-Seq and stained root samples were observed by confocal microscopy.

**Results:**Endophytes and their co-inoculations with ECM fungi showed a plant growth promoting effect.

The endophytes colonized poplar roots with very distinct structures. *Coniochaeta* displayed melanized, extraradicular hyphae and spore-like structures, while *Hyaloscypha* formed intercellular structures.

Inoculation and co-inoculation with ECM fungi highly effected the root transcriptome of poplar. Previously identified ECM-regulated genes were often even higher expressed in co-colonised roots.

**Discussion:**Our results suggest that the two endophytes form different interactions with poplar at the cellular level and promote plant growth with different mechanisms.

**Conclusions:**Using microscopy, we showed how two endophytes co-colonise poplar roots together with ECM fungi and using RNA-Seq, we started to decipher the molecular mechanisms of these poplar-ECM-endophyte interactions.

**Keywords:** ectomycorrhiza, endophyte, poplar, tree-microbe interactions

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## Omics and Ecology

### ECTOMYCORRHIZAL FUNGI MAINTAIN DECOMPOSITION WHEN SAPROTROPHS FAIL – A SOIL METATRANSCRIPTOMICS STUDY IN BOREAL FOREST

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**Introduction/Aim:** Some ectomycorrhizal fungal lineages have retained significant decomposition capabilities during their evolution from saprotrophic ancestors. Still, evidence for direct involvement of ectomycorrhizal fungi in decomposition under field conditions are scarce and circumstantial. We used community-wide sequencing of mRNA (metatranscriptomics) to assess the significance of mycorrhizal decomposers and their interplay with free-living saprotrophs in a Swedish boreal forest.

**Materials and Methods:** We compared soil samples from nutrient poor, pine-dominated plots with samples from more nutrient rich, spruce-dominated plots. Metatranscriptomic analyses targeted gene families involved in oxidation of recalcitrant aromatic polymers (Mn-peroxidases), cellulose hydrolysis (cellobiohydrolases), and mycelial production (glucan synthases, as reference).

**Results:** Transcription of cellulase and glucan synthase genes by saprotrophic basidiomycetes (mainly *Mycena* and *Rhodocollybia* species) was much lower in nitrogen poor plots, indicating strong constraints on saprotrophic growth and activity. In contrast, over-all expression of Mn-peroxidase genes was higher in nitrogen poor plots, with ectomycorrhizal *Cortinarius* and *Russula* species accounting for 60% and 3% of the transcripts, respectively.

**Discussion:** Seemingly, free-living decomposers fail to maintain their role as decomposers under the acidic and nutrient poor conditions that are characteristic of many boreal forests. Instead, symbiosis with ectomycorrhizal decomposers enables the use of current photoassimilates to drive energetically costly oxidation below ground.

**Conclusions:** Ectomycorrhizal decomposers are likely to be of critical importance to maintain organic matter turnover, nutrient cycling and ecosystem productivity in strongly nitrogen limited soils and, thereby, pivotal when boreal forest expands into tundra and alpine heaths in response to climate change.

**Keywords:** Ectomycorrhiza, Metatranscriptomics, Decomposition, Nutrient cycling, Boreal forest

# **Managing Mycorrhizas for Sustainability & Food Security**

**LT-041**

**Managing Mycorrhizas for Sustainability & Food Security**

**RANKINGS OF HOW SUSTAINABLE AGRICULTURAL MANIPULATION PRACTICES MITIGATE GREENHOUSE GAS EMISSIONS**

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**Introduction/Aim:**To mitigate adverse effects of global change, we should reduce greenhouse gas emissions from the agricultural sector. Sustainable management practices hold a lot of promise towards reducing the carbon footprint of agriculture, but they have been rarely assessed comparatively under identical settings.

**Materials and Methods:**We here evaluated how (i) stopping fertilization and (ii) ceasing pesticide applications, (iii) no tillage, (iv) addition of mycorrhizal propagules and (v) experimenting with different cultivars modify N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> emissions over two growth seasons in two rice paddies. We carried out a total of 40 bifactorial experiments and derived over 11,500 records on gas emissions

**Results:**We evaluated both the fitted coefficients and the effect sizes for the manipulations across the 40 experiments. The explanatory power of the experiments varied considerably. Stopping fertilization reduced N<sub>2</sub>O emissions most but the effect declined over time. No tillage reduced N<sub>2</sub>O but increased CO<sub>2</sub> emissions. Mycorrhiza ranked high in reducing CO<sub>2</sub> and CH<sub>4</sub> emissions.

**Discussion:**We are shedding light on the relative importance of agricultural practices in mitigating global change which goes beyond simply demonstrating the directionality of effects.

**Conclusions:**We thus reconcile with our study many past observations on greenhouse gases from agriculture and bring up the issue of reproducibility of snapshot measurements of greenhouse gases.

**Keywords:** Arbuscular mycorrhiza, Carbon footprint, Greenhouse gas emissions, Static chambers, Sustainable agricultural intensification

**LT-042**

**Managing Mycorrhizas for Sustainability & Food Security**

**EXPLORING THE IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI ON RICE GRAIN NUTRITION UNDER AEROBIC PRODUCTION**

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**Introduction/Aim:**Rice *O. sativa* serves as the primary dietary staple for over half of the global population, particularly in developing countries. The escalating water scarcity issue in countries cultivating temperate rice, including Australia, poses a significant concern to stable production. Consequently, the current focus is on transitioning rice cultivation from flooded to aerobic conditions to address climate change and water scarcity on a global scale. To achieve this objective, fundamental research on grain yield and quality under aerobic conditions is imperative. While the associations of arbuscular mycorrhizal (AM) fungi in plant root systems are well-documented, their exploration within the context of Australian aerobic rice production remains limited.

**Materials and Methods:**In this study, we explored how inoculation with the AM fungus *Rhizophagus irregularis* and soil phosphorus (P) fertilisation impacted the grain yield and nutrition of Australian commercial rice cultivars under aerobic conditions.

**Results:**All six rice varieties were well colonised with AM fungi, with an average root length colonised of 50%. However, the impact of AM colonisation on grain biomass and nutrition was plant variety-dependent.

**Discussion:**Generally, AM colonised plants had higher grain yield under low soil P availability than non-colonised plants. While AM rice grain had greater P, magnesium (Mg), and potassium (K) concentrations, they had lower calcium (Ca), zinc (Zn), and iron (Fe) concentrations at low soil P availability.

**Conclusions:**This work suggests that AM fungi could be a potential management strategy in the aerobic production of Australian rice, but requires further research to reduce potential negative impacts on grain Ca, Zn, and Fe concentrations.

**Keywords:** Arbuscular mycorrhizal fungi, rice, grain nutrition, aerobic conditions

**LT-043**

## **Managing Mycorrhizas for Sustainability & Food Security**

### **A GENOME-WIDE ASSOCIATION STUDY OF ARBUSCULAR MYCORRHIZAL SYMBIOSIS IN RICE**

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) form a mutually beneficial symbiosis that could be enhanced in sustainable agroecosystems. Such potential is currently limited by the variable nature of plant host response, mycorrhizal growth responsiveness (MGR). This project aims to determine drivers of genetic variation in host response to AMF-inoculation in *Oryza sativa*, a staple food crop. It is an exciting time to consider such, as there is increasing academic and industry focus on aerobic, water-saving cultivation systems where AM symbioses are prevalent.

**Materials and Methods:** We confirmed foliar abundance of blumenol-C-glycosides as a high throughput marker of AM colonization in diverse rice cultivars. The marker was successfully used to probe natural variation in AM colonization and host response in a panel of 202 indica cultivars from the 3,000 Rice Genomes Project, a diverse germplasm that has not yet been interrogated for AM phenotypes.

**Results:** Natural variation in AM colonization and MGR was identified under controlled glasshouse conditions, and our genome-wide association study (GWAS) mapped candidate genes determining variation.

**Discussion:** Importantly, in addition to glasshouse experiments that determine phenotypes without interference of other factors, cultivar assessments in the field identified natural variation in host response to AMF-inoculation compared to native AMF, corroborating glasshouse results and further evidencing genetic variation in cultivar response to AM symbiosis in rice.

**Conclusions:** Further analyses to identify genetic regions driving such variation are ongoing to ultimately predict cultivars with beneficial response. The collaborative study offers groundwork for future studies assessing the potential for AMF to drive beneficial host response in the rice field.

**Keywords:** arbuscular mycorrhizal symbiosis, rice, GWAS, genetic variation



**LT-044**

## **Managing Mycorrhizas for Sustainability & Food Security**

### **ARBUSCULAR MYCORRHIZA COMMUNITIES AND PHOSPHORUS RUNOFF RISKS OF 12 COVER CROP SPECIES UNDER CONTRASTING SOIL PHOSPHORUS LEVELS**

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**Introduction/Aim:** Cover-cropping is widely used regenerative farming practice that can induce the plant resilience and nutrient uptake benefitting arbuscular mycorrhizal (AM) interactions. Although cover-cropping can prevent soil-erosion-linked P loss from fields, in over-winter conditions where fields go through sequential freeze-thaw-cycles, dissolved P runoff may increase, making cover-cropping a potential risk to water quality. Contribution of different cover crop species to AM community, P runoff risks, and their interaction are not well known.

**Materials and Methods:** A greenhouse experiment with 12 common Finnish cover crops and two contrasting soil P levels was set up to investigate differences in root AM communities with microscopy and ITS2 sequencing, and P content of plants and soil. Total and water-extractable P (WEP) in soil and plants were measured after one and eight freeze-thaw cycles to estimate freeze-thaw-cycle induced P runoff risks.

**Results:** AM diversity varied between cover crops, and soil P level affected AM diversity in a cover crop species specific manner. Whereas soil WEP was mostly unaffected by extended freeze-thaw cycles plant WEP was increased. The highest plant WEP in one-time and extended freeze-thaw cycles were found in cover crop species with the lowest AM richness.

**Discussion:** As previously proposed, this indicates that higher AM diversity may help lowering P runoff risks.

**Conclusions:** These results will benefit crop planning: the correct choose of cover crops can help minimizing P runoff risks and increase crop resilience and nutrient uptake by diversifying AM communities in soil.

**Keywords:** Arbuscular mycorrhiza, cover crop, phosphorus, runoff

LT-045

## Managing Mycorrhizas for Sustainability & Food Security

### IMPACTS OF HUMAN-USE ANTIFUNGALS ON SYMBIOTIC SOIL FUNGI IN THE AGRO-ENVIRONMENT

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**Introduction/Aim:** Unsustainable agricultural practices which degrade soil quality and slow crop production are a threat to increasing food demand globally. Reusing municipal biosolids and promoting arbuscular mycorrhizal (AM) associations in crops to reduce synthetic fertilizer use are practiced in regenerative agriculture but exposes soil to man-made chemicals that enter wastewater treatment works. Here, we evaluate the impact of antifungal pharmaceuticals on AM fungal function.

**Materials and Methods:** We grew mycorrhizal spring onion and lettuce in agricultural soil spiked with three antifungal azole pharmaceuticals at environmentally relevant concentrations and used a combination of radio- and stable isotopes (<sup>14</sup>C, <sup>33</sup>P, <sup>15</sup>N) to track carbon for nutrient exchange between the AM fungi and the plants. We used DNA sequencing to define the microbial communities within roots and soils in both treatments.

**Results:** When exposed to antifungal pharmaceuticals, there was no impact on root colonisation by AM fungi. However, bi-directional exchanges of AM-acquired phosphorus and plant-fixed carbon between symbionts were reduced in all crops alongside changes in bacterial and fungal community composition.

**Discussion:** Our results indicate that antifungal pharmaceuticals have a direct impact on AM function, impacting the wider soil microbiome. This is likely to have wider effects on soil health and function in agro-ecosystems.

**Conclusions:** Our research emphasises the unintended consequences and threats posed by emerging contaminants in soils in terms of impacts on AM function and microbial community composition. This emphasises the pressing need for further research and regulation across potential contaminants of soil systems to determine wider impacts of circular agriculture practices.

**Keywords:** arbuscular mycorrhizal fungi, soil contamination, antifungal azole pharmaceuticals, biosolids, circular agriculture, carbon-for-nutrient exchange

LT-046

## Managing Mycorrhizas for Sustainability & Food Security

### ROCK PHOSPHORUS BIOFERTILISATION IN WILD BLUEBERRY PRODUCTION ON QUEBEC'S NORTH SHORE

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**Introduction/Aim:** Apatite is a biofertilizer used as a source of phosphorus for plants and promotes symbiotic interactions with soil microorganisms. Here, we aimed to evaluate the use of apatite extracted from the subsoil of the Sept-Îles region, micronized and pelletized, as an agricultural biofertilizer for the wild species *Vaccinium angustifolium*.

**Materials and Methods:** Three experiments were performed in the regions of Gallix and Longue-Rive on the Quebec's North Shore in Canada and Sacré-Coeur in Saguenay. Five different treatments were used: i) Apatite; ii) Actisol (poultry manure); iii) Apatite-Actisol mixture; iv) traditional chemical fertilizer; v) unfertilized. Soil chemistry, microbial community parameters, abundance and diversity of ericoid mycorrhizal fungi associated with blueberry roots were evaluated at the end of the 2nd cropping year and compared to the soil parameters of 1st vegetative year of culture. Additionally, a floristic inventory to evaluate the occurrence of invasive plants was performed.

**Results:** Preliminary results indicate a high occurrence of several genera from Heliotales order forming ericoid mycorrhiza, notably *Pezoloma*, *Oidiodendron*, and *Phialocephala*. We also observed a significantly higher pH at Sacré-Coeur when compared with other regions. Mycorrhizal structures were stained and observed. However, the rate of mycorrhization could not be calculated in spite of the adaptation of the protocols.

**Discussion:** We expect that the results may reinforce the idea of promoting the use of biofertilizers as a sustainable alternative for fertilizing wild blueberries along the northern coast of Quebec.

**Conclusions:** In conclusion, this study contributes to the advancement of sustainable agriculture by exploring alternative environmentally friendly fertilizers that can improve soil health.

**Keywords:** Blueberries, phosphorus, fertilizer, microorganisms, sustainable agriculture.

LT-047

## Managing Mycorrhizas for Sustainability & Food Security

### HELP OR HINDRANCE: ARE MYCORRHIZAL FUNGI MAKING OUR CEREALS LESS NUTRITIOUS?

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**Introduction/Aim:** Zinc (Zn) and iron (Fe) deficiencies are widespread throughout the world's agricultural soils. This leads to deficiencies in these essential micronutrients in cereal grains, and ultimately in the humans who rely on cereal-based diets. Arbuscular mycorrhizal fungi (AMF) take up Zn and Fe into cereal crops, so have been proposed as a biofortification tool. However, AMF also take up vast amounts of phosphorus (P) into the plant, leading to greater accumulation of phytate; phytate is an "anti-nutrient" which chelates Zn and Fe in the human gut.

**Materials and Methods:** We conducted three experiments with diverse genotypes of the staple cereals: rice, wheat and sorghum, with or without inoculation with *Rhizophagus irregularis*. We grew the plants to maturity, then determined how inoculation with AMF affected grain yield and Zn, Fe and phytate accumulation. We used those measurements to estimate the effect of AMF on bioavailability of Zn and Fe for human nutrition.

**Results:** There was substantial variation in response to inoculation with *R. irregularis*, both between crop species and between genotypes. AMF inoculation led to increased grain yield in sorghum, without an increase in grain phytate, leading to greater micronutrient bioavailability. This was in contrast with the results in rice and wheat.

**Discussion:** The effect of AMF inoculation on the bioavailability of essential micronutrients in cereal grain was highly dependent on the crop species in question. The P, Zn and Fe availability in the soil was also crucial in determining bioavailability.

**Conclusions:** Management of AMF in cereal cropping should consider effects on crops beyond yield responses.

**Keywords:** Biofortification, hidden hunger, micronutrients, phytate

LT-049

## Managing Mycorrhizas for Sustainability & Food Security

### IMPACT OF WATER AND HEAT STRESS ON MYCORRHIZAL SYMBIOSIS, PLANT BIOMASS OF MILLET (*Pennisetum glaucum* L.) AND MICROBIAL DIVERSITY IN CONTRASTING SOILS

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**Introduction/Aim:** Projected climate changes in the Sahel region are expected to significantly impact plants and soil microorganisms. This study aims to assess the effects of water and heat stress on soil microbial communities, millet plant growth, and mycorrhizal inoculation under these conditions.

**Materials and Methods:** Experiments conducted in a climate chamber examined soil microbial community and millet plant responses to varying water and heat stress levels. Selected strains of AMF were inoculated to evaluate their impact on plant resilience. Microbial diversity, enzymatic activities (dehydrogenase,  $\beta$ -glucosidase, and urease), microbial biomass (measured as soil organic carbon and total nitrogen), and plant mycorrhization rate were analyzed. **Results:** Water and heat stress significantly impacted soil microbial community and millet growth. Enzymatic activities varied, and changes occurred in microbial biomass and mycorrhization intensity. Despite initial positive effects, mycorrhization frequency and intensity decreased under stress, reducing the inoculation's efficacy in improving millet biomass.

**Discussion:** These findings highlight the complex interactions between water and heat stress, soil microbial communities, enzymatic activities, and microbial biomass in shaping plant responses to environmental challenges.

**Conclusions:** This study emphasizes the importance of considering root mycorrhization, enzyme activities, and microbial biomass when evaluating the effects of water and heat stress on soil microbial communities and millet growth.

**Keywords:** Stress hydrique, Stress thermique, Communauté microbienne, Plantes de mil, Inoculation microbienne, Diversité microbienne.

LT-050

## Managing Mycorrhizas for Sustainability & Food Security

### THE SOYBEAN SUGAR TRANSPORTER GMSWEET6 PARTICIPATES IN SUCROSE TRANSPORT TOWARDS FUNGI DURING ARBUSCULAR MYCORRHIZAL SYMBIOSIS

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**Introduction/Aim:**In arbuscular mycorrhizal (AM) symbiosis, sugars in root cortical cells could be exported as glucose or sucrose into peri-arbuscular space for use by AM fungi. However, no sugar transporter has been identified to be involved in sucrose export. An AM-inducible SWEET transporter, GmSWEET6, was functionally characterized in soybean and its role in AM symbiosis was investigated via transgenic plants.

**Materials and Methods:**The expression of GmSWEET6 was enhanced by inoculation with the cooperative fungal strain in both leaves and roots. Heterologous expression in a yeast mutant showed that GmSWEET6 mainly transported sucrose. Transgenic plants overexpressing GmSWEET6 increased sucrose concentration in root exudates.

**Results:**Overexpression or knockdown of GmSWEET6 decreased plant dry weight, P content and sugar concentrations in non-mycorrhizal plants, which were partly recovered in mycorrhizal plants.

**Discussion:**Intriguingly, overexpression of GmSWEET6 increased root P content and decreased the percentage of degraded arbuscules, while knockdown of GmSWEET6 increased root sugar concentrations in RNAi2 plants and the percentage of degraded arbuscules in RNAi1 plants compared with wild-type plants when inoculated with AM fungi.

**Conclusions:**These results in combination with subcellular localization of GmSWEET6 to peri-arbuscular membranes strongly suggest that GmSWEET6 is required for AM symbiosis by mediating sucrose efflux towards fungi.

**Keywords:** arbuscular mycorrhizal fungi, soybean (*Glycine max*), sucrose export, SWEET, symbiosis

## Managing Mycorrhizas for Sustainability & Food Security

### COMBINING METABARCODING AND CULTURE-DEPENDENT APPROACHES: A STRATEGY TOWARDS SCIENCE-BASED HARNESSING OF RHIZOSPHERE MICROBIOTA IN AGRICULTURAL SOILS

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**Introduction/Aim:**The current climate change, together with the continuous use of chemicals is causing severe stresses to crops, and a decrease in field microbial diversity. It is of paramount importance to preserve and foster the beneficial components of the soil microbiota (namely plant growth promoting bacteria and mycorrhizal fungi), possibly increasing their presence in cultivated lands to help crops tolerating such harsh conditions.

**Materials and Methods:**Our current researches range from the study of the mechanisms that govern plant/mycorrhizal interactions to the investigation of the microbial communities associated with cultivated plants through culture-dependent approaches and metabarcoding analyses.

**Results:**In a first set up, two different crops were employed, *Solanum lycopersicum* and *Oryza sativa*. Plants were grown in soils coming from harsh environments. Drought stress was induced and then a recovery phase applied. The metabarcoding results suggest a deep shaping of the microbial community (both bacterial and fungal) upon drought, and indicate that the recovery phase can efficiently restore a higher biodiversity levels. In parallel, the arbuscular mycorrhizal (AM) component from the rhizosphere samples was isolated following the "trap culture" method, and characterized.

**Discussion:**A second experimental set up has been devoted to characterize the Ericoid Mycorrhizal component (ErM) from blueberry orchards. New ericoid fungal strains, belonging to the *Hyaloscypha hepaticicola* species, were isolated and tested for their mycorrhizal abilities.

**Conclusions:***Vaccinium* bulk soil, rhizosphere and endosphere bacterial and fungal biodiversity was analysed, confirming the hypothesis that the soil acts as a 'reservoir' of microbial diversity, from which microorganisms successfully establish themselves in the rhizosphere and endosphere compartments.

**Keywords:** Mycorrhiza, Metabarcoding, Drought Stress, Tomato, Rice, Blueberry

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## Managing Mycorrhizas for Sustainability & Food Security

### INVESTIGATING ENTOMOPATHOGENIC AND ARBUSCULAR MYCORRHIZAL FUNGAL INTERACTIONS IN WHEAT

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) are renowned for promoting plant growth. Besides AMF, plants may also engage in symbiotic relationships with entomopathogenic (i.e., causing disease to insects) fungi (EF). A few studies have uncovered the EF propensity to promote plant growth, meaning EF may have a natural duality as a biopesticide and biofertiliser. However, despite recent studies suggesting promising synergistic effects between AMF and EF, it remains to be tested how efficient the dual application of these two types of fungi is in promoting plant growth, and under what circumstances any benefits to plants are maximised.

**Materials and Methods:** A fully factorial pot experiment using a winter wheat variety under two watering regimes (control vs. water-stress) was used. Pots were established with autoclaved soil with and without addition of AMF (*Rhizophagus irregularis*) and EF (*Beauveria bassiana*, either spraying directly on leaves or by direct inoculation to the soil). A subset of plants was harvested at 3 different key growth stages to measure temporal dynamics. Plant growth rate, flowering phenology and yield were measured.

**Results:** Our results suggest that EF and AMF can affect biomass partition within plants and yield.

**Discussion:** Our study suggests that both type of fungi can affect plant performance/resilience under drought. The synergistic, and sometimes not complimentary effects of AMF and EF highlight the potential for combining fungal treatments.

**Conclusions:** Comparative analysis of dual inoculation with AMF and EF under different conditions is not well understood. Awareness of natural plant promotion is vital for combatting yield shortages and maintaining healthy soils in changing environments.

**Keywords:** *Beauveria bassiana*, entomopathogenic fungi, *Rhizophagus irregularis*, *Triticum aestivum*, plant-fungi interactions, wheat.



## Managing Mycorrhizas for Sustainability & Food Security

### THE POTENTIAL FOR AMF TO FACILITATE AEROBIC RICE PRODUCTION THROUGH WATER AND PHOSPHORUS USE EFFICIENCIES

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**Introduction/Aim:** Majority of rice production is conducted in flooded (anaerobic) soil conditions, but aerobic rice production could lead to several benefits: increased water use efficiency (WUE), reduced methane emissions and reduced loss of applied phosphorus (P). In addition, AMF colonise and function in rice to a greater extent in aerobic than flooded soil, and the rice-AMF association could further increase plant WUE and P use efficiency.

**Materials and Methods:** We used a precision irrigation platform at the Australian Plant Phenomics Facility to maintain watering treatments (60% or 80% of soil field capacity) for the experiment's duration. Four commercial rice varieties were grown in pots, with or without inoculation with *Rhizophagus irregularis*, and with addition of P fertiliser at 10 or 25 mg P/kg soil. The plants were grown to maturity, harvested, and grain yield, WUE and PUE determined.

**Results:** Two rice varieties responded positively to AMF inoculation in terms of grain yield. While the grain yields of all the rice varieties were limited by water availability, P was not limiting to two of the varieties. AMF colonisation was consistently reduced in the high P treatment and was, in most varieties, also reduced with lower water availability (60% field capacity).

**Discussion:** The effect of AMF inoculation on rice varieties grown in aerobic soil conditions was dependent on the rice variety in question.

**Conclusions:** Our results show potential for AMF to increase the water and P use efficiencies of aerobically grown rice and bolster the resilience of rice production to abiotic climate change factors.

**Keywords:** Rice, phosphorus, water use efficiency, high throughput phenotyping, sustainable agriculture

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## **Managing Mycorrhizas for Sustainability & Food Security**

### **EXPLORING THE ROLE OF OSCPK13 IN SYMBIOTIC MYCORRHIZAL ASSOCIATIONS AND PHOSPHATE UPTAKE IN RICE**

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**Introduction/Aim:**Rice, the principle staple food for half the global population, is primarily cultivated under flooded conditions. Employing water-efficient methods and enhancing phosphorus uptake without heavy reliance on fertilizers are essential for minimizing the environmental impact of rice farming. A promising strategy involves fostering mutualistic symbiotic relationships with fungi to enhance nutrient uptake. In plants, symbiosis signaling is calcium dependent and involves a calcium/calmodulin-dependent protein kinase and similarly calcium-dependent protein kinases (CDPKs) phosphorylate downstream targets by calcium binding. This study focusses on elucidating the role of OsCPK13 in AM symbiosis establishment and phosphate (Pi) uptake in rice.

**Materials and Methods:**We aim to grow five OsCPK13 lines, *oscpk13 ko*, OX-OsCPK13, GUS reporter line, cellular mTurquoise2 line and *oscpk13 ko* complemented, in an upland-like system under varying Pi-regimes, with or without "*Rhizophagus irregularis*" presence. Assessments include OsCPK13 spatial and temporal expression, root morphology, Pi uptake, phosphate starvation and AM marker gene levels, and characterization of the interactome of OsCPK13 using TurboID.

**Results:**Preliminary findings indicate that OsCPK13 regulates Pi homeostasis by modulating the abundance and activity of Pi transporters. Interestingly, OsCPK13 overexpression increased susceptibility to "*Magnaporthe oryzae*" infection and previous reports demonstrated shared responses between AMF colonization and pathogen response.

**Discussion:**Therefore, we hypothesize that OsCPK13 mediates calcium-signals elicited by AMFs potentially influencing symbiosis.

**Conclusions:**This study endeavors to explore whether OsCPK13 regulates Pi homeostasis and symbiotic associations, or if AMF colonization is a consequence of Pi-signaling responses.

**Keywords:** sustainable agriculture, calcium signaling, mycorrhizal, nutrient homeostasis

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## Managing Mycorrhizas for Sustainability & Food Security

### IMPACT OF COVER CROPS ON AMF AND PROKARYOTIC DIVERSITY IN CHILEAN VINEYARDS:: A STUDY ON LONG-TERM TRIALS ACROSS CONTRASTING CLIMATIC AREAS

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**Introduction/Aim:** Vineyards implementing sustainable agricultural practices, such as cover crops, encourage soil microbial diversity and can enhance soil chemical parameters. Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with grapevines and play crucial roles in improving their nutrient and water uptake. Moreover, AMF can influence plant secondary compound synthesis and fruit quality. Nevertheless, the interaction cover crops, AMF diversity and grapevines productivity might be affected by climatic parameters that can modulate the output of the symbiosis. This study aims to investigate how cover crop influences AMF abundance and diversity in vineyards in Chile across contrasting climatic areas, and how these can be linked with prokaryotes and grapevine productivity.

**Materials and Methods:** Soil samples were collected from organic vineyards with and without cover crops in Chile across four contrasting climatic areas. Illumina sequencing of AMF and trap plant establishment were carried out. Moreover, soil prokaryotes were characterized and interactions with AMF described by network analyses.

**Results:** Under drought conditions the interaction cover crops, AMF diversity and quality of grapevines was stronger. Cross-domain networks were more complex in abiotic stressed cultivation areas. Grapevine productivity was linked to the abundance of certain AMF and prokaryotes retrieved in hubs in soil microbial networks.

**Discussion:** Cover crops offer numerous benefits, e.g. enhanced soil structure, moisture retention and organic matter addition, creating an environment suitable to soil microorganism functionality.

**Conclusions:** This study contributes to better understand the microbial diversity in cultivated sites in the Chilean Mediterranean region. It has implications for the productivity, sustainability and resilience of viticulture against environmental and agronomic challenges.

**Keywords:** Arbuscular mycorrhizal fungi (AMF), Soil microorganisms, Cover crops, Vineyards, Sustainable agricultural practices

## Managing Mycorrhizas for Sustainability & Food Security

### ARBUSCULAR MYCORRHIZAL FUNGI CONTRIBUTE TO THE ROOT AND RHIZOSPHERE ECONOMICS OF LANDRACES AND MODERN CULTIVARS OF TEMPERATE MAIZE

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**Introduction/Aim:** A holistic understanding of plant strategies to acquire soil resources is pivotal in achieving sustainable food security. However, we lack knowledge about variety-specific root and rhizosphere traits for resource acquisition, their plasticity and adaptation to drought.

**Materials and Methods:** We conducted a greenhouse experiment to phenotype root and rhizosphere traits (mean root diameter, specific root length, root tissue density, root nitrogen content, specific rhizosphere mass, arbuscular mycorrhizal fungi colonization) of 16 landraces and 22 modern cultivars of temperate maize (*Zea mays* L.).

**Results:** Our results demonstrate that landraces and modern cultivars diverge in their root and rhizosphere traits. Although landraces follow a 'do-it-yourself' strategy with high specific root lengths, modern cultivars exhibit an 'outsourcing' strategy with increased mean root diameters and a tendency towards increased root colonization by arbuscular mycorrhizal fungi. We further identified that specific rhizosphere mass indicates an 'outsourcing' strategy. Additionally, landraces were more drought-responsive compared to modern cultivars based on multitrait response indices.

**Discussion:** We suggest that breeding leads to distinct resource acquisition strategies between temperate maize varieties.

Conclusions: Future breeding efforts should increasingly target root and rhizosphere economics, with specific rhizosheath mass serving as a valuable proxy for identifying varieties employing an outsourcing resource acquisition strategy.

**Keywords:** arbuscular mycorrhizal fungi, Index for Adaptive Responses, phenotypic plasticity, rhizosheath, root economics space

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**ARBUSCULAR MYCORRHIZA AND ROOTS ALTER SOIL WATER RETENTION**

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**Introduction/Aim:**The symbiosis of plants with fungal partners can benefit plant water status in drought scenarios. While water flow and retention in soil are strongly driven by soil texture and structure, the presence of roots and hyphae can potentially alter these characteristics. Nevertheless, there is a lack of exploration of experimental evidence.

**Materials and Methods:**We investigated how AMF alter water flow in soil and water status of plants during drought. We sterilized sandy soil and recolonized it only with bacteria. Half of the soil was inoculated with rhizophagus irregularis. Sorghum plants were grown for six weeks in pots with either inoculated soil or the control and then subdued to a dry-down phase, during which plant and soil parameters were measured. Each pot contained also two cylinders filled with the same soil and covered with either a wide or narrow gaze to allow root and hyphal growth, or only hyphal growth, respectively. After harvest we extracted the cylinders and determined water retention and hydraulic conductivity curves.

**Results:**The presence of roots and hyphae changed the soils' water retention and hydraulic conductivity curves, i.e., more water could be sustained in wet soil and during drying without roots and hyphae. In drying soil, hydraulic conductivity was highest in soil with root and hyphae. Plants grown with and without AMF showed no significant difference in their reaction to drought.

**Discussion:**In our pot experiment, drought-adapted sorghum could not benefit from the symbiosis with AMF.

**Conclusions:**We could show soil water retention alterations due to the presence of hyphae and roots.

**Keywords:** Sorghum bicolor, drought, AMF, hydraulic conductivity

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## Managing Mycorrhizas for Sustainability & Food Security

### BIODIVERSITY OF ERICOID MYCORRHIZAL FUNGI IN CULTIVATED AND WILD CRANBERRY (*VACCINIUM MACROCARPON* AIT.) POPULATIONS IN WISCONSIN

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**Introduction/Aim:** Associations with fungal endosymbionts are near-universal for vascular plants, and while cranberries (*Vaccinium macrocarpon*) are no exception, details of their relationship with beneficial root endophytes remains a significant knowledge gap for cranberry agriculture. Ericaceous plants, including cranberry, blueberry, rhododendron and more, form mutualisms with Ericoid mycorrhizal fungi (ErMF), the endophytic root fungi that co-evolved with them in acidic peat bogs.

**Materials and Methods:** In this study, we report the sequencing and metabarcoding analysis of all fungal root endophytes from cranberry root samples across wild and cultivated growth sites in Wisconsin, USA. Roots of *V. macrocarpon* were sampled from seven different locations of wild cranberry growth and from the farms of five cooperating growers. Roots were cleaned, surface-sterilized, and freeze-dried, then sent for Illumina sequencing focusing on the ITS2 region.

**Results:** Preliminary results indicate substantial variation in the cranberry root microbiome between wild and cultivated sites, as well as across farms based on the growing region.

**Discussion:** While we had hypothesized there would be more diversity in the wild sites, cultivated cranberry roots demonstrated 1613 fungal species, as opposed to 572 fungal species in the wild cranberry roots, with 69 species found in both settings. Principal Coordinates Analysis shows that cultivated fungal communities and wild fungal communities cluster separately, demonstrating their overall dissimilarity.

**Conclusions:** Our results lend credence to the notion that agricultural management practices in cranberry may affect the community composition and populations of both the microbiome as a whole and the beneficial microbes that these plants rely on for survival and stress mitigation.

**Keywords:** *Vaccinium macrocarpon*, ericoid mycorrhizal fungi, root endophytes, biodiversity, metabarcoding, next-gen Illumina sequencing

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## Managing Mycorrhizas for Sustainability & Food Security

### MYCORRHIZAL DIVERSITY AND COMPOSITION: ECOSYSTEM DRIVERS AND AGROECOLOGICAL ENGINEERING

Cameron Wagg

Cameron Wagg

**Introduction/Aim:** Mycorrhizal fungi play a central role in carbon and nutrient cycling through their ability to facilitate the transfer of carbon and nutrients between the above and belowground ecosystems through their symbiosis with plants that together supports various ecosystem services. Yet their diversity and composition varies considerably among ecosystems. What are the drivers and how can we ecologically engineer them to support agroecosystem services?

**Materials and Methods:** Root associated fungal communities were assessed from ecosystems across Atlantic Canada varying in climate, soil, and anthropogenic management to determine drivers of mycorrhizal diversity. In a plot study I assessed the potential of crop traits, identity and diversity to promote mycorrhizal fungi in arable agricultural fields.

**Results:** While diversity was greatest in croplands and pastures compared to forests and wetlands, the relative abundance of mycorrhiza to fungal pathogens was the lowest. Plot trials revealed that mycorrhizal abundance and diversity can be enhanced by cover crop composition and their associated traits over greater crop diversity.

**Discussion:** The depletion of mycorrhizal fungi relative to pathogens in croplands is attributed to intensive arable cropping where soils are depleted in soil carbon and have excess soil nitrate compared to unmanaged ecosystems. Specific crops and combinations of them and their traits may be used a tool to promote healthy mycorrhizal communities in managed agricultural ecosystems.

**Conclusions:** Anthropogenic management activities can promote pathogens over mycorrhiza, yet it is possible to engineer more diverse and desirable composition of soil fungal communities through agroecological management, such as through the use of cover crops and their traits.

**Keywords:** Atlantic Canada, Boreal forest, Plant functional traits, Soil health, Arable cropping systems



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## Managing Mycorrhizas for Sustainability & Food Security

### INTERACTIONS BETWEEN INOCULATION WITH ARBUSCULAR MYCORRHIZAL FUNGI AND FERTILIZATION WITH DIFFERENT ORGANIC PRODUCTS IN ROSEMARY PLANTS

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**Introduction/Aim:** The use of organic fertilizers is emerging as paramount amidst the scarcity of mineral nutrients and fossil fuels needed to manufacture and transport inorganic fertilizers. The processing of livestock manure may improve its quality as a fertilizer. Additional crop nutritional benefits can be obtained through root inoculation with arbuscular mycorrhizal fungi (AMF). However, relatively few agronomical studies have been conducted on the combined application of different organic fertilizers and the inoculation of AMF.

We studied the response of plants to the interaction between AMF inoculation and fertilization with a set of organic products, which had the same origin (cow manure) but had been subjected to different processes.

**Materials and Methods:** Rosemary plantlets (160) were used in a factorial (mycorrhizae\*fertilizer) trial in 1L containers with washed and autoclaved river sand. The mycorrhizal factor consisted of inoculation with *Rhizoglyphus irregularis* and without AMF inoculation. The fertilizer factor comprised mineral fertilizer (with and without), as well as bioacidified, digested, a composted mixture of cow manure and olive oil mill wastes, and digestate/compost enriched with dark septate endophytes (DSE; *Cladosporium* sp.). All fertilizers were applied at the beginning of cultivation (170 kg-N·ha<sup>-1</sup>). After 218 days of plant growth under greenhouse conditions, the relative chlorophyll content, stem diameter, plant height, and aerial and root part biomass were measured.

**Results:** Mycorrhized plants assimilated nutrients from all organic fertilizers more efficiently than non-mycorrhized plants and produced higher biomass yields ( $p < 0.05$ ).

**Discussion:** Among non-mycorrhized plants, those fertilized with DSE-enriched digestate also resulted in significantly higher yields, which were analogous to mycorrhized plants.

**Conclusions:** Conclusions

**Keywords:** Arbuscular mycorrhiza, compost, dark septate endophytes, biofertilizer, digestate, *Rosmarinus officinalis*.

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## **Managing Mycorrhizas for Sustainability & Food Security**

### **DEVELOPING A MANAGEMENT QUALITY FRAMEWORK TO PRODUCE MYCORRHIZAL FUNGI INOCULANTS: PHASE 1. QUALITY CONTROL**

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**Introduction/Aim:** Biostimulant market has been growing at fast approval rate in recent years. Policies and initiatives that promote increased crop output and a reduction in the use of chemical pesticides in food production are driving changes in legislation and policy that affect food security. The passage on which these changes are happening, is hampering the opportunity for farmers to source high quality inoculant mycorrhizal fungi (MF)-based products. Infective propagules (IP) products could be as effective as spore-based products, or better at keeping their viability, according to our previous analysis.

**Materials and Methods:** To construct corporate quality standards on the production of MF inoculants and use these results to implement future guidelines as baseline for developing formulations based on MF, a bioassay is being carried out to assess the effectiveness of infection of propagules from 10 coded commercial products available on the area of UK. In short, sorghum seeds treated with a suspension of the different products will be evaluated 6 weeks after treatment and results will be compared.

**Results:** Previous experiments (data not shown) showed differences between two commercial products on their potential to infect sorghum roots after 21d.

**Discussion:** We propose to use the recommendations derived from previous publications and apply a general management quality framework, as proposed by Solomon, et al. (2022) for production of MF inoculants, which in turn, would contribute to deliver high quality commercial products.

**Conclusions:** We hypothesize that, quality of the inoculants will vary greatly within the tested samples. The results and discussion of it will be done on due course.

**Keywords:** Mycorrhizal fungi, commercial product, infectivity, *Glomus* sp, *Rhizophagus irregularis*, stability test.

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## Managing Mycorrhizas for Sustainability & Food Security

### GENETIC VARIATION IN RICE ROOT COLONIZATION BY ARBUSCULAR MYCORRHIZAL FUNGI DEPENDS ON FUNGAL IDENTITY

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**Introduction/Aim:** Harnessing AM fungi has the potential to improve sustainability of rice production, though the efficacy of commercial inoculants remains controversial. A wide genetic variation in rice root colonization with *Rhizophagus irregularis* has been established. However, fewer studies have tested this against different AM fungal species.

**Materials and Methods:** To address these, we conducted a pot experiment with five rice cultivars and three AM fungal species applied singly or as a community and a field experiment with ten rice cultivars under rain-fed conditions with or without commercial inoculant.

**Results:** In both experiments, root colonization differed between host cultivars and AMF. We found contrasting results on plant growth and nutrient uptake in the pot experiments depending on the host and species interaction.

**Discussion:** Rice root colonization rates with mixed and singly applied AMF were inconsistent and dependent on the host cultivar, suggesting host specificity. *C. claroideum* improved rice growth and N uptake, while *R. irregularis*, *F. mosseae*, and a mixed AMF reduced growth, and P and N uptake, independent of colonization rate. Similarly, colonization levels of field-grown rice were cultivar-dependent and varied between time points. Root colonization levels, however, did not correlate with plant growth, yield or nutrient uptake.

**Conclusions:** The wide genetic variation in rice root colonization and plant growth response was host- and species-specific, and the variation is inconsistent with earlier findings. Application of commercial inoculants increased colonization but not growth and nutrient uptake under rainfed conditions. Further research is needed to determine the biological significance of differing colonization rates among rice cultivars.

**Keywords:** AM fungi, P uptake, rice, root colonization, *Rhizophagus irregularis*, *Claroideoglomus claroideum*

## Managing Mycorrhizas for Sustainability & Food Security

### EXPLOITING ARBUSCULAR MYCORRHIZAL FUNGI TO DECELERATE THE NITROGEN CYCLE

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**Introduction/Aim:** Anthropogenic nitrogen (N) inputs into soil now exceeds those from natural pathways, accelerating severe environmental impacts. Nitrification is the major process that results in loss of inorganic ammonium-based fertilizers from agricultural soil, producing nitrate that pollutes water courses, and directly and indirectly produces the potent greenhouse gas nitrous oxide (N<sub>2</sub>O). As arbuscular mycorrhizal fungi (AMF) transfer N from soil to plant, an understanding of their impact on nitrification-related processes under different fertilizer scenarios is required for harnessing their potential to increase nitrogen use efficiency and mitigate pollution.

**Materials and Methods:** To determine interactive impact of AMF, plant and nitrifying prokaryotes, unsterilized agricultural soil mesocosms with low or high (spore-inoculated) AMF-colonized wheat plants were applied with inorganic ammonium sulphate, slow-release ammonium fertilizer or plant-based organic N, together with synthetic nitrification inhibitors (SNIs) to differentiate activity.

**Results:** As expected, soil applied with SNIs had relatively high ammonium and low nitrate concentrations, and abundance of ammonia oxidizers (AO) and N<sub>2</sub>O emissions were lower. Despite predicted niche partitioning for different AO groups related to ammonium N source (inorganic versus organic) there was an overall trend of lower AO abundance and N<sub>2</sub>O emissions in high compared to low AMF-colonized mesocosms for all fertilizers. AO community differences between AMF treatments were found across all lineages.

**Discussion:** As high compared to low AMF-colonized wheat had greater root biomass, but were similar in ammonium uptake, both direct and indirect AMF effects potentially occurred.

**Conclusions:** Findings support that AMF contribute to reducing impact of nitrification derived N pollution under a range of agricultural fertilizer regimens.

**Keywords:** Arbuscular mycorrhizal fungi, Agriculture, Nitrogen cycle, Nitrification, Ammonia oxidizers, Fertilizers

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## Managing Mycorrhizas for Sustainability & Food Security

### THE RESPONSE OF MOTILITY AND BIOFILM FORMATION OF BACTERIA TO THE HYPHOSPHERE ENVIRONMENT AT THE TRANSCRIPTIONAL LEVEL

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with more than 80% of terrestrial plants. AMF produce enormous mycorrhizal networks in the soil. The AMF hyphae secrete carbon sources to soil, making the hyphosphere a unique interface for biological interactions. However, how hyphosphere bacteria chemotaxis respond to exudates and colonize the hyphal surface remain unclear.

**Materials and Methods:** In this study, we used the carrot hair root-AMF in vitro culture system to study the hyphosphere interaction of AMF *Rhizophagus irregularis* MUCL 43194 and the phosphate-solubilizing bacterium *Rahnella aquatilis* (RA) HX2. The genes related to RA flagella synthesis (*fliC*, *fliD*, *fliF*, *motA*, *motB*), chemotaxis (*cheA*, *cheW*, *cheY*, *tap*, *tsr*), biofilm formation (*epsA1*, *epsA2*, *epsB1*, *epsB2*, *epsC*, *epsD*, *epsP1*, *epsP2*, *luxS*, *rcsA*, *rcsB*, *rcsC*, *rcsD*, *rstA*, *rstB*), and phosphorus metabolism (*acp1*, *alp*, *phoB*, *phoP*, *phoR*, *phy*) were selected. After 0.5, 6, 12, 24 and 48 h, the relative expression levels of those genes were analyzed to explore how AMF affected the formation of RA biofilm and the mineralization of organic phosphorus (P) at the transcriptional level.

**Results:** At 6-12 h after inoculation, AMF promoted the expression of *fliC*, *fliD*, *flagellar motA*, *motB*, *epsB1*, *epsD*, and *phoB* in RA. And *epsB2*, *epsC*, *epsP1* and phosphatase gene *phy* were highly expressed in the late stage.

**Discussion:** RA regulated motility, biofilm formation, P metabolism by regulating the expression of related genes.

**Conclusions:** This study indicates that with the influence of hyphae exudates, in hyphosphere RA will move chemotactic and accelerate the formation of biofilm, and begin to mineralize organic phosphorus.

**Keywords:** AM fungi, hyphosphere bacteria, hyphae exudates, bacteria chemotaxis, biofilm

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## Managing Mycorrhizas for Sustainability & Food Security

### INFLUENCE OF THE SELECTED ERICOID MYCORRHIZAL FUNGI ON NUTRIENTS UPTAKE BY BLUEBERRY PLANTS IN RHIZOTRONS UNDER CONTROL CONDITIONS

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**Introduction/Aim:** Blueberries (*Vaccinium corymbosum* L.) originated in the United States and the production and consumption of blueberries have increased sharply in the world. Ericoid mycorrhizae are fungi (EMF) that form a mutualistic relationship with the roots of blueberry plants and can increase their uptake of soil nutrients and crop yield.

The goal of the research was to study beneficial potential of the selected EMF associated with blueberries and the influence of ericoid mycorrhiza on nutrients uptake by blueberry plants.

**Materials and Methods:** The most typical species of EMF (*Hyaloscypha hepaticicola* (D.J Read) and *Oidiodendron maius* (G.L. Barron)) associated with blueberry plant roots was used for the experiment. It was used 'Legacy' and 'Duke' varieties for the experiment, because they are recognized as the most adaptable and spreading varieties in Georgia and in many countries.

**Results:** Research revealed that the inoculation with a selected EMF has beneficial influence to absorb nitrogen, phosphorus, and potassium (NPK) nutrients by 'Duke' variety however in case of 'Legacy' control variant has better absorption percentage than in case of inoculated ones. The results of all cases were not significantly different ( $P > 0.05$ ).

**Discussion:** Several authors reported that EMF favored the nutrients uptakes by blueberry plants from substrate, however the same inoculum may impact different plants in different ways, and plant responses cannot be safely generalized by species across varying ecosystems.

**Conclusions:** In conclusion, further research over a longer time span is needed for unambiguous results.

**Keywords:** *Vaccinium corymbosum*, Ericoid Mycorrhizae, Rhizotrons, Control conditions

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## Managing Mycorrhizas for Sustainability & Food Security

### EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON ROOT COLONIZATION AND GENETIC VARIATIONS OF TEFF GENOTYPES IN TIGRAY, ETHIOPIA

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**Introduction/Aim:** Teff, a significant cereal crop grown in Ethiopia, has shown noteworthy genetic diversity. In order to adapt to resource-limited conditions, it forms a symbiotic relationship with arbuscular mycorrhizal fungi (AMF). In recent times, AMF has become a crucial bio-inoculant in agronomic and plant breeding programs.

**Materials and Methods:** A greenhouse experiment conducted using a Complete Randomized Design (CRD) with ninety selected teff genotypes assessed the genetic variability and level of dependency of teff on AMF based on twelve morphological traits.

**Results:** The results showed that all traits were significantly different among the genotypes and they were divided into six distinct clusters. High genetic divergence was observed among the tested teff genotypes evaluated between AMF inoculated genotypes.

**Discussion:** Teff genotypes with a higher root colonization percentage (RCP) of 54.42 to 72.29% were significantly correlated with root dry matter, root length, shoot biomass yield, and grain yield compared to low RCP (25.03 to 34.32%). The group genotypes having higher genetic variability when colonized with AMF showed wider dependency of teff genotypes on AMF with implications for plant breeding

**Conclusions:** AMF-induced roots in teff genotypes show significant potential for plant breeding programs. Developing varieties that thrive in moisture-stressed and nutrient-deficient areas can enhance crop yield and sustainability in resource-limited regions. These findings can contribute to the development of more resilient crop varieties.

**Keywords:** genotypes, genetic diversity, inter-cluster distance, morphological traits, root colonization

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## Managing Mycorrhizas for Sustainability & Food Security

### EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND NITROGEN LEVELS ON MICRONUTRIENT UPTAKE, NUTRIENT, AND WATER USE EFFICIENCIES OF TEFF

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**Introduction/Aim:** Excessive nitrogen fertilizer during teff cultivation leads to soil degradation and increased production costs. To improve nutrient use efficiency, explore methods such as using arbuscular mycorrhizal fungi, selecting genotypes that optimize applied nutrients, and appropriate nitrogen fertilization rates might be an option.

**Materials and Methods:** A study investigated the effects of using two teff genotypes, improved (Kora) and local (Zezew), with and without AMF, along with five nitrogen fertilizer rates. The study was conducted using a factorial Randomized Complete Block Design (RCBD) under irrigated teff in field conditions.

**Results:** Research indicates that pairing Zezew genotype with AMF at 138 kg ha<sup>-1</sup> N leads to higher crop yields and better nutrient uptake, while lower N fertilizer application can result in higher efficiency.

**Discussion:** Appropriate combination of Zezew teff genotypes with AMF and 138 kg ha<sup>-1</sup> N fertilizer can enhance crop quality and yield on nutrient-deficient soils while reducing production costs due to improved yield, nutrient uptake, and water use efficiency.

**Conclusions:** Using Zezew teff genotypes with AMF and N fertilizer can enhance crop quality and yield on nutrient-deficient soils while reducing the need for further N application and production costs.

**Keywords:** Iron, Zinc, Nutrient uptake, teff genotype, use efficiency



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## Managing Mycorrhizas for Sustainability & Food Security

### GENETIC DIVERSITY BOOSTS ARBUSCULAR MYCORRHIZAL RESPONSIVENESS, GROWTH, AND WATER STRESS RESILIENCE IN LOCAL KENYAN MAIZE GENOTYPES

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**Introduction/Aim:** Climate change and dwindling soil fertility pose significant challenges to smallholder farmers in Africa, who mainly rely on rainfed agriculture with limited farm inputs. To address these challenges and enhance staple crop productivity, particularly maize, there is a critical need for adoption of sustainable and climate-smart agricultural practices. In this study, we investigated the effects of mycorrhizal inoculation and genetic diversity on Kenyan maize varieties' growth and resilience to water stress.

**Materials and Methods:** Twenty maize varieties commonly grown in Kenya were cultivated in a greenhouse and inoculated with *Rhizophagus irregularis* (BEG 44) or *Funneliformis mosseae* (BEG 12). The four most responsive varieties (V8, V9, V10, and V11) were selected for further experimentation in pots, grown either as monovarietal or multivarietal cultures, inoculated with arbuscular mycorrhizal fungi (AMF), and subjected to varying watering regimes (4, 8, or 12 days).

**Results:** Results revealed a significantly higher responsiveness ( $p=0.0001$ ) of local maize varieties to BEG 44 compared to BEG 12. Notably, maize plants in multivarietal cultures and inoculated with AMF exhibited increased plant height, shoot dry weight, root dry weight, and chlorophyll content ( $p=0.0001$ ) compared to non-inoculated maize in monovarietal cultures. Furthermore, multivarietal cultures demonstrated remarkable resilience ( $p=0.0001$ ) to water stress compared to their monovarietal counterparts.

**Discussion:** Our findings highlight the positive impact of AMF inoculation and genetic diversity within multivarietal cultures in enhancing local maize performance.

**Conclusions:** This is attributed to improved nutrient uptake and resilience to water stress, offering a promising solution for boosting maize production and food security in Kenya's agricultural landscape.

**Keywords:** Maize Genetic diversity Arbuscular mycorrhizal fungi (AMF) Resilience Water stress Maize, Genetic diversity, Arbuscular mycorrhizal fungi (AMF), Resilience, Water stress, Kenya

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## Managing Mycorrhizas for Sustainability & Food Security

### A TRADE-OFF BETWEEN SPACE EXPLORATION AND MOBILIZATION OF ORGANIC P VIA ASSOCIATED MICROBIOMES ENABLES NICHE DIFFERENTIATION OF AMF ON THE SAME ROOT

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**Introduction/Aim:** Ecology seeks to explain species coexistence, but experimental tests of mechanisms for coexistence are difficult to conduct. We synthesized an arbuscular mycorrhizal (AM) fungal community with three fungal species that differed in their capacity of foraging for orthophosphate (P) due to differences in soil exploration.

**Materials and Methods:** We tested whether AM fungal species-specific hyphosphere bacterial assemblages recruited by hyphal exudates enabled differentiation among the fungi in the capacity of mobilizing soil organic P (Po).

**Results:** We found that the less efficient space explorer, *Gigaspora margarita*, obtained less <sup>13</sup>C from the plant, whereas it had higher efficiencies in Po mobilization and alkaline phosphatase (ALPase) production per unit C than the two efficient space explorers, *Rhizophagus intraradices* and *Funneliformis mosseae*. Each AM fungus was associated with a distinct alp gene harboring bacterial assemblage, and the alp gene abundance and Po preference of the microbiome associated with the less efficient space explorer were higher than those of the two other species.

**Discussion:** The trade-off between foraging ability and the ability to recruit effective Po mobilizing microbiomes is a mechanism that allows co-existence of AM fungal species in a single plant root and surrounding soil habitat.

**Conclusions:** We conclude that the traits of AM fungal associated bacterial consortia cause niche differentiation.

**Keywords:** arbuscular mycorrhizal fungi, carbon, space exploration, hyphosphere bacterial assemblages, organic phosphorus mobilization

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### THE USE OF MYCORRHIZA IN VINEYARDS TO MITIGATE THE ADVERSE EFFECTS ASSOCIATED TO CLIMATE CHANGE ON GRAPE MATURATION WITH CONSEQUENCES IN MUST QUALITY

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**Introduction/Aim:** Vineyard is a vulnerable crop to climate change, especially in the Mediterranean basin. Under an increasing temperature and drought scenario, vines modify their phenology changing wine oenological, chemistry, and organoleptic characteristics. Particularly, the grapes become less acidic, altering key quality factors as flavor, stability and color.

The project LOWPHWINE comprises 18 public-private entities from clone selection to vineyard management. Our objective is to apply the arbuscular mycorrhiza fungi (AMF) "*Rhizoglyphus irregulare strain*" MV1 to promote metabolic pathways of acidic compounds and plant stress tolerance.

**Materials and Methods:** Field tests were done in two Spanish wineries: Pago de Carraovejas and Barbadillo, and the plant metabolomic profile was analyzed by UHPLC/QTOF-MS.

**Results:** The berries with AMF treatment increased the citric acid, malic acid and succinic acid content in both locations. Besides, in Pago de Carraovejas they showed increased flavonoid content derived from phenylalanine, along with volatile compounds derived from benzene. In Barbadillo, they had a high level of flavonoid and synthesis of jasmonates and tetrapyrroles.

The leaves in Pago de Carraovejas showed an increase in phenolic and prenol lipids whereas in Barbadillo increased phenylalanine, flavonoids, benzene compounds, brassinosteroids and jasmonates.

**Discussion:** The metabolic changes induced by AMF in berries increased their acidity, which is associated with the production of wines with highly valuable organoleptic properties and the storing stability.

AMF also induced antioxidant compounds denoting a higher potential to tackle oxidative stress.

**Conclusions:** "*R. irregulare*" MV1 promoted vine metabolites that are associated with higher wine quality. Besides, it increased metabolites involved in crop stress tolerance.

**Keywords:** Arbuscular mycorrhizal fungi, Climate change, Vineyard, Wine industry, Organic acids, Antioxidants

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### DRIVERS OF MYCORRHIZAL COMMUNITY ASSEMBLY AT THE LANDSCAPE SCALE

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**Introduction/Aim:** Mycorrhizal fungi have key ecological functions in grasslands such as enhancing nutrient acquisition, mediating plant-plant competition and providing resistance against drought and disease. Yet, to manage their functions effectively we must be aware of key drivers of their distribution from both anthropogenic and natural sources. Here, we present results from a nationwide soil survey exploring the relationship between grassland management intensity and soil characteristics on mycorrhizal fungal distribution.

**Materials and Methods:** Soils were collected from 48 English sites representing a range of grassland types. At each site we characterised plant species composition using morphological identification, soil abiotic characteristics, and mycorrhizal fungal community composition through phylogenetic marker gene sequencing.

**Results:** We show how key environmental gradients influence the distribution of mycorrhizal fungi at the national scale and how differences in land management may influence their function and community assembly. Furthermore, we discuss data from a long-term field manipulation experiment showing how mycorrhizal fungi respond in grasslands under contrasting management scenarios.

**Discussion:** We note that mycorrhizal distribution is strongly correlated with soil pH, agreeing with previous results from other studies. Nevertheless, we note that key indicator species also correlate with certain AMF species; yet, grassland type explains variation in community composition not captured by soil abiotic variables and plant community composition.

**Conclusions:** These findings provide insight into how mycorrhiza respond to grassland management which underpins their utility to provide sustainable solutions for healthy agricultural landscapes.

**Keywords:** agriculture, grassland, fungi, soil management, long-term field site, phylogenetic marker gene

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### Managing Mycorrhizas for Sustainability & Food Security

#### ARBUSCULAR MYCORRHIZAL FUNGI DRIVE RAPID SOIL AGGREGATION, SOIL ORGANIC CARBON STORAGE AND MICROBIOME FUNCTIONAL DIVERSITY IN TROPICAL AGRICULTURE

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**Introduction/Aim:**The tropics is the most critical region for food production because of its rapidly growing human population. Tropical agricultural soils show high CO<sub>2</sub> fluxes, making them very large contributors to global CO<sub>2</sub> emissions. Current management practices that increase soil organic carbon (SOC) and aggregation are incredibly slow, taking decades to show effects. Arbuscular mycorrhizal fungi (AMF) channel carbon below-ground, contributing to these processes. However, it's notoriously difficult to measure AMF contribution to these processes in agriculture. Using genetic variation of AMF in tropical field trials, we quantified how much AMF inoculation contributes to SOC accumulation, aggregation and CO<sub>2</sub> efflux.

**Materials and Methods:**We inoculated cassava in conventional farming with genetically variable *Rhizophagus irregularis*. We measured soil aggregation and SOC (at two depths), CO<sub>2</sub> efflux, the soil bacterial metagenome, and crop growth.

**Results:**We found that inoculation of very closely related siblings of AMF led to surprisingly large differences in the formation of critical soil aggregate sizes, SOC and CO<sub>2</sub> efflux in 1 year. AMF inoculation also determined the functional diversity of the soil bacterial metagenome.

**Discussion:**Our results are important for understanding how AMF inoculation significantly contributes to important soil processes: 1. AMF effects on soil aggregation are much more rapid than expected; 2. Closely related AMF strains affect these soil processes in different ways; 3. These fungi drive functional gene diversity of bacterial microbiomes in an unexpected way.

**Conclusions:**Our study demonstrates that applying AMF can quickly lead to greater soil sustainability in large-scale tropical agricultural management in regions of the most rapidly growing populations.

**Keywords:** soil organic carbon, inoculation, agriculture, carbon emissions, aggregation, soil sustainability

**Managing Mycorrhizas for Sustainability & Food Security**

**SUCCESSIONAL DYNAMICS OF FUNGAL COMMUNITIES IN REMEDIATED SOILS FOLLOWING THE REMOVAL OF METAL CONTAMINANTS**

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**Introduction/Aim:**We investigated the importance of plant cover and inoculation for the secondary succession of soil- and plant-root fungal communities in remediated soils where EDTA washing was used to remove metal contaminants.

**Materials and Methods:**Molecular methods were used to quantify the abundance of fungi (qPCR, ITS markers) and to characterise arbuscular mycorrhizal fungal (AMF) communities in roots (18S rRNA, metabarcoding) in two soil types, before and after remediation and with sown "Lolium perenne" or without plant cover.

**Results:**The presence of plants was the main factor determining the abundance of ITS gene copies, which increased in the rhizosphere in most treatments, but remained at low levels in the soil without plants. While soil fungi could be detected at the end of the first season, AMF structures were not found in roots in any of the treatments during the entire first season. However, one year after the start of the experiment, AMF colonisation was detected in roots in most of the treatments. Interestingly, the addition of environmental inoculum (rhizosphere soil) had no positive effect on fungal ITS copy number in the soil during the first growing season, but had a significant effect on the development of the AMF community in roots during the second year, which was more diverse in the inoculated treatments.

**Discussion:**This study demonstrates the importance of the plant and rhizosphere as well as inoculation for the development of fungal communities in treated soil.

**Conclusions:**This has important implications for the revitalisation of low-diversity soils, including soils following the removal of metal contaminants.

**Keywords:** AM fungi, community composition, revitalisation, soil biodiversity, succession

## Managing Mycorrhizas for Sustainability & Food Security

### SPATIAL DISTRIBUTION OF MYCORRHIZAL SPECIES IN SOUTHERN GHANA

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**Introduction/Aim:**Inherently low soil fertility in Ghana leads to low crop yield on farmers' field. Resource-poor farmers are not able to supply the needed mineral fertilizers for improving yield. Access and availability of fertilizers in most rural communities is a challenge. It is therefore imperative to seek sustainable ways of crop production. One of the alternatives is to use microbes. Mycorrhizal fungi play a key role in ecosystem health by providing nutrients to plants and drawing down carbon into soil systems. However, the power of these nutrient-providing fungal networks is under-exploited in Ghana's agriculture where there could be potential food insecurity in future. Ghana's coastal areas which are threatened by erosion are predicted to have diversity of mycorrhizal species. This study therefore sought to: (i) map the biodiversity of mycorrhizal communities in Southern Ghana; and (ii) to determine the species contribution to crop growth.

**Materials and Methods:**Fungal DNA was extracted from thirty-eight soil samples collected from the coastal areas of Ghana and sequenced through mycorrhizal -specific and general fungal primers.

**Results:**This was used to identify the mycorrhizal communities of these regions and to examine whether different mycorrhizal species offer diverse services (salt, drought resistant etc.).

**Discussion:**Having mycorrhizal communities that possess superior symbiotic efficiency compared to industrial standards and mineral fertilizers will be beneficial to smallholder farmers.

**Conclusions:**This study has implications for agricultural practices and the restoration of the coastal forests of Ghana.

**Keywords:** Mycorrhiza, coastal areas, biodiversity, mapping

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### ARBUSCULAR MYCORRHIZAL FUNGI OF NATURAL GRASSLANDS BENEFIT FORAGE CROP GROWTH AND SALINITY TOLERANCE ONLY UNDER CERTAIN CONDITIONS

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**Introduction/Aim:** Agricultural soils contain less diverse and abundant arbuscular mycorrhizal fungi (AMF). Given their potential importance for plant growth, there is interest in using inoculants to restore AMF communities and mycorrhizal functioning. Not all plants benefit from the same AMF, however, and AMF vary greatly among environments, making restoration of mycorrhizal functioning challenging.

**Materials and Methods:** To identify AMF inoculants for salt-affected forage systems, we collected soils from 41 grassland sites in spring or late summer and cultured them using three different trap culture species. We inoculated three forage species of different functional groups: a C3 annual grass, a C3 perennial grass, and a perennial legume under saline and non-saline conditions. We also sequenced the AMF from the field soils to determine whether AMF composition explained the inoculation effects.

**Results:** Inoculation responses depended on sampling time, trap culture and responding species, and greenhouse salinity treatment. Contrary to expectation, the annual grass benefited most from inoculation and spring collected cultures were more beneficial, but also more variable, especially when salt-treated; although this depended on the trap culture species. Inoculation effects were associated with variation in AMF composition and diversity; however, these effects varied greatly among salt treatments, trap species, and response species.

**Discussion:** AMF have the potential to increase growth and salinity tolerance of annual crops; however, there are no uniformly beneficial AMF nor is there a consistent benefit from increasing AMF diversity.

**Conclusions:** A universally beneficial AMF inoculum is unlikely and inoculum development will have to be tailored to specific uses.

**Keywords:** AMF inoculation, agriculture, grasslands, barley, *Medicago sativa*



**Managing Mycorrhizas for Sustainability & Food Security**

**CONSERVATION AGRICULTURE MAY IMPROVE PLANT AND HUMAN HEALTH BY BOLSTERING MYCORRHIZAL NETWORKS**

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**Introduction/Aim:** Many farmers have adopted reduced tillage management practices. While the effectiveness of these practices at reducing soil erosion and enhancing soil health is well documented, the impact of reduced tillage on plant nutritional quality is not well understood and the relationship between tillage intensity and crop yield is inconsistent. Current interest in the role of the fungal derived antioxidant ergothioneine (ERGO) in human health has driven efforts to understand the influence of different crop management practices on the transfer of ERGO from soil to plants and ultimately to human consumption.

**Materials and Methods:** We sampled roots and plant tissue from soybeans (*Glycine max*) and wheat (*Triticum aestivum*) in a long-term (40+ year) side-by-side tillage trial and examined the extent to which moldboard plow (high intensity tillage), chisel/disk (intermediate tillage), and no-till (minimal disturbance) practices affected mycorrhizal colonization, ERGO concentration, mineral nutrient concentration, and yield.

**Results:** We found that high tillage intensity reduced the ERGO concentration of wheat grain by about half and reduced the total ERGO yield per plot of both wheat and soybeans by ~30%. The ERGO concentration of wheat was positively correlated with percent mycorrhizal colonization. Additional benefits of reduced tillage were increased concentration of soybean P, Mg, Cu, Zn, and increased soybean yield.

**Discussion:** We hypothesize that enhanced mycorrhizal colonization in reduced till plots played a key role in plant uptake of both ERGO and mineral nutrients from soils.

**Conclusions:** These results demonstrate mycorrhizal fungi as a possible link between soil health and human health by positively influencing ERGO and mineral nutrient uptake.

**Keywords:** tillage, soil health, conservation agriculture, mycorrhizal fungi, ergothioneine, reduced till

## Managing Mycorrhizas for Sustainability & Food Security

### HOW TO ALTER MYCORRHIZA-RESPONSIVENESS IN PETUNIA

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**Introduction/Aim:** Symbiosis with mutualistic arbuscular mycorrhizal (AM) fungi can lead in an overall improved plant performance. Although this so-called mycorrhiza-responsiveness could be of great importance for sustainable plant production systems, AM fungi are not widely used in agricultural and horticultural practice, because of the unpredictable outcome. As studies have shown, the variation is at least partially influenced by plant genotype, implying that AM-responsiveness could be a breeding target. Conversely, plant performance and thus AM-responsiveness is strongly affected by environmental conditions.

**Materials and Methods:** The environmental influence was investigated using 19 recombinant inbred lines (RILs) derived from the cross *Petunia axillaris* x *Petunia exserta*. Plants were inoculated with *Rhizoglyphus irregulare* and grown under three light and temperature regimes. In a subsequent experiment, three RILs were selected due to their light and temperature-dependent performance. After inoculation, the influence of light intensity, day length and sum of light per day on the AM-responsiveness of the three RILs was examined.

**Results:** It appears that: (i) for similar day length, a higher level of light intensity increases positive AM-responsiveness; (ii) increased light intensity by simultaneously shorted day length does not result in positive AM-responsiveness; and (iii) the genotypic AM-responsiveness is reproducible under similar environmental conditions.

**Discussion:** Although the correlation between the regimes was very low, no negative responses to AM fungi were observed in one of the environmental regimes, suggesting that AM-responsiveness can be genetically and environmentally altered.

**Conclusions:** Genetic and environmental factors influence plant responsiveness to AM fungi, highlighting the potential for targeted breeding and environmental optimization.

**Keywords:** AMF plant interaction, responsiveness, *Rhizoglyphus irregulare*, petunia

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### CHARACTERIZATION OF THE REPRODUCTIVE MODE OF THE DESERT TRUFFLE *TERFEZIA CLAVERYI* CHATIN

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**Introduction/Aim:** *Terfezia claveryi* is an ascomycete that establishes ectendomycorrhizal symbiosis with different *Helianthemum* species. In virtue of this symbiosis, edible hypogeous fruitbodies (desert truffles) are produced. They are regarded as a delicacy and represent a sustainable economic resource for arid and semiarid areas. Nevertheless, *T. claveryi* life-cycle and reproductive mode are still poorly understood. In ascomycetes the presence and distribution of the mating type genes, *MAT1-1-1* and *MAT1-2-1* dictate their reproductive strategies. Here we aim to unveil the structure of *MAT* locus in *T. claveryi* to gain evidence on the genetic control of its fructification.

**Materials and Methods:** Moving from bioinformatic analyses of the sequenced *T. claveryi* strain (T7) we designed a PCR-based approach to screen ascocarps and in vitro isolated strains to characterize the idiomorphs of the *T. claveryi* *MAT* locus.

**Results:** *T. claveryi* is a heterothallic species, its sequenced genome showed the only presence of *MAT1-1-1*. We isolated and characterized the alternative *MAT1-2-1* gene and its entire idiomorph in the *MAT1-1-1* lacking strains. Furthermore, a PCR-based approach has been designed to screen *T. claveryi* strains according to their mating type. **Discussion:** *T. claveryi* *MAT1-1-1* and *MAT1-2-1* genes contain their characteristic  $\alpha$ -box and High Mobility Group (HMG) domains, respectively. The screening of *T. claveryi* strains harbouring opposite mating types from nursery to the field is being carried out to investigate spatiotemporal distributional patterns of this species.

**Conclusions:** The characterization of the reproductive mode of *T. claveryi* and the spatiotemporal dynamics from mycorrhizal plantlets to open field will contribute to optimize management strategies of desert truffle cultivation.

**Keywords:** Desert truffles, *Terfezia claveryi*, mating type, edible mycorrhizal fungi

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## **Managing Mycorrhizas for Sustainability & Food Security**

### **THE USE OF AMF IN COMMERCIAL HORTICULTURE CROPPING SYSTEMS AND INCREASING RESILIENCE IN APPLE ORCHARDS**

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**Introduction/Aim:** Farmers need sustainable production approaches to overcome climate change, increased fertiliser costs and chemical resistance/removal. We investigate how increased abundance of microbes through amendments or management practices can benefit cropping. AMF are known to increase overall plant health and productivity, to alleviate stress, directly and indirectly, from abiotic and biotic factors.

European apple canker, *Neonectria ditissima*, a major disease of apple (*Malus domestica*) currently has little or no mitigation strategies available. Here we address the two biggest threats to apple production, canker disease and unpredictable weather events (drought and waterlogging) in newly planted orchards

**Materials and Methods:** Commercial products (AMF/*Trichoderma*) were applied in either newly established or existing commercial orchards. New orchards, either drought or waterlogging susceptible, were planted +/- amendments. Existing orchards, amendments were applied using a root-pruner or inoculated wildflowers. Assessments will be made over three growing seasons for colonisation, tree establishment, growth and canker.

**Results:** Results from newly planted orchards show some between treatment effects on growth, yield and canker expression, though there's large differences between sites. In existing orchards, we are seeing good wildflower establishment and colonization with AMF. Early results suggest beneficial trends in fruit quality.

**Discussion:** Early results from newly planted orchards show some between treatment effects on growth, yield and canker expression, though there are large differences between sites. In existing orchards, we are seeing good wildflower establishment and colonization with AMF. Early results suggest beneficial trends in fruit quality.

**Conclusions:** The use of microbes poses new challenges to farmers, further understanding is needed to incorporate them into commercial farming systems.

**Keywords:** horticulture, commercial, apple, canker, extreme weather, AMF

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### ASSESSING ARBUSCULAR MYCORRHIZAL FUNGI (AMF) RESPONSIVENESS IN DIFFERENT DURUM WHEAT GENOTYPES

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**Introduction/Aim:** Environmental stresses intensified by climate change are significantly impacting durum wheat (*Triticum turgidum* subsp. *Durum*) production. Arbuscular mycorrhizal fungi (AMF) represent promising bio-based tools to improve wheat tolerance to these stresses. However, AMF can have different effects on crops, depending on plant genotypes. This experiment was conducted to identify which durum wheat genotypes performed better in terms of AMF responsiveness. **Materials and Methods:** Fifteen durum wheat genotypes were inoculated with *Rhizophagus intraradices* and maintained in well-watered condition for 5 months. Root architecture (by WinRHIZO), grain number and mass, AMF colonization, and shoot mass, were evaluated. Data were used to select 8 genotypes showing contrasting results in terms of AMF responsiveness to be used in an experiment under abiotic (water stress and water logging) and biotic (*Fusarium*-infected plants) stress conditions.

**Results:** Mycorrhizal colonization assessment showed that all genotypes were colonized by AMF, with a mycorrhization rate ranging from 44% to 76%. Shoot dry mass ranged from 0.383 g to 0.118 g, underlining the variability within the set of tested genotypes. Additionally, 8 genotypes developed spikes, and 6 out of them produced two or more culms.

**Discussion:** Root traits, including root architecture, exudates, and AMF colonization responsiveness, are pivotal to elucidate the plant response to environmental stresses. Our results provided insights on the variability of durum wheat, highlighting that AMF responsiveness depend on genotype.

**Conclusions:** Based on this first trial, selected genotypes will be assessed under abiotic and biotic stress conditions, to study the signalling and immune mechanisms at molecular level.

**Keywords:** AMF responsiveness, durum wheat, root phenotypic plasticity, WinRHIZO

## Managing Mycorrhizas for Sustainability & Food Security

### ORGANIC MANAGEMENT SHAPES AM FUNGAL COMMUNITY STRUCTURE AND FUNCTION, PARTIALLY REVERSING THE NEGATIVE EFFECTS OF CONVENTIONAL AGRICULTURE

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**Introduction/Aim:** Arbuscular mycorrhizal (AM) fungi present a promising alternative to environmentally damaging fertilisers and pesticides in agroecosystems. However, our understanding of how agricultural practices influence the diversity and functions of AM fungi is limited, posing a significant challenge to their effective use in agriculture.

**Materials and Methods:** We assessed how different AM fungal communities shaped by either organic or conventional farming influence crop outcomes, using *Sorghum bicolor*. Plant growth and development was analysed alongside DNA metabarcoding of AM fungal community composition.

**Results:** We observed that AM fungi from conventional agricultural fields resulted in a pronounced reduction in sorghum biomass (-52%) and a significant delay in flowering compared to the non-AM fungal control. Sorghum biomass was also reduced for fungi from the organic system, but to a lesser extent (-31%) and without a delay in flowering. Organic systems were associated with a large proportion of taxa (49% of VTs) not found in conventional systems, including *Diversispora* and *Archaeospora* spp., but also shared a large proportion of taxa with conventional systems (44% of VTs). Conventional systems had relatively few unique taxa (7% of VTs).

**Discussion:** Conventional agricultural practices favoured less-beneficial AM fungi. Organic management practices reversed this negative effect, supporting more diverse communities, but only partially, likely due to the continued presence of those less beneficial taxa.

**Conclusions:** Our study underscores the pivotal role of agricultural management in shaping the functionality of AM fungal communities for crops. It highlights that organic management may be an effective means of influencing AM fungi, leading to benefits for crop growth and development.

**Keywords:** sustainable agriculture, functional ecology, community structure, food security, arbuscular mycorrhizal fungi

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## Managing Mycorrhizas for Sustainability & Food Security

### ARBUSCULAR MYCORRHIZAL FUNGI DECREASES CHICKPEA ASCOCHYTA BLIGHT SEVERITY

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) were found to decrease Ascochyta blight severity in chickpea (*Cicer arietinum*) crops through the common mycorrhizal network (CMN) and possibly through supporting chickpea defense responses.

**Materials and Methods:** Chickpea plants were grown in an intercrop or monocrop in H-pots that allowed for control over the formation of common mycorrhizal networks (CMNs) using size exclusion mesh. One-gallon pots with single chickpeas and an H-pot donor-receiver relationship were also conducted. Plants were grown in a greenhouse and challenged with *A. rabiei* with visual disease ratings and morphological measurements obtained.

**Results:** There was no significant difference in disease severity in intercropped or monocropped plants ( $p=0.61$ ); however, there was a significant decrease in disease severity ( $p=0.03$ ) in the mesh treatment that permitted the formation of CMNs with no root interaction across both plant pairings. AMF inoculation significantly decreased disease severity ( $p=0.04$ ) in single chickpea plants. There was no significant difference in disease severity ( $p=0.43$ ) between donor and receiver chickpea plants. Overall, there was no significant difference in morphological measurements between plants that were connected by a CMN or inoculated with AMF versus those that were not.

**Discussion:** AMF have shown potential as a disease reduction mechanism in chickpeas. The CMN may provide an increase in nutrients that support the defense system of the plants, or act as a conduit for transference of defense related signaling between plants. Plants inoculated with AMF versus uninoculated may have their defenses primed and/or be better able to fight pathogens with the provision of increased nutrients while providing damage compensation.

**Conclusions:** AMF CMNs play a role in decreasing Ascochyta blight severity in chickpeas and may be an important addition to an integrated pest management plan.

**Keywords:** AMF, chickpea, ascochyta, intercrop, disease, CMN

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**Managing Mycorrhizas for Sustainability & Food Security**

**ZINC CONCENTRATION OF URBAN HORTICULTURAL SOILS IMPACTS THE NUTRITIONAL FUNCTION OF ARBUSCULAR MYCORRHIZA FUNGI**

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**Introduction/Aim:**Arbuscular mycorrhizal (AM) fungi can play an important role in affecting crop health, yield and nutritional value. However, the benefits conferred on host plants by AM fungi can be affected by biotic and abiotic factors, including contamination of soil by heavy metals. Zinc is particularly prevalent in soils used for urban food production due to unregulated use of manures as fertilisers, however the effect of elevated zinc on the functionality of AM symbioses is unknown.

**Materials and Methods:**Using radioisotope tracing in *Pisum sativum*, a commonly grown horticultural crop, we investigated how environmentally-relevant concentrations of zinc in urban horticultural soils affected the exchange of phosphorus and carbon between plants and AM fungi. We measured plant growth and productivity traits to assess the impacts of zinc and AMF on plant health.

**Results:**Our findings indicate that increasing zinc concentrations in soil significantly reduced phosphorus acquisition by plants through AM fungi. However, AM fungal colonization of roots and hyphal density in soil remained unaffected by high zinc levels. Alternative benefits of AM inoculation, such as greater biomass and photosynthetic rates, were observed compared to non-inoculated plants.

**Discussion:**These results suggest that while zinc contamination may diminish mineral nutrient gains via AM colonization, other non-nutritional benefits of AM symbiosis could be more significant in promoting plant growth and yields in urban horticultural settings.

**Conclusions:**Urban horticulture holds promise for improving food security, but impacts of soil characteristics, such as elevated zinc levels, on AM fungi demands a greater understanding in order to unlock the full potential of urban soils.

**Keywords:** Urban horticulture, soil contamination, crops, AMF, Symbiosis



**Managing Mycorrhizas for Sustainability & Food Security**

**ARBUSCULAR MYCORRHIZAL FUNGI ARE IMPACTED BY SOIL COVERING AND ROOTSTOCKS IN AN APPLE ORCHARD**

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**Introduction/Aim:**Arbuscular mycorrhizal fungi (AMF) are root symbionts of apple trees (*Malus domestica*). To mitigate disease pressures, apples are grafted onto rootstocks in this region. The type of apple rootstock may facilitate soil organic carbon capture and impact AMF community composition due to their strong host specificity. This study investigated the impact of soil covering and apple tree rootstock on AMF community composition in roots. We hypothesized that AMF would exhibit strong host specificity dependent on rootstocks and soil covering would have an impact on soil physicochemical parameters, both of which would impact AMF community composition.

**Materials and Methods:**Crop rows were arranged in a split-plot experimental design with soil covering (bark mulch, control, and woolly thyme) in main plots and rootstock treatments (M9 and B9) in split plots (n=6). Root tip samples were collected in June 2022.

**Results:**We found that rootstocks and soil covering did result in differences in AMF community structure (p=0.008). We found higher AMF richness and diversity in B9 rootstocks. We also found two significant indicator species in this rootstock: *Claroideoglossum claroideum* BEG31 and an unidentified Glomeraceae OTU. We found soil covering also impacted AMF communities detected with a significant indicator species associated with each treatment and control, correlating with soil carbon and nutrient values.

**Discussion:**AMF facilitate the uptake of essential nutrients and enhance apple tree resistance to abiotic stresses and thus their community, as influenced by rootstock choice, is impacting the legacy inoculum in soils.

**Conclusions:**Thus rootstock type and ground cover impacts AMF community structure in an apple orchard.

**Keywords:** arbuscular, apple, rootstock, soil cover

**Managing Mycorrhizas for Sustainability & Food Security**

**MOLECULAR BASIS OF NUTRIENT-ACQUISITION STRATEGIES IN THE TROPICAL C4 CROP SUGARCANE**

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**Introduction/Aim:**Sugarcane is one of the major crops in tropical and subtropical regions of the world and shows high productivity, even in nutrient-poor tropical soils. We hypothesized that mycorrhizal function is a key factor that improves fertilizer efficiency in sugarcane production.

**Materials and Methods:**To test this hypothesis, we applied the field-transcriptomics approach to identify soil/plant factors that drive mycorrhizal function. However, the high-polyploidy of sugarcane genomes leads to over- or underestimation of transcript abundance of the multicopy genes in the read counting process, which was overcome by using the monoploid reference sequences reconstructed in reference to the genome of sorghum.

**Results:**Sugarcane roots were collected from fields with different productivity and subjected to RNA-Seq, followed by weighed gene-coexpression analysis, and 25 genetic modules were designated. Among them, two modules containing homologues of the maize mycorrhiza-specific genes, namely mycorrhizal modules, were identified. A biplot of principal component analysis indicated that the expression levels of the mycorrhizal modules were correlated negatively with soil NO<sub>3</sub>-N and leaf N:P and positively with leaf P concentration. Most interestingly, mycorrhizal module expression was significantly correlated with biomass production per unit P-fertilizer input (i.e., P-fertilizer-use efficiency).

**Discussion:**These results suggest that the mycorrhizal pathway in sugarcane is driven mainly by N deficiency relative to P and that increased N acquisition through the pathway improved P-fertilizer-use efficiency, probably via enhancing photosynthetic activity per unit P.

**Conclusions:**Our findings provide new insights into the role of AM symbiosis in the productivity of C4 crops in nutrient-poor tropical soils.

**Keywords:** Sugarcane, Tropical soil, Mycorrhiza, Transcriptomics

## Managing Mycorrhizas for Sustainability & Food Security

### DIFFERENCES IN COLONISATION, SOIL SPORE DENSITY, AND DIVERSITY OF AM FUNGI BETWEEN ORGANIC AND CONVENTIONAL CROP SYSTEMS: A META-ANALYSIS

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**Introduction/Aim:** A meta-analysis is an approach involving numerical analysis of data extracted from studies that have been previously published. The meta-analysis aimed to investigate the effects of organic versus conventional crop management practices on arbuscular mycorrhizal (AM) fungal parameters to meet the challenge of sustainable cropping systems.

**Materials and Methods:** A literature search spanning 1992 to 2023 was conducted using Web of Science, Google Scholar, and Scopus. Eligible papers were collected and analyzed based on study types, participant types, and outcome measurements. Weighted meta-analysis was performed to summarize results using effect sizes, mean effects, standard errors, standard deviations, sample sizes, and number of replications. The standardized mean difference was calculated and then pooled using random effects model.

**Results:** The analysis revealed that organic systems exhibited significantly greater AM fungal root colonization and soil spore density compared to conventional systems. However, there was a non-significant effect on AM fungal diversity. Heterogeneity was observed among studies, especially in spore density. Publication bias was detected, with larger studies showing bias towards publication of results related to AM fungal root colonization and spore density. Mixed effect models indicated that crop type did not significantly affect the impact of organic management on AM fungal parameters, though there was a trend for greater impact on grain crops, particularly wheat, compared to fruit and vegetables.

**Discussion:** Overall, the meta-analysis confirmed that organic farming enhances AM fungal development, potentially contributing to crop stability and resource efficiency in the future.

**Conclusions:** This increased understanding of AM fungi's role in agricultural practices can inform sustainable farming approaches.

**Keywords:** Organic farming, conventional farming, biodynamic, AM fungi, colonisation, diversity

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## Managing Mycorrhizas for Sustainability & Food Security

### GROWTH EFFECTS AND ROOT TRAITS IN THEOBROMA CACAO DUE TO MYCORRHIZAL COMMUNITIES IN PANAMA

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**Introduction/Aim:** One of the most crucial mutualistic root-symbiotic microorganisms is arbuscular mycorrhizal fungi (AMF), enhancing plant immune response and nutrient status by supplying phosphorus and underground water, often the limiting for photosynthesis. A valuable perspective for plant crop development would be to breed for beneficial mutualistic symbiosis. To achieve this, understanding how each partner modifies supporting symbiosis structures is important. We assessed root traits relevant to symbiosis, including diameter (D), root tissue density (RTD), specific root length (SRL), and root N content (N), while evaluating the mycorrhizal responsiveness of the plant in terms of biomass production and mycorrhizal root abundance.

**Materials and Methods:** We grew three selfed-progenies from these clones with four different mycorrhizal communities and negative control. The mycorrhizal communities were from different plantations in Panamá, varying in elevation and proximity to the ocean as well as pH and soil texture, which influence mycorrhizal community composition.

**Results:** Regarding the aboveground biomass production, we found significant interaction between mycorrhizal communities and progenies, and strong main effects from AMF and progenies treatments. Regarding root traits, we found significant differences between progenies and AMF treatments.

**Discussion:** We found a different root economic space framework where RTD and D negatively correlate with SRL. Possible explanations are an overlooked pattern for tropical habitats or a relatively higher importance of intraspecific variation associated to a smaller range of trait variation.

**Conclusions:** This finding strengthens the value of considering key players in the ecological niche when developing new plant cultivars.

**Keywords:** arbuscular mycorrhizal fungi, root traits, growth effects, theobroma cacao

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**RHIZOSPHERE DIALOGUE: THE ROLE OF STRIGOLACTONES AND FLAVONOIDS ON ARBUSCULAR MYCORRHIZAL FUNGI COLONIZATION ACROSS DIFFERENT SOIL FUNGAL RICHNESS**

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**Introduction/Aim:** Plant productivity is related to the composition of arbuscular mycorrhizal fungi (AMF) in the soil, although the mechanisms underlying plant-AMF interactions are not clear. It has been shown that strigolactones (SLs) and flavonoids (FVs), are essential for plant-AMF communication and symbiotic establishment acting as molecular cues in the rhizosphere. This study aimed to evaluate the effect of the exogenous application of SLs and FVs on mycorrhization parameters in tomato plants inoculated with soils containing high and low AMF richness from conserved and disturbed forests, respectively.

**Materials and Methods:** To this end, we conducted a greenhouse experiment with three factors: soil AMF richness (high, low), mycorrhizae (with, without), and signaling molecules (none, SLs, FVs, and SLs+FVs). After six weeks, plants were harvested, and the percentage of mycorrhizal colonization (MC), arbuscules (A) and vesicles (V), as well as plant root and shoot biomass, were measured.

**Results:** MC was positively stimulated by SLs+FVs in plants inoculated with a high AMF richness and by SLs and SLs+FVs in those with a low AMF richness. Additionally, SLs promoted V and A colonization in high and low soil AMF richness, respectively. Root and shoot biomass remained unaffected by both AMF richness and by the exogenous application of SLs and FVs.

**Discussion:** These findings revealed variations in mycorrhization parameters depending on AMF richness and the presence of signaling molecules.

**Conclusions:** Further exploration of species composition in the roots and the production of SLs and FVs by plants will contribute to a deeper understanding of the observed responses.

**Keywords:** strigolactones, flavonoids, arbuscular mycorrhizal fungi, AMF richness

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**RHIZOBIUM SPP. AND MYCORRHIZAL FUNGI AS POTENT BIOCONTROL AGENTS IN THE SOYBEAN (GLYCINE MAX L.) UNDER SEMI-ARID ENVIRONMENT**

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**Introduction/Aim:**The growing human population, especially in emerging nations, poses a severe danger to food security, which microbial inoculants can lessen. In addition, there are issues with the overuse of chemical fertilisers, such as the fact that most of them are rather expensive and contaminate surface and subsurface water, raising the risk of stomach cancer in adults and blue baby syndrome in infants.

**Materials and Methods:**Water regimens of 100, 70, and 40% field capacity, the efficacy of rhizobia species and arbuscular mycorrhizal fungi (AMF) to promote soybean tolerance to drought stress was investigated in this work.

**Results:**Rhizobium spp. (R1+R3) and Rhizobium spp. and mycorrhizal consortium (R1+R3MY) co-inoculation of soybean was found to have significant effects ( $P < 0.05$ ) on the relative water content and electrolyte leakage of soybean leaves, respectively. Proline levels rose primarily in microbially modified soybeans during drought stress. Plants inoculated with R1+R3MY exhibited the highest spore count and mycorrhisation percentage. Compared to the non-inoculated plants, the R1+R3MY treatment stimulated soybean growth at 40% FC. At 40% FC, R1+R3MY inoculum had the biggest effects on soybean pod count, number of seeds, fresh weight of seeds, number of seeds per pod, and seed dry weight; at 70% water stress, R1MY inoculation had a considerable impact on the pod.

**Discussion:**Therefore, it is necessary to use more affordable, environmentally friendly, and helpful biological agents to increase agricultural productivity—especially during dry spells.

**Conclusions:**These findings demonstrated that food insecurity can be addressed biotechnologically by co-inoculating mycorrhizal and rhizobial fungi.

**Keywords:** rhizobia species, arbuscular mycorrhizal fungi (AMF), drought stress, water regimens

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**DISTRIBUTION AND DIVERSITY OF ARBUSCULAR MYCORRHIZAL SPECIES IN BANANA AGROECOSYSTEMS IN THE CANARY ISLANDS**

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**Introduction/Aim:**Banana is the most important crop in the Canary Islands. Its production involves a high water and nutrients requirements, which implies an important environmental challenge. In this context, the activity of the arbuscular mycorrhizal fungi is essential to increase the efficiency of water and nutrient absorption. The aim of this work was to describe the diversity and distribution of mycorrhizal fungi in different banana agroecosystems of Tenerife Island.

**Materials and Methods:**Rhizosphere soil was collected from 14 banana farms located in different agroecosystems on the northern (cooler and wetter) and southern (warmer and drier) slopes of Tenerife. Banana seedlings (obtained by in vitro culture) were grown in each soil sample under greenhouse conditions for 6 months. Subsequently, the number of soil spores (wet sieving method) and the percentage of root colonization (staining) were determined. Mycorrhizal identification was performed by roots DNA extraction and the amplification and sequencing a fragment of the 18S rRNA.

**Results:**Not significant difference were detected in the number of the spores between the northern (1.10 spore/g soil) and southern soils (1.07 spore/g soil), while the percentage of mycorrhizal colonization was significantly higher in the southern slope (18.8 % at the northern soils and 30.6% at the southern soils). Four species of the Glomeraceae section were identified: *Glomus sinuosum*, *Rhizophagus irregularis*, *R. intraradices* and *R. arabicus*.

**Discussion:**The predominant species on both slopes of the island was *G. sinuosum*.

**Conclusions:**This is the first work to report the diversity of mycorrhizal species in Tenerife banana soils from different production areas.

**Keywords:** Banana, Canary Island, mycorrhizal, soil, agroecosystem, diversity

## Managing Mycorrhizas for Sustainability & Food Security

### DRAMATIC EFFECTS OF COMMON AGRICULTURAL PRACTICES ON ARBUSCULAR MYCORRHIZAL FUNGAL MEDIATED SOIL AGGREGATION

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**Introduction/Aim:** Arbuscular mycorrhizal fungal (AMF) hyphae and hyphal exudates contribute to soil aggregation. This has been demonstrated to a large extent in experiments where mycorrhizal fungi are removed from the soil. This is however a rare situation in nature as well as in agricultural soils. Here we therefore aimed to manipulate the AMF abundance by practices common in agriculture.

**Materials and Methods:** Explicitly, we allowed pre-sieved soil to be re-aggregated below 10 different common crop plant species of which five were mycorrhizal and five non-mycorrhizal species. In a second experiment we challenged fresh agricultural soil with increasing dosages of fungicides in two rotations of clover as a mycorrhizal host plant. Different mesh compartments allowed us to study aggregation both in the presence of roots as in root-free soil. We measured soil wet stability, hyphal length as well as a range of plant, soil and mycorrhizal parameters.

**Results:** Plant species clearly had an impact on aggregate size-distributions and specific mycorrhizal and non-mycorrhizal hosts where among the treatments with largest and smallest soil aggregation respectively but a general mycorrhizal effect was not clear. In contrast, the effect of two commonly used fungicides, Axozystrobin and Prothioconazole was very clear with a simple dose-response decrease of soil wet aggregate stability with higher fungicide load.

**Discussion:** Factors and relevance behind the manipulations and observed effects are discussed.

**Conclusions:** In conclusion, continued use of pesticides and non-mycorrhizal plants may compromise soil stability in agricultural soils.

**Keywords:** arbuscular mycorrhizal fungi, soil stability, soil aggregation, hyphae, host species, fungicides



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## Managing Mycorrhizas for Sustainability & Food Security

### IMPACT OF FIELD TURNOVER ON ABUNDANCE OF ARBUSCULAR MYCORRHIZAL FUNGI

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**Introduction/Aim:** Some growers use bioinoculants, including arbuscular mycorrhizal fungi (AMF), as part of an integrated pest management strategy, but bioinoculants may not be needed. In the raspberry (*Rubus ideaus*) industry, field turnover typically involves tilling, fumigating, and re-planting. We asked if AMF abundances are reduced during field turnover, and if so, how long it takes them to recover.

**Materials and Methods:** We sampled soil and roots of 20 cultivars across five fields of different ages over two years. We measured AMF abundance via spore density (sucrose gradient method), root colonization (trypan blue, 200x), and viable propagule density via root colonization of *Sorghum sudanese* grown in 1:1 field soil:sand in the greenhouse. We also measured AMF abundance before and after fumigation.

**Results:** Across all genotypes, field colonization increased from 9% in first-year fields to 42% in third-year fields ( $F_{2,108} = 24.0$ ,  $p < 0.001$ ). Spore and viable propagule densities followed a similar pattern. Fumigation lowered viable propagule density ~50% ( $t_{36} = 2.9$ ,  $p < 0.01$ ). Mature fields field colonization was 47% while viable propagule density dropped ~50% from third-year fields.

**Discussion:** AMF abundance in newly turned fields was low, which may be due at least in part to fumigation. AMF abundance does recover during the first three years, but if growers anticipate environmental stressors during the plant establishment, they may want to consider bioinoculants. Viable AMF propagules could be collected from second and third year fields, where densities peaked.

**Conclusions:** AMF abundance in raspberry fields is dramatically reduced with turnover, but recovers within three years.

**Keywords:** fumigation, tilling, *Rubus ideaus*, raspberry, mycorrhizal infectivity potential

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## **Managing Mycorrhizas for Sustainability & Food Security**

### **THE RESPONSE TO ARBUSCULAR MYCORRHIZAL FUNGI IN FIELD GROWN MAIZE**

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) are ubiquitous, forming symbiotic relationships with the major crop species. Studies in controlled conditions have demonstrated the potential of AMF. It is difficult, however, to estimate benefit in the field, not least because of the lack of AMF-free controls.

**Materials and Methods:** We have used maize genetic mutants to generate AMF incompatible plants that can be used as a baseline against which to evaluate the impact of AM symbiosis. To estimate the AMF effect and characterize the impact of host genetic variation, we have selectively incorporated AMF-incompatibility into genetic mapping populations for field evaluation.

**Results:** We have estimated AMF to contribute up to ~30% of total yield in medium input fields. However, using high resolution soil maps, we have found the AMF effect to vary dramatically in response to heterogeneity in soil fertility. Genetic mapping has identified variants linked to differential performance in the presence or absence of AM symbiosis. We also identified antagonistic effects such that an allele beneficial to mycorrhizal plants is deleterious in the absence of the symbiosis.

**Discussion:** Conditional genetic variants are analogous to previously described direct and symbiotic nutrient uptake pathways. Similarly, conditional variants support the distinction between mycorrhizal benefit and dependence.

**Conclusions:** We present an experimental design allowing a robust estimation of host mycorrhizal response in the field. Evidence of plant genetic trade-offs between performance with and without AMF indicates the importance of tailoring crops to the AMF “environment”. Our approach is applicable to other crops and scalable to larger yield trials.

**Keywords:** Mycorrhizal Growth Response, Genetic Mapping, Genetic Architecture, Dependence, Benefit, Maize

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## Managing Mycorrhizas for Sustainability & Food Security

### IS ARBUSCULAR MYCORRHIZAL FUNGAL ADDITION BENEFICIAL TO POTATO SYSTEMS? A META-ANALYSIS

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**Introduction/Aim:** The application of arbuscular mycorrhizal (AM) fungi has been reported to confer multiple agronomic benefits to crop plants including cereals, vegetables, and fruit trees, as well as to improve soil structure and health. However, much less is known whether these benefits translate in potato agri-systems.

**Materials and Methods:** We conducted a meta-analysis by reviewing a total of 106 independent experimental studies from 37 peer reviewed publications to assess the beneficial effects of AM fungi addition on potato plant's parameter (plant mass, nutrient content and yield). Experimental conditions (pot vs. field experiment), inoculation method (single taxon or multiple taxa), source of the AMF inoculum (commercial vs. not), and plant cultivar used were included as moderators.

**Results:** Our results show that AM fungal addition is overall beneficial in potato agri-systems as it increased plant mass and nutrient contents, as well as tuber number and yield. The only significant moderator in our analyses was potato cultivar.

**Discussion:** Our findings agree to several meta-analysis reporting positive effects of AM fungi on wheat and other food cereal crops, showing the feasibility of AM fungal inoculation in enhancing also potato production.

**Conclusions:** AM fungal additions can be beneficial to potato systems. Our results further suggest that whether studies were performed in the field or in pot settings did not influence the results, and highlight the importance of considering plant cultivar, as this was an important significant moderator explaining our results.

**Keywords:** *Solanum tuberosum*, meta-analysis, potatoes, arbuscular mycorrhizal fungi.

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## Managing Mycorrhizas for Sustainability & Food Security

### MAKING VISIBLE THE INVISIBLE: MYCORRHIZAL THREADS FROM THE GARDEN TO THE STUDENT COMMUNITY

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**Introduction/Aim:**The agroforestry university garden (HUA) of the National School of Higher Studies of UNAM-Unidad Morelia (ENES-Morelia) is a space that invites us to interact between human beings, plants, animals, soil and microorganisms. The objective was to carry out an awareness exercise towards the communities of arbuscular mycorrhizal fungi (AMF) that inhabit the garden using scientific, pedagogical and artistic tools.

**Materials and Methods:**Soil and root samples were taken from five plants in the orchard (*Zea mays*, *Amaranthus* sp., *Physalis* sp., *Morus nigra* and *Prunus serotina*). The roots were stained with trypan blue to observe mycorrhizal colonization. Microscopic photographs of the characteristic mycorrhizal structures were taken and printed on fabric. The images were embroidered collectively at the HUA.

**Results:**All the species analysed showed mycorrhizal colonization. The embroidery sessions brought together 20 students from diverse disciplines such as: Environmental Sciences, Agroforestry Sciences, Intercultural Literature, Music and New Technologies and Art History.

**Discussion:**The exercise generated spaces for discussion and reflection around those seemingly invisible beings that give vitality to the Garden and what it produces. Later the embroideries were exhibited in the University library.

**Conclusions:**Generating these spaces for reflection among a diverse student community allows us to relate scientific knowledge, personal temperaments and the invisible imaginaries that inhabit the garden.

**Keywords:** AMF, Community, Threads, Science communication

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**Managing Mycorrhizas for Sustainability & Food Security**

**NATURAL FARMING: CONSEQUENCES ON SOIL PROPERTIES, INCLUDING ARBUSCULAR MYCORRHIZAL FUNGI OF ACID-INFERTILE SOIL OF NORTHEAST INDIA**

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**Introduction/Aim:**Changes in the chemical properties of the soil surface layer of shifting cultivation, a form of natural farming in a sub-tropical and humid ecoregion of Northeast India, were investigated.

**Materials and Methods:**Soil samples obtained from different sites spanning the entire cultivation cycle and fallow periods viz., five, ten, and twenty years were documented for the changes in soil properties.

**Results:**Results suggest that a slight decrease in pH in the soil surface layer occurred during the fallow periods, with the content of organic carbon seemingly increasing with the rise in the fallow period. Organic carbon (1.3%) and available nitrogen (477.3 kg/ha) were the highest in twenty years' fallow. The levels of available phosphorus, exchangeable calcium, potassium, and pH of the soil were lowest during the five-year fallow period. Exchangeable calcium was noticeably abundant in the long fallow period. Burned soils had lower organic matter and nitrogen than unburned soils, higher calcium and potassium, and nearly unchanged pH and available phosphorus were recognized. Arbuscular mycorrhizal spore density was maximum (140-671 spores per g of soil (d.w.) in the twenty-year fallow site.. Spore density was highest in the upper slope, relatively followed by the middle and bottom slopes.

**Discussion:**The surface layer accumulation of organic matter during the long fallow period was the driver for mycorrhizal proliferation.

**Conclusions:**From the preceding results, it can be concluded that some soil properties in fallows less than ten years are less favourable for shifting cultivation even when the beneficial effects of burning and adding ash are taken into account.

**Keywords:** Shifting cultivation, arbuscular mycorrhizal fungi, soil properties, acid soil

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## Managing Mycorrhizas for Sustainability & Food Security

### IDENTIFYING MYCORRHIZA ASSOCIATED TRAITS FOR IMPROVED GROWTH AND DROUGHT TOLERANCE OF FORAGE LEGUMES

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**Introduction/Aim:** Plant responses to arbuscular mycorrhizal fungi (AMF) vary among plant genotypes, potentially driven by intraspecific trait variation in plants. Despite many studies on how AMF colonization and root traits affect nutrient foraging and ecological function, the relationship among these traits within plant species is underexplored. We collected intact mature plants from breeding nurseries of two perennial forage legume species to identify which mycorrhizal traits are correlated to forage plants' performance.

**Materials and Methods:** For this purpose, excavated plants were reestablished in pots in the greenhouse, where we exposed them to three different treatments: fertilizer (control), no fertilizer (low nutrients), and drought. After four months, we harvested the plants. For each plant, we estimated before and after treatment mycorrhizal colonization, plant biomass, average root diameter, specific root length, root tissue density.

**Results:** AMF colonization was negatively affected when water, but not nutrients, was limited. However, plants that were initially highly colonized by AMF hyphae produced more biomass in the drought treatment than less colonized individuals. Plants with thicker roots were also more drought tolerant, with less shoot dieback.

**Discussion:** Our results suggest that intraspecific variation in extent of root colonization and root diameter may determine the drought tolerance in forage legumes and possibly important traits for future breeding efforts. However, these characteristics may vary according to plant species as host preference is one of the primary things for AMF dependency in plants.

**Conclusions:** Both AMF colonization and root diameter are important traits affecting drought tolerance within forage legumes.

**Keywords:** Mycorrhiza associated traits, Root colonization, Forage legumes, Drought tolerance

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## Managing Mycorrhizas for Sustainability & Food Security

### OPTIMISING CEREAL MYCORRHIZAS FOR SUSTAINABLE AGRICULTURE – FROM GENETICS TO AGRONOMY

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**Introduction/Aim:** Targeting plant genes which control arbuscular mycorrhizal symbioses could allow improved nutrient capture and reduced fertiliser demand - a huge opportunity for enhancing agricultural sustainability and reducing environmental impact worldwide. Plant genes critical to mycorrhizal signalling and control of the symbiosis have been characterised in controlled conditions, while the effects of these genes in a real-world agricultural setting are virtually unknown.

**Materials and Methods:** In multi-site, multi-year field trials, we are testing genetically engineered barley genotypes showing reduced, abolished, or increased arbuscular mycorrhizal colonisation. Comparing against wild-type controls we can determine for the first time the effects of mycorrhizal symbiosis on barley in this farming system, in addition to the effects of increased mycorrhizal colonisation.

**Results:** Collecting datasets of agronomic traits, soil microbial ecology, elemental composition and gas fluxes will provide a detailed physiological investigation of this symbiosis in the field.

**Discussion:** The initial results of a sampling survey of UK farms will also be presented. We have identified strong effects of soil management on mycorrhizal fungal abundance in winter wheat crops nationwide.

**Conclusions:** Ongoing work characterising the root-associated microbiome in these survey samples will also be discussed.

**Keywords:** arbuscular mycorrhiza, genetic engineering, barley, field trials

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## Managing Mycorrhizas for Sustainability & Food Security

### ARBUSCULAR MYCORRHIZAL FUNGI ENHANCE POTATO CROP YIELD AND NUTRITIONAL QUALITY

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**Introduction/Aim:** Agricultural application of arbuscular mycorrhizal fungi inoculum as a biofertilizer aims to increase the level of root colonization, which in turn enhances plant access to soil nutrients, improves sustainability, and has been demonstrated to result in yield increases of ~15% for a variety of crops. Due to their popularity and widespread consumption in the United States, and the documented mycorrhizal crop yield response from prior studies, we selected potatoes *Solanum tuberosum* for further evaluation of mycorrhizal impact on nutritional quality through inoculation trials.

**Materials and Methods:** A controlled inoculation study in pots, and field trials at two sites were conducted in 2022 and 2023 to investigate the nutritional benefits of mycorrhizal inoculation within the context of indigenous communities of mycorrhizal fungi and other soil microorganisms.

**Results:** We observed yield increases of up to >20%, up to two-fold increases in antioxidant activity, and up to nine-fold increases in the amino acid ergothioneine in tubers harvested from inoculated potato plants.

**Discussion:** In addition to enhanced crop yields, mycorrhizal colonization alters plant physiology and has been shown to increase the level of antioxidants and other beneficial secondary metabolites in edible plant tissues of multiple crops. Inoculation of potatoes and other crops has been demonstrated to increase the concentration of ergothioneine, a diet-derived antioxidant produced by only fungi and certain bacteria that is known to prevent age-related diseases in humans.

**Conclusions:** These experiments demonstrate how agricultural practices providing benefits to soil health enhance the nutritional quality of food and have implications for improving human health and reducing disease.

**Keywords:** agriculture, biofertilizer, inoculation, nutrition



**Managing Mycorrhizas for Sustainability & Food Security**

**ARBUSCULAR MYCORRHIZAL FUNGI CAN REDUCE MICROPLASTICS ACCUMULATION IN ABOVEGROUND EDIBLE PARTS OF PLANTS**

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**Introduction/Aim:**Microplastics pose a threat to soil health and food security, raising concerns about their impact on plants. Understanding how these particles interact in the rhizosphere, especially their potential entry into plants, is crucial. Arbuscular mycorrhizal (AM) fungi, a ubiquitous species in soil, establish symbiotic relationships with a vast majority of terrestrial plants. Therefore, studying the ability of AM fungi to protect plants from microplastic damage is essential.

**Materials and Methods:**Lettuce (*Lactuca sativa*) was grown with and without inoculation of the AM fungus *Rhizophagus irregularis*. The plants were exposed to five different levels of polymethyl methacrylate (PMMA) soil pollution, ranging from 0% to 0.4% (measured as the mass ratio of pollutant to soil).

**Results:**It demonstrated that a significant reduction of the PMMA accumulation in the aboveground edible parts of mycorrhizal plants. However, the presence of AM symbiosis did not have a significant impact on the uptake of microplastics by plants from the soil.

**Discussion:**This decrease of microplastics in aboveground part can be attributed to the immobilization of PMMA particles by AM vesicles and intraradical fungal hyphae. Moreover, AM colonization was found to disrupt PMMA aggregates within the vessels, potentially impeding particle movement. Additionally, the chemical composition of PMMA remained unchanged during the transportation process, indicating that mycorrhizal fungi did not alter its chemical properties.

**Conclusions:**Our study provides evidence of the immobilization of microplastics by AM fungi, offering a more profound understanding of microplastic behavior in AM-associated plants. Furthermore, it suggests the potential application of AM fungi in plant cultivation under microplastic contamination.

**Keywords:** mycorrhizal plants, microplastic allocation, immobilization, transformation, accumulation

## Managing Mycorrhizas for Sustainability & Food Security

### COMPARATIVE STUDY: EVALUATION OF MAIZE MYCORRHIZATION IN SENEGAL AND UGANDA

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**Introduction/Aim:**Maize (*Zea mays*) cultivation is vital for food security and livelihoods worldwide, often requiring significant chemical fertilizer inputs. This study explores the potential of mycorrhizal symbiosis as an alternative approach to enhance maize production sustainably.

**Materials and Methods:**On farm studies conducted in Senegal and Uganda investigated the role of native arbuscular mycorrhizal fungi (AMF) in maize cultivation within diverse agroecological contexts.

**Results:**In Senegal, research focused on mycorrhizal fungi's impact on groundnut and maize intercropping and indigenous strains' potential to enhance crop growth. Similarly, Uganda's studies examined mycorrhizal fungi's effectiveness in various agroforestry and green manure systems, highlighting its role in improving soil fertility and crop resilience. The results showed that AMF inoculum combined with 50% NPK realized higher maize yields than the full NPK dose alone and the control.

**Discussion:**The findings showed the importance of local soil conditions and management practices in optimizing mycorrhizal symbiosis benefits for maize production. Tailored approaches integrating indigenous knowledge are essential for maximizing these benefits.

**Conclusions:**Comparative analysis reveals diverse effects of mycorrhizal symbiosis on maize cultivation in Senegal and Uganda. Harnessing the potential of mycorrhizal fungi offers sustainable solutions to enhance maize productivity, contributing to food security, environmental sustainability, and reducing inorganic fertilizer use.

**Keywords:** Maize, Mycorrhizal symbiosis, Sustainable agriculture, Soil fertility, Crop productivity

## Managing Mycorrhizas for Sustainability & Food Security

### SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI FROM TWO URBAN GARDEN COMPOSTS

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**Introduction/Aim:**The application of organic amendments and arbuscular mycorrhizal fungi (AMF) inoculation are common practices for sustainable crop production. However, it is necessary to learn about the abundance and diversity of AMF found in organic amendments to define proper management practices. The aim of this study was to find if AMF spores present in two urban-garden composts (Huerto Tlatelolco, HT; and Huerto Roma Verde, HR) were affected during the composting process.

**Materials and Methods:**The HT pile was made with greenhouse substrate, plant residues, 21-day old compost, food waste, and straw, with a 2-day turning frequency during the first 25 days. The HR pile was made with sawdust, food waste, poultry manure, vegetal residues, and dry leaves, without turning.

**Results:**The piles reached 61°C between day 1 and 2. Ambient temperature was recorded at days 34 and 54 for HR and HT piles, respectively. Both composts had acceptable values of moisture, organic matter, pH, electrical conductivity, and germination index according to Mexican standards. AMF spore abundance was significantly higher in HR (281±38 spores per 50 g compost) than in HT (32±9 spores per 50 g compost).

**Discussion:**This could be due to the presence of soil in poultry manure and lack of disturbance of associated with turning of the pile.

**Conclusions:**Our results suggest that urban-garden compost may become a source for AMF inoculum depending on compost composition which should be taken into consideration when applying it for agricultural usage. We are currently working on the identification of AMF spore diversity in these composts.

**Keywords:** food waste, city, sustainability, soil, fertility

**Managing Mycorrhizas for Sustainability & Food Security**

**THE ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI IN GRASSLAND RESTORATION: ENHANCING REGROWTH OF RESEEDING SPECIES AFTER DEFOLIATION**

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) establish mutualistic symbiosis with most grassland plants and determine the natural succession restoration of degraded grassland ecosystems. However, whether and how AMF affect reseeded species growth is less clear. The aim of this study was to investigate the effects of AMF on reseeded species at different growth periods and unravel mechanisms of AMF altering plant regrowth after defoliation.

**Materials and Methods:** Greenhouse study 1. We investigated biomass and mycorrhizal growth response (MGR) of five reseeded species (*Bromus inermis*, *Agropyron cristatum*, *Leymus chinensis*, *Poa pratensis*, *Medicago falcata*) under two inoculation treatments (with or without AMF) at three growth periods, seedling, jointing and jointing after defoliation.

Greenhouse study 2. We measured plant regrowth and root transcriptome under two defoliation intensities (3-cm and 7-cm stubble height) and two same inoculations.

**Results:** Biomass of *B. inermis* and *L. chinensis* at seedling and jointing periods were increased by AMF inoculation. MGR of four grass species were positive at each period and the lowest MGR were observed at jointing period after defoliation. Furthermore, regrowth of *M. falcata* was promoted by AMF inoculation, but AMF only support *A. cristatum* regrowth when faced with heavy defoliation.

**Discussion:** Transcriptome sequencing demonstrates high host specificity for reseeded species genetically responding to AMF inoculation after defoliation, and AMF may support shoot regrowth through the phenylpropanoid biosynthesis pathway.

**Conclusions:** Our study shows that the symbiotic relationship between AMF and plants is species-specific, and the benefit of plant-AMF symbiosis changes depending on plant growth periods and management practices.

**Keywords:** Reseeded species, Growth period, Mycorrhizal growth response, defoliation, Regrowth, Transcriptome

## Managing Mycorrhizas for Sustainability & Food Security

### INVESTIGATING EFFECTS OF MYCORRHIZA INOCULATION ON THE ESTABLISHMENT AND GROWTH CHARACTERISTICS OF SOME PLANTS IN BAHAR KISH RANGELAND, QUCHAN, IRAN

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**Introduction/Aim:** This research was aimed at investigating the possibility of improving seedling establishment and growth on four rangeland plant species *Ziziphora clinopodioides* Lam, *Thymus vulgaris* L, *Bromus kopetdaghensis* Drobv, and *Medicago sativa* L via their root inoculation with the arbuscular mycorrhizal fungi, in Baharkish rangelands, Quchan, Iran.

**Materials and Methods:** First, the seeds of all species were sown under greenhouse conditions. After one month, the seedling was inoculated with the mycorrhiza species and transplanted into small pots. After four weeks, pots were transported into the field. Seedlings were planted in a randomized blocks design, with 3 replications (12 plots in each block) and factorial combination in an area of about 3600 square meters.

**Results:** Results of the first year measurements indicated high colonization of *Ziziphora* and *Thymus* with *G. mosseae* whereas high values of *Bromus* and *Medicago* with *G. intraradices*. For both years, the seedling establishment rate and growth of all species were significantly higher for the inoculated than the control treatment.

**Discussion:** The survival morphological and growth characteristics of all plants decreased with the diminished rainfall and the increase in temperature in the second year compared to the first year. Despite this, in the second year, the plants inoculated with mycorrhizal species were more successful than the control treatment. In two years, root colonization rate and seedling survival rates were higher for *Ziziphora* and *Bromus* as compared with *Thymus* and *Medicago*.

**Conclusions:** *G. intraradices* can be used as an efficient biological fertilizer for increasing the establishment of *Ziziphora*, *Bromus*, and *Medicago* in semiarid and Baharkish rangelands.

**Keywords:** Biofertilizer, *Bromus kopetdaghensis*, *Medicago sativa*, *Thymus vulgaris*, Semi-arid rangelands and *Ziziphora clinopodioides*.

# Environmental Change

**O-025**

**Environmental Change**

**ARBUSCULAR MYCORRHIZA AND MULTIPLE FACTORS OF GLOBAL CHANGE**

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**Introduction/Aim:** Multiple factors of global environmental change affect terrestrial ecosystems concurrently; however, we know very little about soil biota responses to such joint effects.

**Materials and Methods:** This is unfortunate, since we know from a recent meta-analysis that mycorrhiza benefits plants in response to major global change factors (GCFs).

**Results:** Building on previous work in soil microcosms, in a greenhouse and a 2-year field experiment (in grassland), we explored the effects of up to ten GCFs on arbuscular mycorrhiza, using a random sampling from a factor pool approach to generate a gradient in the number of GCFs from zero to ten.

**Discussion:** We find that an increasing number of GCFs negatively affects plant biomass in the field and in the greenhouse experiment, with similar patterns also observed for mycorrhiza.

**Conclusions:** Future work on the effects of global change on mycorrhiza should consider the concurrent action of a number of drivers, as a focus on just one or two factors likely underestimates the true impacts of global change.

**Keywords:** AM fungi, global change, soil pollution, drought; environmental change

O-026

## Environmental Change

### FACULTATIVE MYCORRHIZAL STATUS HELPS PLANTS NATURALIZE TO VARIOUS ENVIRONMENTS

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**Introduction/Aim:**The naturalization of alien species has become one of the most important global issues. Mycorrhizal fungi play a key role during this process. Previous studies found facultative mycorrhizal (FM) plants can naturalize to more locations. However, it is still unclear whether FM status can help plants adapt to a broader range of environments.

**Materials and Methods:**To explore how FM helps plant naturalization, we collected the occurrence records of 4,793 naturalized species and corresponding environmental information, including climate and soil information. We calculated the Mahalanobis distance of species distribution environments using methods such as principal component analysis and random forest.

**Results:**Our results showed that the environmental variations among the occurrence points of FM plants are notably higher compared to other statuses of mycorrhizal plants. We classified the global environment and found that FM plants occur among the most diverse environmental types, followed by obligately mycorrhizal plants. The niche width of FM plants is significantly greater than other plants in terms of soil physical and chemical properties, such as available phosphorus, pH, total nitrogen, and fine earth fraction bulk density. However, the promotion of climate niche is limited. FM plants can improve their tolerance of temperature and precipitation changes, but can not help plants naturalize to regions with extreme climates throughout the year.

**Discussion:**FM plants can naturalize to all environments except for those with an annual mean temperature below  $-20^{\circ}\text{C}$  and zero rainfall in the driest month.

**Conclusions:**Our research contributes to a deeper understanding of the effect of mycorrhizal symbiotics on non-native species' naturalization.

**Keywords:** mycorrhizal status, naturalized plants, facultative mycorrhizal, niche width



O-027

## Environmental Change

### RHIZOSPHERE INTERACTIONS BETWEEN FIRE, INVASIVE GRASSES, TREE SEEDLINGS, AND ECTOMYCORRHIZAL FUNGI: IMPLICATIONS FOR RESTORATION

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**Introduction/Aim:**The frequency and severity of wildfires has increased globally in recent decades, with potentially lasting effects on woodland regeneration and mycorrhizal fungal communities. In the western United States, the recovery of pinyon pine (*Pinus edulis*) and juniper (*Juniperus osteosperma*) woodland trees and their fungal symbionts following disturbance is becoming increasingly uncertain.

**Materials and Methods:**We used soil from stand-replacing wildfires in pinyon-juniper woodlands to examine interactions between fire history, introduced grasses, pinyon seedling growth, and ectomycorrhizae. We also tested the use of live soil inoculum in combination with other restoration techniques for replanting tree seedlings into burn scars.

**Results:**Even decades after fire, ectomycorrhizal fungal inoculum levels are reduced by burning, with altered fungal communities that are less beneficial for seedling growth than the communities of intact woodlands. An invasive grass that colonizes burned landscapes, *Bromus tectorum*, reduced ectomycorrhizal abundance and seedling growth, both when grown with pinyon seedlings and also when used to pre-condition soils. In the field, seedlings that received soil from intact woodlands were more likely to survive and showed reduced water stress.

**Discussion:**This work indicates that both wildfire and the non-native grasses that establish after wildfire leave legacies that alter ectomycorrhizal fungal communities with negative consequences for their host plants and ecosystem recovery.

**Conclusions:**Under changing climate conditions, reintroduction of beneficial soil biota and attention to site vegetation history may improve forest restoration outcomes.

**Keywords:** Ectomycorrhizal fungi, fire, invasive species, *Pinus edulis*, restoration

O-028

## Environmental Change

### PLANT BIODIVERSITY, SOIL BIODIVERSITY, AND THE IMPACT OF DROUGHT ON THE COMPOSITION AND DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) are crucial for plant and ecosystem functioning. However, we know little about the influence of soil biodiversity, plant biodiversity, and environmental stressors and their combined effects on AMF diversity and community composition within plant roots. Various aspects of plant ecological strategies, particularly their degree of dependence on AMF, may further shape AMF diversity within roots.

**Materials and Methods:** Here, we conducted a full factorial mesocosm experiment involving soil biodiversity, plant biodiversity, and drought. We characterized the ecological strategy of 16 herbaceous plant species, both above and belowground, and analysed root AMF composition and diversity at two time points.

**Results:** Drought, as well as low soil and plant biodiversity, negatively impacted AMF diversity within roots. Notably, high soil biodiversity partially mitigated the adverse effects of drought on AMF diversity. The ecological strategy of plant species, especially their reliance on AMF, strongly controlled the AMF diversity and composition in roots. Finally, AMF diversity showed large variation over time, albeit less so for plant species heavily reliant on AMF.

**Discussion:** Overall, our findings suggest that ecosystems undergoing losses in above and belowground biodiversity, as well as experiencing climate extremes, may lose the capacity to ensure diverse AMF colonization of plant roots. Furthermore, it indicates that plant species heavily reliant on AMF exert greater control over AMF composition and may be more vulnerable as the soil AMF diversity declines.

**Conclusions:** We show that the coincidence of drought and belowground biodiversity loss amplifies detrimental effects on AMF diversity in roots, potentially compromising the functionality of the symbiosis.

**Keywords:** soil biodiversity, plant biodiversity, AMF, mycorrhizal dependency, drought, root traits

O-029

## Environmental Change

### MYCORRHIZAL COLONIZATION, AND ROOT AND SOIL MICROBIOMES UNDER AN ACACIA AND EUCALYPTUS SPECIES ACROSS A RAINFALL GRADIENT IN SOUTHERN AUSTRALIA

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**Introduction/Aim:**The climate of south-eastern Australia is becoming warmer, drier, and the frequency, duration, and severity of drought is predicted to increase in the coming decades. It is of great importance to understand the response of plant-soil systems to changes in precipitation and water availability. Here we study patterns of root colonization by mycorrhizal fungi and endophytes together with bacterial and fungal communities under an Acacia and Eucalyptus tree species across a precipitation gradient.

**Materials and Methods:**We used a natural precipitation gradient of 545 to 1327 mm per year in south-eastern Australia, we sampled roots and soil from 35 individual trees of *Acacia dealbata* and *Eucalyptus obliqua* (70 trees total) across 7 sites during summer. We analyzed root colonization rates of ectomycorrhizal fungi (ECM), arbuscular mycorrhizal fungi (AM), and dark septate endophytes (DSE) microscopically, and fungal (ITS) and bacterial (16S) communities using metabarcoding.

**Results:**Our preliminary results so far suggest that ECM colonization is negatively associated with precipitation, yet the relative abundance of different ECM taxa respond differentially to precipitation and soil moisture, there are also differences depending on tree species.

**Discussion:**Both root-associated fungi and bacteria may contribute significantly to drought tolerance in trees, particularly mycorrhizal fungi, our results contribute to a growing body of evidence that ECM associations in general appear to be sensitive to changes in precipitation and water availability, but certain ECM taxa, and other microbial and symbiotic guilds may be more tolerant to drier conditions.

**Conclusions:**It is important to understand the nuances of drought responses in plant-soil systems.

**Keywords:** Ectomycorrhiza, arbuscular mycorrhiza, dark septate endophytes, fungi, bacteria, drought

O-030

## Environmental Change

### LONGITUDINAL VARIATION OF ARBUSCULAR MYCORRHIZA FUNGI DIVERSITY IN FOREST AND GRASSLAND ACROSS GERMANY

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) are key players in terrestrial ecosystems and can be threatened by land use intensification. Thus, the long-term characterization of land use effects on AMF is important.

We assessed the spatiotemporal variation of AMF in forests and grasslands along a land use gradient from 2011 to 2023 within the Biodiversity Exploratories, a long-term and large-scale research platform with three study regions across Germany.

**Materials and Methods:** The soils of 150 grassland and 150 forest plots were sampled five times at three years intervals since 2011. To assess the abundance and diversity of AMF amplicon sequencing of the fungal SSU rDNA was conducted.

**Results:** Throughout the years, Shannon and Simpson indexes were consistently higher in grasslands compared to forests while there was no difference in Pielou evenness. Indicator AMF discriminated between ecosystems along the longitudinal series. PERMANOVA showed a significant effect of ecosystem time and land use intensity on species abundance.

**Discussion:** Our results agree with previous studies and provide information on the longitudinal variation of AMF. The observation of a signature group of AMF that differentiate grassland and forest mirrors the AM fungal plant specialization. The longitudinal series adds information to the previously observed effect of land use intensity on AMF.

**Conclusions:** Understanding the relationship of mycorrhizal associations in grassland and forest biomes and land use intensity is the first step towards elucidating the role of AMF in ecosystem functioning. Moreover, this knowledge would contribute to developing approaches to protect biodiversity and sustainably maintain existing and new agroecosystems in future scenarios under climate change.

**Keywords:** Land use Intensity, Arbuscular Mycorrhizae Fungi, Longitudinal variation, Forest, Grassland

**O-031**

**Environmental Change**

**TEMPORAL AND SPATIAL PRODUCTIVITY AND FUNCTIONAL ECOLOGY OF ARBUSCULAR MYCORRHIZAL FUNGI UNDER DROUGHT**

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**Introduction/Aim:** To predict how grassland ecosystems will respond to drought it is necessary to consider the response of arbuscular mycorrhizal (AM) fungi, that not only contribute to key ecosystem processes but confer drought tolerance in plants. However, novel approaches are required to study the functional ecology of AM fungi in natural systems.

**Materials and Methods:** To assess the response of AM fungi to drought, we measured AM fungal traits and allocation in the soil and roots at a long-term rainfall manipulation experiment in eastern Australia. We installed mesh bags and root in-growth cores at various incubations to capture the temporal and spatial aspects of AM fungal turnover, measuring AM fungal productivity (hyphal biomass, hyphal turnover, spore number), morphological traits (hyphal width, spore size and spore wall thickness) and % root length colonised.

**Results:** To date, AM fungi were observed to colonise mesh bags from four weeks with longer incubations resulting in a greater production of hyphal biomass and spores, especially during periods of noticeable plant production. This included fungi from Gigasporaceae, along with other taxa yet to be identified. Assessment of fungal traits is ongoing at the time of writing.

**Discussion:** Potential strategies regarding the allocation and abundance of fungal biomass and traits could suggest a preference for a life history mode or function related to nutrient or water exchange and transport, or survival and persistence.

**Conclusions:** By evaluating the sensitivity or adaptive ability of AM fungi under drought, this study contributes to a wider discourse on the impacts and resilience of current and future droughted systems.

**Keywords:** traits, fungal ecology, arbuscular mycorrhizal fungi, global change

O-032

## Environmental Change

### THE IMPACTS OF NITROGEN AND PHOSPHORUS FERTILISATION ON THE SYMBIOSIS BETWEEN ECTOMYCORRHIZAL FUNGUS AND *PINUS SYLVESTRIS*

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**Introduction/Aim:**Inorganic nitrogen deposition has been linked to shifts in ectomycorrhizal (EcM) fungal and plant community composition; the deposition of nitrogen may have resulted in the forests becoming phosphorus limited. In phosphorus-limited forest systems, there is evidence that distinct phosphorus sources are partitioned between plants depending on their mycorrhizal associations. This study aims to discern whether phosphorus partitioning occurs between EcM fungal species in nitrogen-limited forests and to use these findings to predict how these changes impact nutrient cycling.

**Materials and Methods:**Fungal isolates obtained from non-fertilised and nitrogen-fertilised soil were grown in monocultures and given access to distinct phosphorus sources. *Pinus sylvestris* seedlings were germinated and grown for 6 months in soils inoculated with the microbial community from non-fertilised soil, nitrogen-fertilised soil, or nitrogen and phosphorus-fertilised soil.

**Results:**Differences in phosphatase and phytase enzyme activity between fungal isolates were detected. Seedlings were observed to have distinct leaf traits and EcM symbionts dependent on soil inoculum, and the soils were tested for differences in nutrient content resulting from the introduction of the distinct microbial communities.

**Discussion:**The preliminary results indicate that EcM species show some preference for phosphorus sources in lab conditions, and the inoculum impacts seedling access to key soil nutrients.

**Conclusions:**Nutrient fertilisation results in distinct microbial communities that have different abilities to access nutrients for *Pinus sylvestris* seedlings, and the community composition of EcM fungi may significantly impact nutrient cycling at the ecosystem level.

**Keywords:** ectomycorrhizal fungi, boreal, pinus, nutrient cycling, nitrogen, phosphorus

O-033

## Environmental Change

### HOW DO MYCORRHIZAE INFLUENCE MIGRATION OF TEMPERATE TREES INTO BOREAL FORESTS UNDER CLIMATE AND DISTURBANCE REGIME CHANGES?

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**Introduction/Aim:** Climate change, through changes in growing season, temperature, and precipitation patterns, is shifting the distributions of forest tree and fungal communities. Meanwhile, stand replacing disturbances like fire, which burns organic layers, and harvest which mechanically disturbs and compacts soil, can alter fungal communities and are changing in frequency and severity with climate change. These disturbances may play an important role in facilitating migration of temperate trees into historically boreal zones, particularly for early successional species.

**Materials and Methods:** Here, we review the current state of knowledge at the nexus of climate induced tree migration, biotic and abiotic disturbance, and mycorrhizal fungi to help predict changes to boreal forest composition and function.

**Results:** While mycorrhizal fungi typically enhance tree establishment and growth, and can help trees resist climate related stressors (e.g. drought, N deposition, salt, flooding) they can also alter species interactions like competition and facilitation. It remains unclear what role mycorrhizal relationships with trees play in facilitating or constraining migration of temperate trees. For instance, boreal trees are predominantly ectomycorrhizal, whereas temperate trees more readily form arbuscular mycorrhizal relationships. Both climate change and disturbances can reduce the diversity of fungal communities, reduce carbon fixation, decrease ectomycorrhizal to arbuscular mycorrhizal ratios, and alter ecological functions like productivity and nutrient cycling. However results can vary by disturbance severity, climate variables, and geographic location.

**Discussion:** We address dominant research approaches to research design, and discuss implications for boreal forest resilience, management, and carbon balance.

**Conclusions:** Finally, we suggest a research agenda to address key unanswered questions.

**Keywords:** Disturbance, Climate Change, Biogeography, Boreal-Temperate Forest Ecotone, Migration

O-034

## Environmental Change

### RELATING FUNDAMENTAL TO REALIZED NICHES OF ARBUSCULAR FUNGI ALONG A TEMPERATURE GRADIENT

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**Introduction/Aim:** While the realized niche can be derived from a species' distribution in different habitats, the fundamental niche is approached more difficultly, because it requires experimental investigation of responses to environmental gradients. Knowing parameters of fundamental niches of arbuscular mycorrhizal species, however, would be a valuable tool for predictions of the species' responses to changing abiotic environments, important e.g. in relation to expected climate change.

**Materials and Methods:** As temperature was recently identified as one of the most important abiotic predictors of the distribution of arbuscular mycorrhizal fungi (AMF), we conducted growth chamber experiments with four plant species and eleven AMF isolates to assess their fundamental niche along a temperature gradient. The GlobalAMFungi database was used to preselect the AMF based on their putative preference for colder or warmer environments. Development of their intraradical and extraradical phase along a gradient of temperature was scored after two months of cultivation.

**Results:** We observed clear differentiation of AMF in sensitivity to increasing temperature: e.g. *Gigaspora rosea*, *Rhizophagus manihotis* and *Racocetra fulgida* had higher intraradical colonization in 40°C, whereas it was much lower in 25°C and they did not establish at all in 5°C. In contrast, e.g. *Diversispora epigagea*, *Claroideoglossum claroideum* or *Archaeospora schenkii* displayed very low colonization in 5°C, proliferated best in 25°C, while had lower or even zero root colonization in 40°C.

**Discussion:** To our knowledge, fundamental niche assessment in AMF is new to science.

**Conclusions:** Our results hint that different AMF species will respond very differently to global changes, which could influence their future distribution.

**Keywords:** temperature, experiment, arbuscular mycorrhiza, species, niche



O-035

## Environmental Change

### EFFECTS OF ELEVATED TEMPERATURE ON ERICOID MYCORRHIZAL GROWTH RESPONSE

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**Introduction/Aim:**Ericoid mycorrhizas (ERM) form symbiotic relationships with ericaceous plants, including economically relevant species like blueberries and rhododendrons. Nonetheless, they are particularly understudied within mycorrhizal ecology. ERM play an important role in the nutrient cycle of wetlands, including heathlands and peatlands, and support the large carbon storage capacity of these systems. In the context of climate change, these wetlands are warming at accelerated rates, which raises the question: Do higher temperatures affect the growth benefits (i.e. carbon production) that plants derive from ERM?

**Materials and Methods:**To address this knowledge gap, we are growing five wetland plant species paired with seven ERM fungal species in climate chambers at ambient and elevated temperatures, based on predictions for 2100 in the boreal and temperate biomes of the focal species. After 4 months, we will measure plant biomass and assess fungal colonization using traditional microscopy techniques.

**Results:**Results will show how climate warming influences the growth benefits that ericoid plants obtain from their fungal symbionts, and whether some plant-fungal pairings are more susceptible than others.

**Discussion:**This study will help to understand how wetland plant growth, and thus carbon sequestration, could be affected if fungal benefits are altered or lost under future warming scenarios. Moreover, increased knowledge of the factors influencing ERM associations will be relevant for improving agricultural practices for ericaceous plants.

**Conclusions:**This study is among the first to quantify climate change effects on mycorrhizal growth benefits in ericoid systems and will provide critical knowledge for understanding how wetland carbon dynamics may change in the future.

**Keywords:** Ericoid mycorrhizal fungi, climate change, temperature, restoration, wetland systems

O-036

## Environmental Change

### VARIATION IN REGIONAL MYCORRHIZAL PLANT INVASION STRATEGIES: A GLOBAL PERSPECTIVE

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**Introduction/Aim:** Plant invasions pose a significant threat to global biodiversity, dramatically altering habitats and disrupting ecosystem dynamics. Therefore, understanding the drivers of successful plant invasion is crucial for effective management. Invasive plant establishment is influenced by traits such as rapid growth rate, high reproductive and high dispersal capacity. However, the role of mycorrhizal associations in determining plant invasion success is increasingly recognised. While mycorrhizal invasive plants may outcompete native flora by leveraging enhanced nutrient acquisition and stress tolerance conferred by mycorrhizal symbioses, non-mycorrhizal plants may achieve higher invasion success due to independence from the mycorrhizal partners. Despite studies investigating the success of one strategy over the other, results remain inconclusive, with research overwhelmingly focused on European ecosystems.

**Materials and Methods:** In our study, we aim to test for regional patterns of mycorrhizal strategy using data collected across different regions worldwide. We analyse mycorrhizal strategies of invasive plants in combination with region-specific climatic, edaphic, and biogeographic factors and disturbance regimes to pinpoint the drivers of regional invasion strategies.

**Results:** Previous work has found evidence for regional variation in mycorrhizal plant invasion strategies between the US and Europe, underscoring the need for broad-scale investigations on invasion dynamics. Our study builds upon this, expanding its scope to explore global variations in mycorrhizal plant invasion strategies across diverse regions.

**Discussion:** Our study enhances understanding of mycorrhizal associations in plant invasion success on a global scale.

**Conclusions:** Results of our research are vital for identifying high-risk areas and plants for devising targeted prevention measures.

**Keywords:** Mycorrhizal Fungi, Plant Invasion Ecology, Mycorrhizal Strategy, Regional Variation, Disturbance Intensity, Global Study

LT-051

## Environmental Change

### THE EFFECT OF ANTHROPOGENIC NITROGEN DEPOSITION COUPLED WITH STAND-REPLACING FOREST FIRES ON MYCORRHIZAL FUNGI DIVERSITY

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**Introduction/Aim:** Mycorrhizal fungi enhance the ability of ecosystems to drawdown carbon, but are sensitive to anthropogenic nitrogen deposition and increasing forest fires. As these fungal communities play a large role in plant succession, nutrient cycling, etc., they help determine rate of carbon sequestration and stability of soil carbon pools.

**Materials and Methods:** We used the established critical load (CL) of nitrogen for mycorrhizae mapped over recent forest fire perimeters to select sites that occur inside and outside of burn perimeters, and above and below the CL of nitrogen in Yosemite National Park (Washburn/Lukens-Fire, 9000ha), Kings Canyon NP (KNP-Fire, 36,000ha), and Rocky Mountain NP (East Troublesome-Fire, 75,000ha). In each site, we collected soil samples for mycorrhizal fungi and nutrient analyses in summer 2023.

**Results:** We found soil N was very limiting within stand replacing fire, regardless of mapped anthropogenic N deposition totals. Overall, in Kings Canyon, total carbon was significantly higher in areas designated as below the CL for mycorrhizae than those above. In greenhouse inoculation studies, *Pinus contorta* inoculated with soil from burned sites in Rocky Mountain has significantly reduced germination compared to controls and unburned plots.

**Discussion:** Dominant pine species have been slow to reestablish in these parks. Post-fire management greatly influences critical ecological functioning and total carbon sequestration. Our data shows large variation across parks and megaburns in total C and N, but some trends within parks exist, leading to further microbial/nutrient analyses.

**Conclusions:** To restore and maintain critical ecological functioning we need a better understanding of complex stoichiometric interactions of forest ecosystems under climate change.

**Keywords:** forest fires, anthropogenic nitrogen deposition, mycorrhizae, soil carbon

**LT-052**

**Environmental Change**

**BIOTIC NOVELTY INDEX AS A MEASURE OF THE IMPACT OF INVASIVE PLANT SPECIES ON FUNGAL COMMUNITIES IN SOIL**

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**Introduction/Aim:** Multiple mechanisms can enable species to spread outside their native range. According to Darwin's Conundrum, successful novel species can be either closely related to native species, allowing them to take advantage of mutualistic organisms in their new range, or success derives from differences that enable escape from specialised pathogens

**Materials and Methods:** The Biotic Novelty Index (BNI) has recently been proposed to quantify the differences between native and invading plant species, considering the mismatch of plant traits and coexistence time. We use invasion gradients of two widespread aliens, *Reynoutria japonica* and *Solidago canadensis*, to test if BNI can be used to predict the shift these plant species cause in soil communities.

**Results:** We focus on the dynamics of different guilds of soil fungi, e.g. mycorrhizal and pathogenic fungi. We test the hypothesis that increasing BNI is related to decreasing fungal diversity across both invasive plant species.

**Discussion:** Comparing the species, *S. canadensis*, which has closely related plant species in the native flora and establishes communities with lower BNI, brings about a weaker shift than *R. japonica*.

**Conclusions:** Altogether, the work tests the applicability of the new approach for generalising the effect of invasive plant species on other trophic levels across plant species and habitats.

**Keywords:** invasive species, fungal ecology, biotic novelty, plant traits

LT-053

## Environmental Change

### LATITUDE PATTERN OF WARMING-INDUCED FRUITING PHENOLOGY SHIFT AFFECTS GLOBAL SUPPLY CHAIN OF PRIZED MATSUTAKE MUSHROOM

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**Introduction/Aim:** Timing of supply in the market is decisive to the price of gourmet wild edible mushrooms, which is the result of spatial variation of fruiting phenology across their global distribution range. Growing researches inferred varying patterns of fungal fruiting phenology using collective herbarium records along with a few site-specific field observations opened our understanding of these neglected organisms. Knowledge derived from direct evidence is, however, yet missing on the temporal and spatial variation of fruiting phenology for a given species under the impact of climate change. **Materials and Methods:** We conducted a direct fruiting observation of a prized matsutake (*Tricholoma matsutake*) at 38 sites across its global distribution range during 1966-2023.

**Results:** We found a general pattern of warm induced delayed first fruiting day and shortened fruiting duration of the global prized matsutake mushroom across its distribution range over the past 58 years, where the rate of change is latitude-dependent. The average rate of delay was 5.60 days/decade with the highest rate occurring at low-latitude. One-month temperature prior to first fruiting day was the major factor causing the shift. The sensitivity of first fruiting day to temperature also latitude-dependent, with the highest sensitivity in low-latitudes.

**Discussion:** This first large network with 38-on-site observations provided an explicit proof of climate impact on the displacement of fungal phenology and its global spatial variation.

**Conclusions:** Our findings are transformable to the international adaptation of supply chain on the trade of this delicatized mushroom.

**Keywords:** Mushroom fruiting phenology, Latitudinal pattern, Climate warming, Temperature sensitivity

LT-054

## Environmental Change

### EFFECTS OF REDUCED SNOW COVER ON SOIL FUNGI AND PLANT ROOTS IN BOREAL CONIFEROUS FOREST

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**Introduction/Aim:** Many regions have experienced a decrease in winter snow cover depth and duration due to the ongoing global warming. This affects microbial activities and the survival of plant roots, thereby influencing the soil organic C and N pools. Understanding microbial responses to varying snowpack conditions is important for evaluating the effects of climate changes on forest ecosystems. This study aims to assess how absence of snowpack affects the soil nutrients, pine root growth, and fungal communities in soil and roots of pine and shrub.

**Materials and Methods:** The experiment consists of two treatments: a control site with undisturbed snow cover and a snowless site where snow was prevented from covering the soil surface using shelters. Fine roots of Scots pine (*Pinus sylvestris* L.), along with rhizosphere soil and bulk soil, were collected for C, N and DNA analyses in 2022, following two winters of snow exclusion. The roots of ericoid dwarf shrub *Vaccinium myrtillus* were collected to evaluate the microbial interaction between shrub and pine roots.

**Results:** Soil respiration, serving as an indicator of microbial activities, showed a lower level in the snowless site after snow had melted away. Fungal growth, determined by soil ergosterol, was significantly reduced under snow exclusion. The absence of snowpack enhanced the pine root growth in autumn. To study the fungal community, fungal Internal Transcribed Spacer (ITS2) sequences were obtained from isolated DNA by Illumina NovaSeq platform. The results will be presented in the poster.

**Discussion:** It will be presented in the poster.

**Conclusions:** It will be presented in the poster.

**Keywords:** snow exclusion, root growth, fungal community, Scots pine, *Vaccinium myrtillus*

LT-055

## Environmental Change

### HOW DO SPECIFIC MYCORRHIZAL SYMBIOSES AFFECT THE PRIMING OF SOIL ORGANIC MATTER IN UPLAND AND ARCTIC SOILS?

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**Introduction/Aim:** Due to changes in climate, increased vegetation productivity and shifting vegetation types in northern latitudes has been thought to result in increased net carbon sequestration from the atmosphere; however, this largely overlooks the potential fate of pre-existing soil organic carbon stocks and decomposition processes. In this project, we examine how ecto-, ericoid, and non-mycorrhizal guilds affect the priming of organic rich soils in tree-line ecosystems.

**Materials and Methods:** We are applying novel natural abundance (and depleted) radiocarbon (<sup>14</sup>C), together with metagenomics, enzyme assays and soil organic matter chemistry, to quantify and understand mycorrhizosphere effects on soil organic matter stocks in situ and in vitro. Specifically, we are pairing trees and shrubs with mycorrhizal partners to see how they decompose heath and sedge dominated organic carbon rich substrates.

**Results:** Deciduous trees and shrubs are hypothesised to have the highest priming rates and highest fluxes of carbon dioxide that originate from soil organic matter. Additionally, high rates of priming are related to specific mycorrhizal taxa which are associated with substantial oxidative enzyme activity. For the ectomycorrhizal symbiosis, we will use isolated *Cortinarius* (higher potential oxidative capacity) and *Laccaria* species (lower oxidative enzyme activity) as inoculum with pine and birch as the hosts.

**Discussion:** Our projects uses novel field and laboratory approaches to examine ecologically relevant soil processes to examine the fate of pre-existing soil organic carbon stocks.

**Conclusions:** We are still developing how different ericoid and ectomycorrhizal partners decompose older soil organic matter.

**Keywords:** ectomycorrhizal fungi, ericoid mycorrhizal fungi, soil organic matter, decomposition, subarctic, tree planting

LT-056

## Environmental Change

### ARBUSCULAR MYCORRHIZAL FUNGI IN NORTH SEA SALT MARSHES UNDER GLOBAL CHANGE

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**Introduction/Aim:** Salt marshes form the transition from marine to terrestrial habitats in the temperate zone. Global change pose extraordinary threats to such ecosystems, as they are directly affected by rising sea level and temperature. The aim of the presented research is to gain knowledge on the arbuscular mycorrhizal fungi (AMF) abundance and community in salt marshes and their changes induced by experimental warming.

**Materials and Methods:** To achieve this, we collected samples from the Hamburger Hallig at the North Sea coast of Germany along the elevational gradient typical for salt marshes, both from sites grazed by sheep and from ungrazed sites, and from the MERIT experiment (Marsh Ecosystem Response to Increased Temperature).

**Results:** First results show a clear trend along the elevational gradient, with the highest abundance of AMF in roots (analyzed by microscopy and qPCR) in the high marsh zone, occasionally flooded during storm surges, followed by the low marsh at mid elevation and the (pioneer) zone located below mean high tide and thus flooded regularly twice a day. Increased salinity seems to affect AMF abundance negatively, while sheep grazing has no strong effect. Experimental warming (+ 3°C) seems to negatively affect the occurrence of AMF in the plants from the high marsh.

**Discussion:** Further (particularly the massive parallel sequencing) analyses still need to be finalized, but the initial results show that AMF are present, especially in the high marsh zone.

**Conclusions:** Analyses on the influence of other environmental factors on AMF community composition and function will allow insights relevant to their future conservation and protection.

**Keywords:** arbuscular mycorrhizal fungi, salt marshes, experimental warming, diversity, community profiling, quantification



LT-057

## Environmental Change

### LONG-TERM INDIVIDUAL-BASED POPULATION MONITORING REVEALS COMPLEX ENVIRONMENTAL AND HOST-SPECIFIC RESPONSES TO GLOBAL WARMING IN A KEY FOREST ENGINEER

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**Introduction/Aim:** European beech forests face increasing risks with global change. While the beech microbiome directly magnifies their resilience and resistance to climate and pests disturbances, a considerable knowledge gap surrounds the potential effect of global change on their symbiotic organisms themselves. *Boletus edulis* is probably the most prized mushroom forming ectomycorrhizal (EcM) fungus world-wide. In Europe, it forms very common associations with beech trees and is considered a very favorable mutualist based on its gene repertoire.

**Materials and Methods:** A decade long exhaustive mushroom sampling was used to test the year-around climate and host effect on individual (=genet) productivity, in 15 sites from German beech woodlands. We used an innovative approach combining Integrated Laplace Approximation (INLA) spatio-Bayesian models with microsatellites to identify every genet.

**Results:** The best predictors of annual mushroom production were temperature and precipitation during summer and fall, with a negative effect of temperature and a positive effect of precipitation. Interestingly, winter and early spring temperature and precipitation could also predict mushroom production, but in the opposite direction. Large host trees seem to buffer these effects, being associated with the most productive genets, while the majority of unproductive genets are very sensible to climate variations.

**Discussion:** Our approach with identified unique genets highlights the need to consider winter weather along with the host age when predicting EcM climate response.

**Conclusions:** Altogether, our results raise additional concern for the future of beech forests in Germany, demonstrating that warmer and drier summers are severely affecting a keystone beech symbiont.

**Keywords:** Global change, Beech forests, Ectomycorrhiza, *Boletus edulis*, productivity, INLA

LT-058

## Environmental Change

### A COMPARISON OF THE REALIZED AND FUNDAMENTAL NICHES OF SELECTED ARBUSCULAR MYCORRHIZAL FUNGAL SPECIES ALONG A NITROGEN GRADIENT

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) responses to environmental change are often predicted based on realized niches (observed distributions). However, these niches may not capture the full potential of AMF species due to limitations from biotic interactions.

This study investigates the fundamental niches (full growth potential) of AMF species compared to their realized niches along a nitrogen gradient, a factor highly impacted by human activity.

**Materials and Methods:** We utilized the GlobalAMFungi database to estimate realized niches of selected AMF taxa and conducted controlled greenhouse experiments to assess their fundamental niches along a nitrogen gradient. After eight weeks of continuous application of nutrient solution with six different nitrogen concentrations (in form of ammonium nitrate), we monitored root colonization and other structural traits of AMF and also host plant growth.

**Results:** Preliminary results suggest that AMF species exhibit varying preferences along the nitrogen gradient. For instance, *Rhizophagus irregularis* thrived even at high nitrogen levels detrimental to host plants and other AMF species. However, only certain AMF species, under specific nitrogen treatments, showed positive effects on host plant growth.

**Discussion:** Understanding the fundamental niche of AMF along the nitrogen gradient and comparing it with realized niches can enhance predictions of how global changes, such as nitrogen deposition, affect AMF distribution and community composition.

**Conclusions:** This study sheds light on the intricate dynamics between AMF, host plants and nitrogen availability, crucial for ecosystem management in the face of anthropogenic impacts.

**Keywords:** arbuscular mycorrhizal fungi, climate change, nitrogen deposition, fundamental niche, realized niche

LT-059

## Environmental Change

### WARMING-INDUCED EFFECTS ON THE ERICOID MYCORRHIZAL SYMBIOSIS OF THE BOREAL DWARF SHRUB *VACCINIUM VITIS-IDAEA* IN A 2.5-YEAR TRANSLOCATION EXPERIMENT

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**Introduction/Aim:** In boreal forests, understory shrubs rely on symbiotic soil microorganisms such as ericoid mycorrhizal (ErM) fungi to overcome nutrient limitations. Although future warming may alter plant nutrient demand and supply, the effects of climate warming on shrub-ErM interactions remain uncertain. We hypothesised that warming, simulated by translocation, modifies the interactions between *Vaccinium vitis-idaea* and ErM fungi due to accelerated nutrient mineralization by (I) a reduced assimilate flow of *V. vitis-idaea* to soil microbes and (II) a subsequent decrease in the abundance of ErM fungi in plant roots and soil.

**Materials and Methods:** To test these hypotheses, we translocated individuals of *V. vitis-idaea* and their surrounding soil in cores from North to South Finland (+4 °C mean annual temperature). The cores were excavated after 2.5 years, and the plants were pulse-labelled with <sup>13</sup>C<sub>2</sub>. We tracked the incorporation of <sup>13</sup>C into soil microbial biomass after chloroform fumigation extraction. ErM fungal abundance and root infection were assessed using phospholipid fatty acids, DNA extraction, and root staining.

**Results:** According to our hypotheses, the assimilate flux to microbes in the mineral soil was significantly reduced. We also observed a narrower ratio of fungi/bacteria and a lower root colonization by ErM fungi after translocation, yet not significant.

**Discussion:** These findings imply that *V. vitis-idaea* rely less on ErM symbiosis after simulated warming of +4 °C mean annual temperature, which may be due to increased nutrient mobilization by other soil microbes than symbionts.

**Conclusions:** This could reduce the need for plant investment in ErM under global warming, which might influence soil C sequestration.

**Keywords:** Ericoid Mycorrhiza, Carbon, Global Warming, Stable Isotopes

LT-060

## Environmental Change

### A TUNNING TOOL FOR ADAPTATION OF ARBUSCULAR MYCORRHIZAL FUNGI TO HARSH ENVIRONMENTS

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**Introduction/Aim:**AMF show resilience to harsh environments, including nutrient-poor soils, high salinity, extreme temperatures, and pollution. The HORIZON-MSCA-MYTUNETOOL project(2023-2027) focuses on adapting AMF to unhealthy environments for soil remediation and ecological restoration while understanding their adaptive mechanisms using molecular genetics tools.

**Materials and Methods:**In our project, we successively expose *in vitro* grown *R. irregularis* to increasingly harsh environments – in this case, heavy metals (HM). Over successive generations, we are observing whether fungal life history traits improve under high HM concentrations, indicating acclimation or adaptation. For acclimation, we expect an epigenetic response, while true adaptation would have an underlying genetic basis. The HM-tolerant *R. irregularis* strains will be tested in HM-contaminated sites to test their survival in planta and to see if those fungi could be effective inoculums.

**Results:**The phenotype of *R. irregularis* showed significant differences between HM exposure and controls. Molecular genetics tools are being used to analyze any genetic or epigenetic changes associated with metal tolerance and detoxification, aiding in unraveling AMF tolerance mechanisms.

**Discussion:**To successfully use AMF across diverse harsh environments, such tool enabling customized adaptation or acclimation of fungal strains to harsh conditions and understanding the mechanism is essential. Applications of HM-tolerant AMF would be ideal for soil remediation and ecological restoration. This project will bridge gaps in understanding AMF adaptation mechanisms to environmental stress, clarifying genetic or epigenetic bases of tolerance. It establishes an adaptable system applicable to studying other adverse environments and provides an eco-friendly AMF tool for promoting plant growth and ecological functioning in HM-contaminated sites.

**Conclusions:**

**Keywords:** arbuscular mycorrhizal fungi, heavy metals, adaptation, acclimation, soil remediation

LT-061

## Environmental Change

### EXPLORING THE ADAPTIVE PHENOTYPES OF *SUILLUS* SPECIES IN RADIONUCLIDE CONTAMINATED AREA OF FUKUSHIMA - JAPAN

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**Introduction/Aim:** The genus *Suillus* is a model for the study of ectomycorrhizal ecology and evolution. Type species, *Suillus luteus* is a fungal pioneer that is particularly abundant in disturbed areas, including polluted environments. In this study, we aim to catalogue *S. luteus* phenotypes present in radionuclide (Cs137) contaminated soils and nearby non-polluted sites of Fukushima Prefecture next to assessing the variety in phenotypes within closely related species *Suillus granulatus*.

**Materials and Methods:** Every strain has been exposed to an increasing gradient of stable cesium and gamma-irradiation. Morphometric parameters have been analyzed and gene expression has been studied. The potassium solubilization capabilities of the strains has been also evaluated.

**Results:** All collected isolates of both species were able to grow at high concentrations of Cs (0, 50, 100, 300, 500 mg/L) and doses of gamma-irradiation. (0, 70, 140, 540, 2140 mGy/h).

**Discussion:** This suggests the absence of a specific adaptation towards Cs137. However, the dose-response analyses showed that strains of *S. luteus* applied different strategies to cope with the presence of the Cs. Additionally, the gene expression analysis indicates that there is high intraspecific variability in responses of ROS scavenging enzymes regardless of the isolation site. The difference related to the isolation site is shown in the capability to solubilize K, and in the production of siderophores.

**Conclusions:** All together, these results show the complexity of local adaptations and suggest them to be translated in non-obvious traits. Whether these traits could support pine growth in radionuclide polluted areas remains to be tested.

**Keywords:** radiocesium, local adaptation, radionuclide tolerance, {*Suillus luteus*}

LT-062

## Environmental Change

### ENVIRONMENTAL DEPENDENCY OF ECTOMYCORRHIZAL FUNGI AS SOIL ORGANIC MATTER OXIDIZERS

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**Introduction/Aim:** Boreal forest soils are global carbon (C) sinks, where the dynamics of soil organic matter (SOM) are significantly influenced by ectomycorrhizal (ECM) fungi. Despite the strong correlation between ECM fungal community composition and soil C storage being well-documented, the underlying mechanisms remain elusive.

**Materials and Methods:** Here, we conducted controlled pure culture experiments to elucidate the mechanisms through which ECM fungi regulate SOM dynamics in response to varying inorganic nitrogen (N) availability, both in interspecific non-competitive and competitive conditions.

**Results:** Our findings show that ECM species typically prevalent in N-poor soils exhibit enhanced capacity for SOM decay, while ECM species dominating in N-rich soils display a preference for ammonia-N utilization, coupled with limited capacity to modify SOM. Further, we found that increased inorganic N availability alleviates N limitation for all ECM species but exacerbates their C limitation, as evidenced by decreased N compound decomposition and increased C compound decomposition.

**Discussion:** In competitive conditions, ECM species with heightened SOM decomposition potential facilitate the persistence of less proficient SOM decomposers, although all ECM species demonstrated a preference for C over N, intensifying the decomposition of relatively labile C compounds.

**Conclusions:** This study underscores the dynamic interplay between ECM fungal nutritional requirements and the nutritional environment of their habitat, offering insights for optimizing ECM-mediated C sequestration in the context of environmental change.

**Keywords:** ectomycorrhizal fungi, soil organic matter dynamics, inorganic nitrogen availability, interspecific competition

**LT-063**

**Environmental Change**

**EIGHT-YEAR SIMULATED DROUGHT TO INFER THE IMPACT OF CLIMATE CHANGE ON  
ECTOMYCORRHIZAL AND SOIL MICROBIAL COMMUNITIES IN ALPINE SPRUCE AND LARCH FOREST**

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**Introduction/Aim:**Climate change will lead to increased drought frequencies and intensities in the immediate future. Understanding how drought effects will impact (sub-)alpine ecosystems and mountain forests is desperately needed.

**Materials and Methods:**Here, we sampled soil cores at the end of a long-term rain-out experiment (8 years), to investigate the soil communities in an alpine mixed forest with spruces (*Picea abies*), larch (*Larix decidua*), and swiss stone pine (*Pinus cembra*) in Austria. We focused on ectomycorrhizal (ECM) communities, which were investigated by ECM morphotyping and molecular identification by Sanger sequencing. For a more comprehensive, and inclusive understanding of cumulative effects of drought on soil microbial communities, soil fungal and bacterial communities were investigated by community-based metagenomics.

**Results:**We expect to detect a clear difference in ECM colonization pattern, with more drought tolerant species present in the rain-out plots. It can be concluded that ECM species present in control plots but not in rainout plots are more susceptible to drought.

**Discussion:**The gained information on ECM as well as fungal and bacterial communities will be correlated to growth and stress response parameters which have been assessed for trees at these sites.

**Conclusions:**In this way, the feedback effect of soil microbial and ECM communities on the resilience of trees in alpine environments to drought stress will be deduced.

**Keywords:** mountain forest, global change, ectomycorrhiza, alpine ecosystem

LT-064

## Environmental Change

### ECTOMYCORRHIZAL FUNGI FORM LONG-LIVED MYCELIA IN THE BOREAL FOREST

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**Introduction/Aim:** While spatial coverage of belowground fungal community description is expanding to the global level, temporal turnover in biomass and community composition at the local scale is largely unknown. This is despite the facts that biomes vary largely in the duration and magnitude of seasonality and that contrasting fungal guilds are likely to respond differently to environmental cues, with potential consequences for ecosystem process rates.

**Materials and Methods:** To evaluate seasonality of contrasting fungal guilds, we monitored soil temperature and moisture continuously and sampled soils monthly over three years along a local fertility gradient in a boreal forest. We analysed the composition of total and active fungal communities by DNA- and RNA-based ITS-metabarcoding and evaluated mycelial biomass (ergosterol, quantitative PCR), productivity and turnover (ingrowth bags), and sporocarp production.

**Results:** Fungal communities (total and active) varied more between high- and low-fertility plots than over time. The Basidiomycota/Ascomycota ratio was higher in active- than in total communities, but this was driven by a larger proportion saprotrophic Basidiomycota in fall and winter and more ectomycorrhizal fungi during summer.

**Discussion:** The surprising stability of local ectomycorrhizal fungal communities throughout the three years suggests that mycelia are long-lived and spatially competitive, potentially due to continued root-derived carbon supply during the cold-season. More variable abundance of certain saprotrophic fungi may relate to seasonality in mycelial biomass turnover, necromass decomposition and sporocarp production.

**Conclusions:** Contrasting seasonality patterns of different fungal guilds suggest that time-shifts in their activities induced by changing climatic conditions may affect the ecosystem-level carbon feedback.

**Keywords:** ectomycorrhiza, fungal ecological guilds, boreal forest, seasonality, mycelial biomass dynamics, moisture and temperature responses



LT-065

## Environmental Change

### RECOVERY AND RESISTANCE OF SOIL FUNGAL COMMUNITIES IN ECOLOGICAL AND CONVENTIONAL GRASSLANDS UNDER ALTERED RAIN REGIMES

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**Introduction/Aim:** Assess the impact of altered rain regimes on the recovery and the resistance of soil fungal communities from conventional and ecological managed grasslands.

**Materials and Methods:** Intact soil monoliths from conventionally and ecologically managed grasslands across Europe were subjected to altered rain regimes (dry, wet, intermittent wet/dry and control). Soil fungal and arbuscular mycorrhizal fungal (AMF) communities were characterized using Illumina-MiSeq at three time points: before rain regime, after rain regime (resistance period), and after a recovery period.

**Results:** Management resulted in distinct soil fungal communities. Rain regimes influenced the AMF community structure after the resistance period, whereas had no effect on the pathogen or saprotroph communities. The dry regime resulted in the most distinct AMF community with greater AMF diversity. Conversely, after the recovery period, the wet regime resulted in reduced AMF diversity.

Conventional management enhanced soil fungal communities' recovery to rain regimes. Independently of grassland management, soil fungal communities were more resistant to the wet regime. Changes in the recovery and the resilience of the overall fungal community related to changes on the saprotroph community. Whereas, the resistance and the recovery of the AMF communities did not differ among rain regimes or grassland management.

**Discussion:** AMF community changes, particularly after the dry rain regime, may potentially aid the plant community to cope with drought. Higher recovery rate of conventional managed grasslands suggests greater adaptability of soil fungal communities to climate change.

**Conclusions:** To understand functional implications of soil fungal resistance and recovery it is necessary to link fungal community changes with ecosystem functioning.

**Keywords:** Climate change, ecosystems resistance and recovery, soil fungal communities, droughts and floods, organic and conventional grassland management

## Environmental Change

### SOME LIKE IT COLD: CHANGES IN SOIL FUNGAL COMMUNITIES ALONG A CLIMATIC GRADIENT

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**Introduction/Aim:** Climate change affects all ecosystem components, including soils. Soil fungi profoundly impact plant health, carbon cycling, and soil carbon balance, making it crucial to understand how climate change will influence their communities. In addition to direct effects, climate change can indirectly affect soil fungi through shifts in soil chemistry and vegetation structure. Here we assess whether soil fungal communities within the same ecosystem change with climate conditions.

**Materials and Methods:** We selected three mountain National Parks (NP) located along a latitudinal gradient within Spain. Within each NP, we sampled six elevation gradients covering the altitudinal distribution range of *Pinus sylvestris* forests. Three replicate plots were located at the lower, middle, and upper ends of the distribution range. At each plot, vegetation structure was characterized, and soil physicochemical properties and soil fungal communities (via metabarcoding) were described.

**Results:** The soil fungal communities differed among the National Parks, reflecting their distinct biogeographical conditions, forest structure, and soils. Still, similar patterns were observed within each NP, with significant differences in diversity and composition of the soil fungal communities along the climatic gradient associated with elevation.

**Discussion:** The changes observed were sometimes site-specific, suggesting that local environmental conditions and limiting factors may determine the direction and magnitude of changes in diversity and composition.

**Conclusions:** The ongoing rapid climatic change poses an important challenge of uncertain dimensions, particularly in the steep gradients of mountain areas. Future climatic conditions will likely alter soil fungal communities and their roles as decomposers, mutualists, or pathogens, with unknown consequences for ecosystem function.

**Keywords:** climate change, ectomycorrhizal fungi, Pinus forest, altitudinal gradient, vegetation structure, soil chemistry

**P-177**

## **Environmental Change**

### **IMPACT OF POSTFIRE MANAGEMENT ON BIODIVERSITY OF ECTOMYCORRHIZAL FUNGI**

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**Introduction/Aim:** Forest management practices need to evolve with the changing climate and land managers need information on what restoration techniques support the health and functionality of forests after megafires. We examine how different postfire management practices in salvage logging, aerial reseeded, and unassisted, natural succession following a stand replacing burn in mixed alpine forest impacts diversity of mycorrhizae biodiversity.

**Materials and Methods:** In Fall 2023, we collected soil cores to a 10cm depth from six paired plots in three regions of the Ashley National Forest within the East Fork Fire that were experiencing three different treatments: 1) unassisted, 2) salvage logged, and 3) aerial reseeded. From each soil core, we measured mycorrhizal fungi diversity and soil nutrients.

**Results:** Salvage logged and unassisted plots had poor revegetation three years following fire. In comparison, aerial seeding plots, while having greater than 50% bare ground, had many native forbs established. Preliminary data on soil nutrient analyses show that soils from unassisted burns were lower in pH and organic matter. Fungal DNA has been extracted and amplicon library prep completed, sequencing and analyses are still underway.

**Discussion:** The East Fork Fire burned 35,000ha in 2020. The following years were above average precipitation resulting in large soil erosion, nutrient loss, and lack of seedling establishment. *Pinus contorta*, the dominant species, may fail to recolonize with a reduced species soil community.

**Conclusions:** Forests after megafires require key species to regain ecosystem functionality. Understanding of how management practices impact mycorrhizal diversity can help inform future management decisions after burns.

**Keywords:** Ectomycorrhizal fungi, Wildfire, Forest Management Practices, Coniferous Forest

P-178

## Environmental Change

### **PINUS CONTORTA SEEDLINGS RESPONSE TO MYCORRHIZAL INOCULUM FROM RECENT, STAND-REPLACING FOREST FIRES BELOW AND ABOVE THE CRITICAL LOAD OF NITROGEN**

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**Introduction/Aim:**We examined the response of *Pinus contorta* seedlings to soil inoculum from recent stand-replacing forest fires that occurred below and above the critical load of atmospheric nitrogen deposition for coniferous mycorrhizae.

**Materials and Methods:**Individuals of *Pinus contorta* were grown from seed in a controlled greenhouse environment. We filled sterile D-pots with sterile soil (control) or sterile soil with inoculum collected across Yosemite, Rocky Mountain, and Sequoia/Kings Canyon National Parks (USA). Soils were collected in burned, unburned, and mapped high and low nitrogen in a full factorial design resulting in 10 treatments each with 10 replicas for a total of 100 pots. After 6 months, we destructively harvested *P. contorta* and aboveground biomass and mycorrhizal root colonization data are being collected.

**Results:**While preliminary *P. contorta* seedling biomass estimates are not significantly different across sites, germination data shows that pots inoculated with soil from the two burned sites, containing both high and low levels of nitrogen, in Rocky Mountain National Park, required more gibberellic acid to break dormancy compared to control pots.

**Discussion:**Failure to germinate, or delayed germination, in key species can have large impacts on ecosystem recovery and functioning. In Sequoia/Kings Canyon scientists are using giant sequoia seedling plugs in restoration efforts for the first time in park history due to reduced seedling establishment postfire.

**Conclusions:**Providing supplemental information can inform forest managers on the potential use of soil plugs with mycorrhizal symbionts to encourage regrowth of iconic forests and restore imperative terrestrial carbon sinks, an integral component of remedying climate change.

**Keywords:** forest fire, nitrogen deposition, mycorrhizae, seedling establishment

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## Environmental Change

### INVESTIGATING SIGNATURES OF LOCAL ADAPTATION IN THE UBIQUITOUS FOREST ECTOMYCORRHIZAL FUNGUS *CENOCOCCUM GEOPHILUM*

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**Introduction/Aim:** Increased levels of temperature- and drought-induced stress on forest ecosystems have led to a rise in tree mortality in Europe in recent years. Yet little is known about the genetic mechanisms underlying climate adaptation of their associated ectomycorrhizal fungi.

**Materials and Methods:** Here, we focus on the ubiquitous ectomycorrhizal fungus *Cenococcum geophilum*, characterized as one of the most abundant and often dominant ectomycorrhizal species, having an exceptionally wide host-range and drought-resistance. We will use in situ measurements to characterize soil and atmospheric environmental conditions at twelve sampling sites along a European transect. After strain-isolation, we will perform whole genome sequencing at individual level and search for significant genotype-environment associations. Finally, we will identify genomic regions involved in local adaptation by assessing the interplay between gene variants and the environment.

**Results:** We expect that gene variants encoding for aquaporin-related genes to be associated with soil water potential and soil chemistry. In addition, we expect complex gene pathways involved in the adaptation to drought with functions related to melanin production and other desiccation-resistance traits.

**Discussion:** Co-adaptation between host and symbiont populations complicates the search for genetic signatures of local adaptation. However, new statistical models enable us to disentangle these confounding factors and thus investigate genomics of natural ectomycorrhizal fungal populations.

**Conclusions:** Gaining a better understanding of the impact of extreme climatic events on mycorrhizal fungi is essential to assess processes of environmental adaptation and the underlying gene variants involved in natural selection.

**Keywords:** adaptation, *Cenococcum*, drought-resistance, ecology, ectomycorrhiza

P-180

## Environmental Change

### EFFECTS OF CLEAR-CUT FORESTRY ON THE ERICOID MYCORRHIZAL FUNGAL COMMUNITY IN *VACCINIUM MYRTILLUS* ROOTS

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**Introduction/Aim:** Clear-cutting and subsequent replanting of tree seedlings has emerged as the most common forestry practice of Northern European spruce forests. This practice, transforming natural forests into stands of even-aged trees, has potential impacts on belowground fungal communities. The ericaceous shrub *Vaccinium myrtillus* dominates the boreal forest floor vegetation and forms symbiotic relationships with ericoid mycorrhizal (ErM) fungi, both known to play an important role in soil organic matter dynamics. The abundance of *V. myrtillus* has decreased due to clear-cut forestry, but corresponding effects on ErM fungal communities are not well understood. We study how clear-cutting affects the diversity and composition of ErM fungi associated with *V. myrtillus* using a DNA metabarcoding approach. We also assess the influence of other co-variables, such as soil nutrients and climate.

**Materials and Methods:** We collected *V. myrtillus* plants from 12 paired plots of near-natural forests not clear-cut, and mature but earlier clear-cut spruce forests in South-Eastern Norway. ErM fungal taxa were identified by ITS2 metabarcoding.

**Results:** Our results will include data on forestry-induced changes in the diversity and composition of ErM fungi associated with roots of *V. myrtillus*, as well as correlations between other biotic and abiotic variables and the ErM communities.

**Discussion:** Paralleling the decrease in *V. myrtillus* caused by clear-cut forestry, we expect to find a decrease in the diversity of ErM fungi in the previously clear-cut forests.

**Conclusions:** Our study will provide insights that contribute to our understanding of the long-term effects of extensive forest management practices on belowground fungal biodiversity and ecosystem functioning.

**Keywords:** boreal forest, Ericaceae, ericoid mycorrhiza, fungal diversity, metabarcoding

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## **Environmental Change**

### **BIOGEOGRAPHIC PATTERNS OF ECTOMYCORRHIZAL FUNGI IN EUROPE**

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**Introduction/Aim:**The distance decay in community similarity is a fundamental concept in biogeography and conservation biology. Likewise, ecoregions are useful biogeographic units for conservation that are well supported across plants and animals, but apparently not for fungi.

**Materials and Methods:**Here we test the relevance of these concepts for ectomycorrhizal (ECM) fungi in central and northern Europe. We collected ~ 24,000 ectomycorrhizas from 129 forest monitoring plots in 18 countries, using a standardised protocol and representing 1,350 operational taxonomic units. We tested if ECM fungi communities follow a distance decay pattern and if they adhere to ecoregion boundaries, using three complementary methods. We also quantified the effects of host tree and fungal taxonomy on the observed biogeographical distributions.

**Results:**The similarity between ECM fungi communities exhibits a negative exponential relationship with geographic distance. Ecoregions predict large-scale ECM fungal biodiversity patterns, independently from climate or host tree. Basidiomycetes and epigeous fungi with short-distance dispersal show the best agreement with ecoregion boundaries.

**Discussion:**It is important to compare communities associated to the same host tree species, and to include a measure of fungal abundance to observe clear biogeographic patterns in ECM fungi.

**Conclusions:**Ecoregions and distance decay curves are useful tools for conservation decision-making encompassing fungi.

**Keywords:** bioregions, forest, microbiome, species distribution, species turnover

**Environmental Change**

**EFFECT OF AMMONIA EMISSIONS ON SOIL BIOCHEMISTRY AND FUNGAL COMMUNITIES UNDER A TREE SHELTERBELT**

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**Introduction/Aim:** Excess ammonia (NH<sub>3</sub>) is a key driver of biodiversity loss. Trees planted around NH<sub>3</sub> sources absorb emissions, but little is known about the subsequent effects on soil function and microbial communities. This study quantifies the impact of NH<sub>3</sub> emissions on soil biogeochemistry and fungal communities in a tree shelterbelt near a point source.

**Materials and Methods:** We measured tree growth and concomitant soil properties under a mixed shelterbelt downwind of poultry housing units on a farm in Cumbria, UK. We used amplicon sequencing to assess the abundance and diversity of soil fungi.

**Results:** Additional NH<sub>3</sub> inputs acidified the soil ( $p < 0.001$ ) and increased soil organic carbon content in the shelterbelt ( $p < 0.05$ ). The relative abundance of arbuscular mycorrhizal (AM) fungi was reduced ( $p < 0.05$ ) and the soil fungal community shifted ( $p < 0.05$ ) under high NH<sub>3</sub> emissions. Changes in soil fungal communities were most strongly correlated with soil pH, C/N ratio, and phosphate availability ( $p < 0.05$ ).

**Discussion:** Reductions in the relative abundance of AM fungi are likely due to lower plant requirements for N supplied by mycorrhizas, concomitantly reducing tree C allocation belowground. High NH<sub>3</sub> emissions may be acting on the fungal community indirectly, through changing phosphate availability, soil pH, and the C/N ratio.

**Conclusions:** We show that NH<sub>3</sub> inputs significantly alter soil biogeochemistry, reducing the relative abundance of AM fungi, and restructuring soil fungal communities in tree shelterbelt ecosystems.

**Keywords:** Arbuscular mycorrhizal fungi, soil microbial communities, ammonia emissions, soil acidification, soil biogeochemistry



**Environmental Change**

**GRADIENTS IN PEATLAND MYCORRHIZAL TYPES, AND THE GEOGRAPHIC DISTRIBUTIONS OF PEATLAND ERICOID MYCORRHIZAL FUNGI**

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**Introduction/Aim:**Peatlands are carbon accumulating wetlands, including acidic nutrient-poor bogs to circumneutral mineral-rich fens. The abiotic conditions and vegetation that vary among water-logged peatland habitats present unique environments for fungal diversity and a gradient of conditions that may affect mycorrhiza functioning.

**Materials and Methods:**We used a metabarcode dataset from across Earth's peatlands to test if the relative abundance of mycorrhizal fungal types (ecto [EMF], ericoid [ErMF], arbuscular [AMF]) track peatland habitat gradients, and to examine the distributions of ErMF lineages.

**Results:**Mycorrhizal fungi shifted along the bog-fen gradient, with ErMF dominant in bogs and poor fens, and AMF more frequent in rich fens. EMF were common in moderate and low pH peatlands, but were partial to drier sites with abundant woody hosts. These patterns were most distinct across northern peatlands. Known ErMF lineages showed broad distributions across the Northern Hemisphere, where they were more species rich than in the Southern Hemisphere.

**Discussion:**Dominant mycorrhizal types shifted along peatland gradients in ways broadly analogous to mycorrhiza distributions in uplands, but are set apart by the variation in saturation among peatland sites. Our results highlight the broad distributions of many ErMF taxa, while the dearth of ErMF in the Southern Hemisphere may be driven by the lower abundance of Ericaceae in these sites or a lack of taxonomic description of Southern Hemisphere ErMF.

**Conclusions:**This work suggests the possibility for developing a holistic framework for understanding gradients in peatland mycorrhiza types from fungal and plant perspectives, and emphasizes the need for work with Southern Hemisphere ErMF.

**Keywords:** bog, ericoid mycorrhiza, fen, ectomycorrhiza, arbuscular mycorrhiza, peatlands

**Environmental Change**

**EXAMINING THE IMPACT OF PRECIPITATION ON PLANT – MYCORRHIZAL ASSOCIATIONS USING POPLAR AS A MODEL SYSTEM**

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**Introduction/Aim:**Environmental stress significantly affects plant growth and alters interactions between plants and microorganisms. A subset of the microbial community, mycorrhizal fungi, plays a role in ecosystems and these interactions can enhance host stress tolerance. Approximately, 90% of woody plant species can be colonized by either Arbuscular Mycorrhizal (AMF) or Ectomycorrhizal (EMF) fungi. These fungi provide benefits such as nutrient exchange, enhancing plant resistance to pathogens, and increasing drought tolerance.

In this project, we ask how variation in precipitation regime impacts mycorrhizal diversity, community composition, abundance, and colonization of *Populus deltoides* (eastern cottonwood) and *Populus trichocarpa* (black cottonwood). *Populus* species are known to associate with AMF and EMF, this unique genus of trees to assess switching between mycorrhizal types within ecosystems across precipitation gradients.

**Materials and Methods:**We selected eight geographically contrasting sites located in Texas and Washington, with different annual precipitation values, to examine the role of precipitation on *Populus* – mycorrhizal dynamics. We collected rhizosphere and root samples from five poplar trees at each site during the fall of 2023. We leveraged staining techniques to assess EMF and AMF colonization and abundance within poplar roots, and we used high-throughput amplicon sequencing to assess changes in mycorrhizal diversity and composition.

**Results:**We observed variation in mycorrhizal colonization and composition across these sites.

**Discussion:**Previous studies have reported that EMF root-tip colonization was inversely correlated with mean annual precipitation, however, relative abundance of Basidiomycete and Ascomycete EMF was invariant across the gradient.

**Conclusions:**Future experiments will examine the role individual mycorrhizal taxa play in host stress tolerance.

**Keywords:** Mycorrhizal fungi, poplar, precipitation, gradient, drought, metagenomics

**Environmental Change**

**TREELINE TREES ARE MORE NUTRIENT-LIMITED THAN TREES AT THE VALLEY BOTTOM IN THE WESTERN BROOKS RANGE OF ALASKA**

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**Introduction/Aim:**Treeline advance has implications for carbon sequestration at the high-latitude forest-tundra interface. The mechanism constraining tree growth at treeline remains controversial. The carbon (C)-sink limitation hypothesis argues that photosynthesis can occur at a wider range of temperatures than cell division, and thus growth at treeline is directly limited by cold temperatures. Alternatively, the C-source limitation hypothesis argues that photosynthetic capacity is limited, thereby limiting growth, most likely from nutrient limitation.

**Materials and Methods:**To test for greater nutrient limitation at treeline, fertilizer was applied to white spruce (*Picea glauca*) trees at an upland treeline, in an adjoining forest, and on a riverside terrace in northwestern Alaska. Air temperature varied little across the sites, but growing season soils were colder at the treeline and forest than the terrace.

**Results:**Growth declined progressively from terrace to forest to treeline. After three years of applying nitrogen-phosphorous-potassium (NPK) fertilizer, growth increased strongly, with greater proportional increases observed at the treeline and forest. The proportion of root length colonized by ectomycorrhizal fungi was greatest on the terrace, where soils are warmest and tree growth is greatest. NPK fertilization led to an overall decrease in ectomycorrhizal fungal colonization.

**Discussion:**These results support the C-source limitation hypothesis and show that growth at the treeline and in the forest is more nutrient-limited than on the lower elevation terrace.

**Conclusions:**Nutrient uptake at treeline could be diminished by the effects of cold soils on microbial activity and soil nutrient availability, root growth, water uptake, reduced ectomycorrhizal fungal foraging efficiency, and/or reduced ectomycorrhizal colonization.

**Keywords:** Treeline, Ectomycorrhiza, Nutrient-Limitation, Carbon-Cycle

## Environmental Change

### EFFECTS OF TEMPERATURE AND NEIGHBOURING PLANT SPECIES ON THE ARBUSCULAR AND ECTOMYCORRHIZAL COMMUNITY STRUCTURE IN TREE SEEDLINGS

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**Introduction/Aim:**We have suggested that low temperature is a direct limiting factor for the distribution of arbuscular mycorrhizas (AM) in cool regions, whilst ectomycorrhizas (EM) are more tolerant to cold. However, the presence of compatible fungal propagules affects the success of mycorrhiza types. The interactions of temperature and availability of propagules are poorly known.

**Hypotheses:** 1. Mycorrhizal-fungal diversity in a seedling is higher if its neighbour has the same mycorrhiza type (AM or EM);

2. Low temperature reduces the diversity of AM but not of EM fungi (AMF, EMF).

**Materials and Methods:**Pairs of seedlings were grown in pots in soil from the field. *Acer platanoides* (AM), *Thuja occidentalis* (AM) and *Pinus contorta* (EM) were paired with different AM and EM tree species. The ambient temperatures during the second growing season were 21/16 and 15/10°C, day/night. Roots were harvested for DNA extraction. AMF sequences were amplified using WANDA and AML2, and EMF with (ITS)ITS2 regions, and sequenced on Illumina MiSeq platform. OTUs were assigned to taxa using the naïve Bayesian classifier and MOTHR-formatted UNITE database.

**Results:**Shannon index for *P. contorta* roots was lower at 16°C than at 21°C. EM diversity was highest when the two seedlings were of the same species. AM data analysis is in progress at the time of abstract writing.

**Discussion:**Comparisons between EM and AM community structure in relation to temperature and neighbours will be presented.

**Conclusions:**The results increase the understanding of the role of competition in mycorrhiza distributions.

**Keywords:** arbuscular, competition, ectomycorrhiza, neighbour, temperature, tree

# **Molecular Biology & Physiology**

O-037

**Molecular Biology & Physiology**

**MUCOROMYCOTINA 'FINE ROOT ENDOPHYTES' DO NOT REQUIRE THE COMMON SYMBIOTIC PATHWAY TO FORM NUTRITIONAL MUTUALISM WITH PLANTS**

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**Introduction/Aim:** Mucoromycotina 'fine root endophytes' (MFRE), a group of non-Glomeromycotinian mycorrhiza-forming fungi, have recently been shown to form nutritional mutualism with flowering plants, but the molecular mechanisms by which carbon(C)-for-nutrient exchange occurs in these symbioses remain undescribed. It is unclear whether the set of genes that orchestrates mycorrhization by Glomeromycotinian arbuscular mycorrhizal (AM) fungi are involved in MFRE colonisation.

**Materials and Methods:** Using mutant lines in the model plant host *Medicago truncatula*, we investigated the role of AM-symbiosis regulating genes in the phosphorous (P) / nitrogen (N)-for-C exchanges observed in plant-MFRE symbioses. With axenic experimental systems and radio- and stable-isotope tracers, we measured movement of <sup>15</sup>N, <sup>33</sup>P and <sup>14</sup>C across mutants impaired in genes involved in the calcium oscillation (DMI2), immune suppression to permit AM inter-radical hyphal colonisation (DMI3) and arbuscule formation (RAM1) in AM symbioses.

**Results:** While we found little evidence that *dmi2-1* and *ram1-1* mutations impaired nutrient exchange between MFRE and host plants, there was reduced C allocation to MFRE extraradical hyphae by *dmi3-1* mutants compared to wild type. Further scrutiny through metabolomic profiling of colonised roots via ultra-performance liquid chromatography-mass spectrometry indicated an MFRE-induced shift in root metabolites.

**Discussion:** Our results suggest the CSP is not necessary for MFRE colonisation and symbiotic function, but DMI3 - a gene with roles outside of the CSP in microbial-interaction and defence signalling - does.

**Conclusions:** This means MFRE symbioses are regulated by alternative and currently unidentified mechanisms.

**Keywords:** Mucoromycotina, *Medicago truncatula*, metabolomics, AM, Symbiosis

O-038

## Molecular Biology & Physiology

### ECTOMYCORRHIZA-INDUCED SYSTEMIC DEFENSES INVOLVE CERK-DEPENDENT AND -INDEPENDENT PATHWAYS

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**Introduction/Aim:**The interaction of mycorrhizal fungi with plant roots has beneficial effects for plant nutrition and defenses. For the exchange of nutrients, formation of a symbiotic structure is required, whereas other benefits may occur via interactions without a functional mycorrhiza. For example, volatile compounds released by mycorrhizal fungi without direct plant contact are involved in the recruitment of plant defenses. In non-host interactions, the ectomycorrhizal fungus *Laccaria bicolor* induced systemic defenses in *A. thaliana* in a CHITIN-ELICITOR-RECEPTOR Kinase (CERK)-dependent manner.

Here, we asked whether ectomycorrhizal systemic defense activation in its host species poplar (*Populus x canescens*) is dependent on CERK and involves volatile signaling

**Materials and Methods:**We generated poplar CRISPR/Cas9 mutant lines, in which the chitin-inducible oxidative burst was abolished.

**Results:**The transgenic lines showed normal growth and mycorrhizal colonization. Wildtype poplars showed significant changes in leaf transcriptional profiles after mycorrhizal colonization of roots in comparison with non-mycorrhizal poplars. The *cerk* mutants lacked an array of transcriptional responses related to immune signaling, while activation of flavonoid metabolism persisted in leaves of mycorrhizal wildtype and *cerk* lines. The flavonoid metabolism of leaves was also activated, when a direct contact between *L. bicolor* and poplar roots was prevented.

**Discussion:**The comparison of leaf transcriptomes of wildtype poplars with and without *L. bicolor* contact and of *cerk* mutants with and without mycorrhiza underpinned that the volatile-induced response was independent from CERK signaling.

**Conclusions:**Our study highlights the existence of CERK-dependent and -independent signaling pathways in a mycorrhizal host species and contributes to our understanding of plant-fungal signal transmission.

**Keywords:** stress, omics, mycorrhization, LysM receptor kinase, volatile organic compounds,

O-039

**Molecular Biology & Physiology**

**EVOLUTION AND FUNCTION OF RECEPTOR KINASES IN ARBUSCULAR MYCORRHIZAL SYMBIOSIS**

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**Introduction/Aim:** Evolution of symbiosis with arbuscular mycorrhizal fungi (AMF) was one of the key innovations that enabled the successful conquest of land plants. In this mutually beneficial symbiosis, fungi-delivered mineral nutrients are exchanged for photosynthates in elaborate, highly-branched arbuscules. To detect micro-organisms and non-self molecules, plants have a complex immune surveillance system including receptor kinases located on the plasma membrane. However, we do not fully understand how the early detection of the symbiotic fungus via receptor kinases lead to appropriate signal transduction, as well as the types of signals and receptors involved in the processes. At the same time, signalling activated by the fungus also reprogrammes plant root development to produce more lateral roots, resulting in increased surface area for symbiotic interactions.

**Materials and Methods:** We generated and analysed a suite of receptor mutants to study plant fungal signalling in a wide range of species: rice, barley and Medicago. These mutants were analysed for their levels of arbuscular mycorrhizal colonisation, calcium spiking responses, root architecture responses, and defence responses.

**Results:** We discovered that a set of plant receptors are involved in symbiosis establishment, a conserved signal-receptor system in activating lateral organs in response to chitin molecules, and for the first time, a LysM-receptor kinase mutant fully blocked in mycorrhizal colonisation.

**Discussion:** Tracing the evolution of these receptors also revealed insights on the origins and deep conservation of their symbiosis signalling functions early in land plant evolution.

**Conclusions:** Our work demonstrates a conserved role of these receptor kinases in orchestrating symbiosis signalling and root development in land plants.

**Keywords:** signalling, receptor kinases, root architecture, genetics



O-040

**Molecular Biology & Physiology**

**A CYTOPLASMIC SIGNALLING PATHWAY REGULATING ARBUSCULAR MYCORRHIZAL SYMBIOSIS IN *LOTUS JAPONICUS***

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**Introduction/Aim:**The symbiosis between land plants and arbuscular mycorrhizal (AM) fungi is regulated by evolutionary conserved genes present in all host plants (e.g. *Lotus japonicus*). This group of genes comprises the Common Symbiosis Signalling Pathway (CSSP), which can be divided into three modules: (1) signalling, (2) transcription, and (3) activation. In the signalling module, transmembrane receptor kinases (Myc factor receptors and SymRK) play a key role in the early recognition and accommodation of the fungal symbiont. However, the transduction of the signal downstream of the initial processes at the plasma membrane is still unclear.

**Materials and Methods:**We use comparative transcriptomics followed by orthology analysis and validate the results with a combination of reverse genetics and molecular approaches.

**Results:**We identified *LjKINa*, a receptor-like cytoplasmic kinase (RLCK) anchored to the plasma membrane by a N-myristoylation signal. Orthologs of LjKINa are conserved and co-regulated in AM-host and non-host plants. We show that LjKINa interacts at the transcriptional and posttranscriptional levels with conserved members of the CSSP. Compared to wild type, loss of function of *LjKINa* resulted in transiently reduced development of arbuscules and hyphae and repressed expression of AM marker genes. We also show that LjKINa interacts with a cytoplasmic kinase of unknown function. The biological significance of this interaction for AM symbiosis is currently being investigated.

**Discussion:**Together, these results suggest that LjKINa functions in the early stages of AM development.

**Conclusions:**In summary, our results reveal a cytoplasmic AM signaling pathway downstream of the CSSP that is required to sustain the development of AM symbiosis.

**Keywords:** Mycorrhiza, symbiosis, common symbiosis signaling pathway (CSSP), receptor-like cytoplasmic kinase (RLCK)

O-041

## Molecular Biology & Physiology

### THRIVING TOGETHER: UNRAVELING NUTRIENT DYNAMICS REGULATING ERICOID MYCORRHIZAL SYMBIOSIS

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**Introduction/Aim:**Ericoid Mycorrhizae (ErM) form symbiotic associations between ascomycetous fungi, such as *Rhizoscyphus ericae*, and plants like those in the order Ericales and leafy liverworts such as *Calypogeia fissa*. Our study establishes an ErM symbiotic system between *C. fissa* and *R. ericae*, aiming to create a model system for ErM research and understand its physiological and molecular mechanisms.

**Materials and Methods:**Axenic propagation of *C. fissa* and *R. ericae* following inoculation experiments.

Nutrient supply experiments with varying Nitrogen (N) and Phosphorus (P) concentrations. Quantitative phenotyping and imaging techniques.

**Results:**We found that P starvation significantly promoted the colonization of *C. fissa* rhizoids and growth benefits by *R. ericae*. Colonization and growth benefits were abolished under ample P and N deficiency. Combined P and N deficiencies resulted in the highest colonization and growth benefits for *C. fissa*.

**Discussion:**Similarly to other mycorrhizae like AMS, ErM establishment in *C. fissa* requires P-starvation. Unlike AMS, ErM inhibition occurs under conditions of ample P and moderate to low N. Our results contribute to a more comprehensive understanding of the role of ErM in plant resilience in low N soils, suggesting a regulatory interaction between N and P that influences the establishment of ErM.

**Conclusions:**The establishment of ErM in *C. fissa* is favored by P-starvation and it is regulated by N supply.

A full permissive state for the establishment of ErM is reached in P- and N-starvation conditions.

N-starvation is not required for the establishment of ErM.

N-starvation hinders the establishment of ErM in *C. fissa* in high P conditions.

**Keywords:** *Calypogeia fissa*, *Rhizoscyphus ericae*, Ericoid mycorrhizae, nitrogen and phosphate

O-042

**Molecular Biology & Physiology**

**UNRAVELLING THE MOLECULAR MECHANISMS BEHIND THE SYNERGISTIC EFFECT OF THE ARBUSCULAR MYCORRHIZAL AND RHIZOBIAL SYMBIOSES IN LEGUMES**

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**Introduction/Aim:** Legumes form symbiotic associations with arbuscular mycorrhizal (AM) fungi and rhizobia. So far, the physiological and transcriptomic changes in legumes upon colonization with either a single or two root symbionts is still unclear.

**Materials and Methods:** We inoculated *Vicia faba* plants with either one or two root symbionts and assessed plant performance, physiological parameters and the transcriptome in roots and nodules at flowering stage.

**Results:** The tripartite association was characterized by an increase of the number of nodules and arbuscules, and a higher plant carbon and nitrogen content compared to single inoculations. Gene expression analysis revealed that genes belonging to the carbon and phosphate starvation pathways were enriched in roots in response to AM fungus and rhizobia inoculation, respectively. In response to co-inoculation, pathways related to nutrient stress were down-regulated, whereas pathways related to auxin metabolism, transcription and metabolic processes were up-regulated in roots. Network analysis highlighted key mRNAs and ncRNAs involved in the regulation of both symbioses, including transporters and transcription factors.

**Discussion:** Physiological and transcriptomic analyses revealed that co-inoculation synergistically improved phosphorus, nitrogen, and carbon nutrition under low phosphorus conditions.

**Conclusions:** For the first time, transcriptomic analyses showed the molecular mechanisms associated to synergistic interactions between AM fungi and rhizobia and their effects upon plant growth and nutrition.

**Keywords:** Arbuscular mycorrhizal symbiosis, Rhizobial symbiosis, *Vicia faba*, Synergistic effect, Transcriptomic analysis

**O-043**

**Molecular Biology & Physiology**

**THE ROLE OF PHOSPHOINOSITIDES IN ARBUSCULAR MYCORRHIZAL SYMBIOSIS**

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**Introduction/Aim:** Phosphoinositides (PIPs) are a family of membrane lipids with a polar inositol head group that can be modified at various positions, giving rise to a range of PIP species. These are distributed throughout the cell and are important for membrane compartment identity. During colonization of plant cells with filamentous microbes such as arbuscular mycorrhizal (AM) fungi, new membranes are formed around colonization structures, resulting in the redistribution of PIPs. The PIP composition of these interfaces has been investigated in the roots of *Medicago truncatula*, which undergoes Arum-type mycorrhizal colonisation consisting of intercellular hyphae and arbuscules. However, I aim to characterise PIP localisation and function in *Nicotiana benthamiana* which displays an intermediate morphology, characterised by inter- and intracellular hyphae and arbuscules.

**Materials and Methods:** Stable transgenic lines of *N. benthamiana* were generated and characterized, primarily through confocal microscopy, during colonization by AM fungi and the oomycete pathogen *Phytophthora palmivora*.

**Results:** PI4P and PI(4,5)P<sub>2</sub> PIP species were found around both AM hyphae and arbuscules, with PI(4,5)P<sub>2</sub> predominantly localized at the tips of these structures (in contrast to *Medicago*), while PI4P showed uniform distribution. The PIP composition of membranes surrounding pathogen structures, with or without AM colonization, was also characterized. The function of PI(4,5)P<sub>2</sub> was investigated using depletion driven chemically or via a mycorrhiza-inducible promoter.

**Discussion:** This establishment of the PI line marker set and PI(4,5)P<sub>2</sub> depletion lines in *N. benthamiana* expands the toolkit for investigating phosphoinositides.

**Conclusions:** The tip localization of PI(4,5)P<sub>2</sub> suggests a role in the polarised growth of these membranes, highlighting its potential role in plant-microbe interactions.

**Keywords:** Phosphoinositides, periarbuscular membrane, *Nicotiana benthamiana*, trafficking, biosensors

O-044

**Molecular Biology & Physiology**

**SPORES-ON-A-CHIP: A NEW MICROFLUIDIC PLATFORM FOR INVESTIGATIONS ON ANASTOMOSIS IN ASYMBIOTIC HYPHAE OF ARBUSCULAR MYCORRHIZAL FUNGI**

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**Introduction/Aim:** Studying the fungal partner in the AM symbiosis at the cellular level is hampered by the difficulty to observe the fungus growing in soil. We exploit microfluidic technology to overcome this issue. Our approach creates microenvironments using transparent polymers, mimicking key aspects of AMF's natural habitat (e.g., micro-obstacles or chemical gradients), while still allowing brightfield and fluorescence microscopy analyses to be performed.

**Materials and Methods:** We imaged and studied behaviour of asymbiotic AMF hyphae using in-house fabricated PDMS microdevices, manufactured from bespoke, self-made silicon master moulds.

**Results:** The previously designed "*AMF-SporeChip*" allowed us to study the physical aspects of hyphal interactions within arrays of obstacles. This led to the discovery of highly dynamic hyphal responses upon mechanical collision, including reversible cytoplasmic retraction. We have since developed a variation of the original device in order to advance our understanding of anastomosis events among AMF isolates. The new devices encourage anastomosis by allowing two different strains to be inoculated at the same time and results provide a proof-of-concept of anastomosis formation at higher percentage than previously reported.

**Discussion:** The "*AMF-SporeChip*" has proven to be a powerful tool for studying AM fungal hyphae and helped reveal interesting new insights, which have been difficult to confirm at high resolution in past work. In continuation, after several iterative rounds of development and testing, the anastomosis device is now ready for a systematic study of anastomosis behaviour in AMF to unravel potential connections between phenotype and genotype.

**Conclusions:** Our microfluidic devices further aid our quest to decipher the ecology of AMF.

**Keywords:** Arbuscular Mycorrhizal Fungi, Microfluidics, Anastomosis, Space Navigation, Microscopy

O-045

## Molecular Biology & Physiology

### TRANSCRIPTIONAL LANDSCAPE OF PRE-SYMBIOTIC SIGNALLING IN RICE

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**Introduction/Aim:** Plants interact with a plethora of organisms in the rhizosphere, with outcomes that range from detrimental to beneficial. Arbuscular mycorrhizal (AM) symbiosis is the most ubiquitous of the beneficial interactions. However, how plants molecularly distinguish AM from parasitic fungi, especially when both produce the same kind of chitin derivatives, is still not fully understood.

**Materials and Methods:** We investigated the transcriptomes of a suite of mutants perturbed at distinct steps of AM symbiosis, exposed to a range of Microbe Associated Molecular Patterns and fungal exudates from beneficial *Rhizophagus irregularis* and pathogenic *Magnaporthe oryzae*, as well as to signals from a physically separated *R. irregularis*.

**Results:** The wild-type transcriptional response to chitinaceous molecules and fungal exudates showed a surprising lack of specificity. Mutants shifted the transcriptional landscape, even in mock conditions, towards defence. Surprisingly, independent of each mutant's deficiency, they all displayed a wild-type-like response to physically separated AMF, which was not the case when the fungus was allowed to contact the roots, suggesting that symbiotic fungal recognition occurs upon contact.

**Discussion:** Unexpectedly, some perception mutants, as well as a nutrient exchange mutant, when exposed to AMF signals, show a remarkable downregulation of genes associated with phosphate starvation, which also occurs under *flg22* treatment.

**Conclusions:** Our results show that the pre-contact plant's response to AMF is much less specific than previously considered, suggesting specificity is achieved mostly upon contact. Results also suggest that a symbiotic perception pathway counteracts the immunity-dependent repression of phosphate starvation, a process where a symbiotic nutrient transporter has a potential new signalling role.

**Keywords:** transcriptomics, symbiosis, defence, phosphate starvation, chitin, fungi.

O-046

## Molecular Biology & Physiology

### **ARABIDOPSIS THALIANA FORMS A MYCORRHIZAL SYMBIOSIS WITH MUCOROMYCOTINA 'FINE ROOT ENDOPHYTES'**

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**Introduction/Aim:** Mucoromycotina 'fine root endophytes' (MFRE) form functional mycorrhizal symbioses with diverse land plants, commonly co-colonising the same hosts as arbuscular mycorrhizal (AM) fungi. Unlike most plants, the Brassicaceous genetic model plant *Arabidopsis thaliana* is not known to host AM fungi, its genome having lost many core symbiosis signalling pathway genes characteristic of AM symbioses. Despite this, MFRE colonises the roots of *A. thaliana* but the functional significance of such associations is unknown. We investigated whether *A. thaliana* engages in carbon-for-nutrient exchange with MFRE.

**Materials and Methods:** We grew wild type *A. thaliana* (Col-0) in a monoaxenic plate system and inoculated plants with the MFRE isolate "Lyc-1" (isolated from *Lycopodiella innundata* and belonging to the Densosporaceae). We imaged root colonisation by MFRE and tracked movement of radio- ( $^{33}\text{P}$ ,  $^{14}\text{C}$ ) and stable isotope ( $^{15}\text{N}$ ) tracers between symbionts.

**Results:** We observed extensive colonisation of the *A. thaliana* root system, with typical MFRE colonisation structures (swellings, fan-like hyphal branching, hyphal coils) being formed within plant root cells. We detected significant bi-directional exchange of fungal-acquired  $^{15}\text{N}$  and  $^{33}\text{P}$  for photosynthetically-fixed carbon.

**Discussion:** Although classically believed to be non-mycorrhizal, *A. thaliana* forms nutritionally mutualistic symbioses with MFRE, suggesting an alternative signalling pathway to the "common" symbiotic signalling pathway may be active during MFRE symbioses. Current research focuses on transcriptomic and reverse genetics approaches to identify new key regulators of this plant-fungus symbiosis.

**Conclusions:** Our findings have important implications for the evolutionary history of mycorrhizas. This experimental system allows further exploration of the molecular mechanisms underpinning MFRE and other mycorrhizal symbioses in plants.

**Keywords:** Mucoromycotina 'Fine Root Endophytes', Mutualism, Nutrition, Arabidopsis, Densosporaceae, Brassicaceae.

**O-047**

**Molecular Biology & Physiology**

**MULTI-DAY TIME-LAPSE IMAGING OF ARBUSCULAR MYCORRHIZAL SYMBIOSIS PROVIDES INSIGHTS INTO COORDINATION AND REGULATION OF SYMBIOTIC NUTRIENT EXCHANGE**

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**Introduction/Aim:**The partnership between arbuscular mycorrhizal (AM) fungi and their host plants is spatially heterogeneous and temporally dynamic. Different fungal individuals and their various symbiotic structures can all be simultaneously, but asynchronously, developing and collapsing within a growing root system. We wished to follow underground AM processes over time at high resolution to capture the spatiotemporal context that is intrinsic to AM symbiosis.

**Materials and Methods:**To investigate the dynamics of colonisation progression, arbuscule development and nutrient exchange, the AMSlide was developed: a non-invasive, time-lapse, mycorrhiza imaging system. By combining the AMSlide with genetically-encoded fluorescent reporter rice lines and confocal laser scanning microscopy, symbiotic structures and their associated nutrient transporters were observed over timescales from minutes to weeks without disturbing plant or fungal partners.

**Results:**This approach captured distinct expression timings and localisations of phosphate uptake transporters, ammonium uptake transporters, and lipid exporters throughout unexpectedly variable arbuscule lifespans. For example, lipid exporter expression was seen to precede phosphate uptake transporter expression, and all three transporters occupied overlapping but contrasting domains in mature arbuscules. While the spatiotemporal dynamics of the nutrient transporters appeared consistent between arbuscules, the abundances of these transporters in the peri-arbuscular membrane were variable and responsive to nutrient availability.

**Discussion:**These results reveal fine-scale coordination of bidirectional nutrient transport at the arbuscules, as well as evidence for arbuscule-level regulation of reciprocal carbon-for-mineral exchange.

**Conclusions:**This work uncovers the unpredictability of arbuscule developmental trajectories, lifespans, and functionality, highlighting the importance of non-destructive monitoring approaches in advancing our understanding of AM symbiosis.

**Keywords:** arbuscule, dynamics, nutrient exchange, live-imaging, time-lapse, microscopy



**O-048**

**Molecular Biology & Physiology**

**INSIGHTS INTO MUCOROMYCOTINA "FINE ROOT ENDOPHYTE" BEHAVIOUR USING MICROFLUIDIC TECHNOLOGY**

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**Introduction/Aim:** Mucoromycotina fine root endophytes (MFRE) are a globally widespread but poorly understood group of mycorrhizal fungi with saprotrophic capabilities. Studying MFRE foraging behaviour confers a unique opportunity to understand how the tradeoff between saprotrophic and mutualistic lifestyles influences mycelial morphology.

**Materials and Methods:** We set out to investigate MFRE mycelial morphology of two MFRE isolates (Lyc1 and Lyc2) using microfluidic technology, a platform for manipulating microscale volumes of fluids that has expanded from chemical applications to the investigation of biological material. We manufactured custom-made microfluidic devices using soft- and photolithography techniques, designed to confine the growth of MFRE mycelium. From these devices, both high-resolution static images of mycelial morphology, and dynamic timelapses of growth behaviour can be obtained.

**Results:** We recorded growth behaviour of MFRE isolates in microfluidic devices using timelapse imaging and developed mycelial morphology quantification pipelines using publicly available software. Extensive branching was observed in sugar-rich media and upon collision with custom manufactured obstacles. The average hyphal growth rate of the Lyc2 isolate was measured at 48µm/hour.

**Discussion:** Our data acquisition and quantification methodologies offer the opportunity to investigate novel questions about the relationship between fungal growth behaviour and lifestyle in MFRE. In particular, for contrasting with Glomeromycotina arbuscular mycorrhizal fungi (AMF) which are obligate biotrophs, and can co-colonise plant roots with MFRE members.

**Conclusions:** We demonstrate proof-of-concept that microfluidic devices are useful tools to study the behaviour of MFRE isolates at the microscale. Future work will utilise microfluidic devices to visually monitor the progress of both mycorrhizal and saprotrophic development in real time.

**Keywords:** Mucoromycotina, microfluidics, behaviour, morphology, Glomeromycotina

O-049

## Molecular Biology & Physiology

### ARBUSCULAR MYCORRHIZAL FUNGI AND SEED PRIMING WITH NATURAL COMPOUNDS FOR IMPROVING TOMATO TOLERANCE TO WATER DEFICIT AND SALT STRESS

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**Introduction/Aim:**The Mediterranean region is particularly vulnerable to adverse environmental conditions, which will significantly affect the yield of major agricultural crops, including tomato. It is known that arbuscular mycorrhizal fungi (AMF) help host plants to endure different stressful conditions. In addition, seed priming could enhance plant ability to tolerate them. Here, we assessed the combined effect of AMF and seed priming (using natural compounds) in improving plant tolerance to abiotic stresses.

**Materials and Methods:**Priming of tomato seeds (Moneymaker variety) using natural compounds was performed, in combination with AMF (*Funneliformis mosseae*) inoculation under different stress conditions (water deficit and salt stress). Effect of the different treatments on plant fitness was studied using an integrated approach that included ecophysiology, hormonal analysis, transcriptomics, and non-targeted metabolomics.

**Results:**Analyzed parameters showed a clear effect of the AMF independent of seed priming under non-stressed and stressed conditions. Under stressed conditions, inoculation with AMF influenced eco-physiological parameters, improving photosynthetic activity. Transcript profiles, and accumulation of hormones and metabolites, were also influenced by the presence of AMF both under non-stressed and stressed conditions.

**Discussion:**A significant interaction between seed priming and AM fungal inoculum was detected, highlighting a differential transcriptomic and metabolic remodeling in stressed plants. The study revealed how AMF interact with seeds treated with natural compounds, shedding light on the molecular and physiological mechanisms controlling this interplay under water and salt stress conditions.

**Conclusions:**Results will provide novel information useful for developing seed priming systems using natural products together with AM fungal inoculation.

**Keywords:** AMF, seed priming, tomato, abiotic stress, plant tolerance

O-050

**Molecular Biology & Physiology**

**EXPLORING THE ROLE OF CROSS-KINGDOM RNA INTERFERENCE IN ARBUSCULAR MYCORRHIZAL SYMBIOSIS**

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**Introduction/Aim:**The establishment of arbuscular mycorrhizal symbiosis (AMS) requires intricate signalling processes, including the exchange of signals between partners; it has recently been proposed that these include small RNAs (sRNAs) acting in cross-kingdom RNA interference (ckRNAi). ckRNAi has been described in pathogenic plant-microbe interactions, with sRNA transport mediated by extracellular vesicles (EVs) (Cai et al., 2018); more recent evidence suggests its occurrence in mutualistic endosymbioses, including AMS (Silvestri et al., 2019). However, the mechanism of ckRNAi in AMS remains largely unexplored.

**Materials and Methods:**We used sRNAseq to identify *Medicago truncatula* sRNAs that are significantly upregulated during AMS, with predicted targets in the genome of *Rhizophagus irregularis*. Target gene and sRNA expression was analysed using qPCR, whilst mechanisms of sRNA transport are being explored through RNAseq of EV samples. We also present methodology currently being used to identify *Medicago* sRNAs bound to *Rhizophagus* RNAi machinery.

**Results:**Our results from gene and sRNA expression analysis indicate a downregulation of predicted targets correlating with the upregulation of the corresponding sRNA, suggesting the occurrence of ckRNAi. We have also preliminarily identified the presence of candidate sRNAs in EVs.

**Discussion:**Currently, our results point towards the targeting of *Rhizophagus* genes by *Medicago* sRNAs during AMS, although it remains to be demonstrated that this results from sRNA-bound *Rhizophagus* RNAi machinery directing transcript cleavage. Intriguingly, *Rhizophagus* targets include fatty acid metabolism genes, suggesting that the host may be using ckRNAi to modulate nutrient exchange.

**Conclusions:**These results suggest the occurrence of ckRNAi in AMS and point towards its pivotal role in symbiotic communication.

**Keywords:** Arbuscular mycorrhizal symbiosis, Cross-kingdom RNAi, small RNAs, plant-fungal communication

O-051

## Molecular Biology & Physiology

### UNRAVELLING THE ROLE OF MYCORRHIZAL SMALL SECRETED PROTEINS IN COLONISATION AND HOST SPECIFICITY

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**Introduction/Aim:** Ectomycorrhizal fungi employ effector-like mycorrhizal small secreted proteins (MiSSPs) to bypass plant defence mechanisms and establish symbiotic relationships with plant roots. Despite their significance, the exact mechanisms by which MiSSPs interact with plant pathways remain largely unexplored. *Pisolithus* is a globally important genus of ectomycorrhizal fungi where each species has distinct host ranges. A well-known example is *Pisolithus microcarpus*, which primarily colonises *Eucalyptus* sp. In this study, we aimed to identify and characterise MiSSPs that play a role in the host specificity of *Pisolithus*.

**Materials and Methods:** We screened a range of eucalypts for colonisation potential with different *P. microcarpus* isolates. Subsequently, we performed *in silico* analysis and biochemical work with MiSSPs to better understand their mechanism of action.

**Results:** Contrary to previous assumptions, our findings reveal a broader host range for *P. microcarpus*, colonising other eucalypt genera more efficiently compared to *Eucalyptus* itself. Focusing on one MiSSP family highly regulated during colonisation, we identified specific amino acids that facilitate their interaction with plant host proteins.

**Discussion:** The expanded host range suggests a greater potential for *P. microcarpus* to interact with a diverse array of plant species within ecosystems. Moreover, the MiSSP-plant interaction sheds light on the mechanisms by which *P. microcarpus* circumvents plant immune signalling to establish symbiotic relationships.

**Conclusions:** Elucidating the mode of action across a broader range of MiSSPs not only opens new avenues for biotechnological exploration, but also paves the way for targeted interventions that can bolster plant resilience against environmental stressors through plant inoculation.

**Keywords:** ectomycorrhizal fungi, effector proteins, small secreted proteins, host specificity, *Pisolithus*, eucalypt

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## Molecular Biology & Physiology

### WHAT SOIL NITROGEN SUBSTRATES ARE ACCESSIBLE TO MUCOROMYCOTINA 'FINE ROOT ENDOPHYTES'?

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**Introduction/Aim:** Mucoromycotina 'fine root endophytes' (MFRE) have recently undergone a taxonomic revision, separating them from arbuscular mycorrhizal fungi (AMF). Like AMF, MFRE form nutritional mutualisms with plants, but potentially play a more prominent role in plant nitrogen uptake. Unlike AMF, MFRE are culturable in the absence of a host, suggesting they are facultatively saprotrophic. However, the range of soil nutrient sources accessible to MFRE remains unknown.

**Materials and Methods:** To identify which forms of nitrogen commonly found in soils are accessible to MFRE, liquid cultures of the fungus were supplied with a range of nitrogenous substrates including organic and inorganic sources. Following incubation, MFRE colonies were imaged and weighed.

**Results:** MFRE supplemented with ammonium, tri-alanine and N-acetyl-D-glucosamine exhibited both increased biomass and colony size compared to the control treatment. Urea and bovine serum only augmented colony size, but to a lesser extent than the previous three nitrogen forms. Nitrate did not elicit any growth response.

**Discussion:** Our results suggest MFRE can access nutrients from a range of organic sources. The mechanisms facilitating assimilation of the sources tested here are unknown, although extracellular catabolic agents and transport proteins seem likely. Unlike AMF, nitrate - a commonly preferred source of nitrogen across both plant and fungal kingdoms - appears unavailable to MFRE.

**Conclusions:** MFRE appear to possess a dynamic saprotrophic lifestyle which could benefit them, and consequently their plant hosts, in habitats where inorganic nitrogen is scarce. This provides a physiological and ecological niche for MFRE, distinct from AMF, explaining their cosmopolitan distribution.

**Keywords:** Mucoromycotina, nitrogen, saprotrophy, culturing

P-160

**Molecular Biology & Physiology**

**CHARACTERIZATION OF 4 "MEDICAGO" ANNEXINS AS EXTRACELLULAR VESICLE BIOMARKERS DURING ARBUSCULAR MYCORRHIZA SYMBIOSIS**

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**Introduction/Aim:** Extracellular vesicles are under high characterization for their roles in intra and inter species communication, making of them interesting candidates in arbuscular mycorrhiza context. This study aims to isolate and characterize extracellular vesicles released at the periarbuscular membrane at arbuscules stage by identifying protein biomarkers. Using mass spectrometry, 9 "Medicago" annexins were more detected in extracellular vesicles coming from colonized roots compared to non-inoculated roots. Here, MTR-5g063670, MTR-276s0050, MtANN1 and MtANN2 were further characterized as strong candidates for extracellular vesicle biomarkers.

**Materials and Methods:** Phylogeny, Western Blot, GUS reporter lines

**Results:** MTR-5g063670/MTR-276s0050 were the closest orthologs of "Arabidopsis" annexin 1 and 2, well-described extracellular vesicle biomarkers in plant-pathogen interactions, whereas MtANN1/2 were closer to "Arabidopsis" annexin 8. Moreover, their promoter activations were arbuscule-specific supporting the hypothesis that they play a role in extracellular vesicles release at the periarbuscular membrane.

**Discussion:** As MTR-5g063670 and MTR-276s0050 were orthologs of "Arabidopsis" annexin 1/2, we hypothesized that those 2 annexins could have a similar role in extracellular biogenesis by stabilizing sRNAs loading. Validating them as extracellular vesicle biomarkers could allow to purify specific subpopulations of vesicles and study their internal cargoes. Conversely, MtANN1/2 seem to label another extracellular vesicles subpopulation which by phylogeny analysis seemed symbiosis specific.

**Conclusions:** MTR-5g063670 and MTR-276s0050, as "Arabidopsis" annexin 1/2 orthologs were really strong extracellular vesicle biomarkers candidates in arbuscular mycorrhiza whereas MtANN1/2 could label a symbiotic specific subpopulation of vesicles.

**Keywords:** Annexins, extracellular vesicle, arbuscule, phospholipid binding protein

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## Molecular Biology & Physiology

### ECTOMYCORRHIZAL FUNGI AND ORGANIC NITROGEN MINING – INSIGHTS FROM MICROBIOLOGY?

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**Introduction/Aim:**The ectomycorrhizal genus *Cortinarius* has the genetic potential to produce class II peroxidases, enzymes that are associated with lignin degradation by saprotrophic fungi. Since ectomycorrhizal fungi typically rely on their host trees for carbon (C), it is believed that *Cortinarius* produces peroxidases to mine for nitrogen (N) from soil organic matter (SOM). *Cortinarius* decomposition activities has been mostly studied through bridging fungal community composition with soil peroxidase activities and soil organic C stocks.

**Materials and Methods:**However, these methods also pointed out *Mycena*, an abundant saprotrophic genus in boreal forests, as the main producer of class II peroxidases in-situ. Given the contrasting lifestyles of *Cortinarius* and *Mycena*, disentangling their respective contribution in SOM decomposition could have substantial consequences of C and nutrient cycles in forest ecosystems. To directly determine *Cortinarius* and *Mycena* decomposition potential, we phenotyped several species of both genus in-vitro for traits related to organic N mining.

**Results:**We evaluated parameters such as biomass production, enzyme activity and total N in mycelium when grown on different simple and complex N sources.

**Discussion:**We also compared *Cortinarius* and *Mycena* species with two outgroups: one ectomycorrhizal and one ericoid mycorrhizal fungi, both including strains well-known for their ability to degrade organic nitrogen sources.

**Conclusions:**Ultimately, these results will help incorporating ectomycorrhizal fungi in soil biogeochemical models of forest ecosystems.

**Keywords:** decomposition, nitrogen mining, soil organic matter, class II peroxidases, ectomycorrhizal fungi, saprotrophic fungi

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## Molecular Biology & Physiology

### REVISITING CLASSIC MYCORRHIZAL PAPERS REVEALS INCONSISTENCY IN METHODOLOGY

#### DESCRIPTION: A CASE STUDY

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**Introduction/Aim:**Has the reproducibility crisis struck mycorrhizal research? Here, we revisited classic papers from 1986–2004 in which ectomycorrhizas were synthesised on *Pinus sylvestris* roots for microcosm experiments. We found inconsistencies in the methodology that appear to have been reproduced throughout the years and that led to different results than expected when the same methodology was followed.

**Materials and Methods:**We examined methods for synthesising ectomycorrhizas on *P. sylvestris* for microcosm research presented in publications from 1986–2004 and replicated these techniques. Mycorrhizas were synthesised aseptically for 8 weeks in Petri dishes with 1:4 peat:vermiculite moistened with MMN-G, 16 h daylight, 15/10 °C day/night.

**Results:**Few papers described their methodology in sufficient detail, and there was enormous variation in techniques employed for the same basic process. For example, where the proportion of peat:vermiculite used was disclosed, it varied from 1:3–1:10. Our attempt to replicate this technique led to seedlings with excessive root growth despite 63% mycorrhization.

**Discussion:**A lack of precise information on ectomycorrhizal synthesis prevented accurate reproducibility. The proportion of peat:vermiculite is correctly described in only one study. We followed the instructions of most of the papers and their stated methods led to a completely different balance between roots and fungal growth compared with those in prior studies, despite the good infection rate.

**Conclusions:**While some variability is expected when growing organisms in symbiosis, to enable replication, studies must accurately describe the methods used. Our concern is that several classic papers inverted the proportion of peat:vermiculite used and described, e.g., 1:4 instead of 4:1, leading to reproducibility errors.

**Keywords:** ectomycorrhizas, reproducibility, synthesis of mycorrhizas, methodology, microcosms



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## Molecular Biology & Physiology

### GENE EXPRESSION OF THE ECTOMYCORRHIZAL FUNGUS *TRUNCOCOLUMELLA CITRINA*: VARIATION ACROSS DEVELOPMENTAL TIME AND PHYSIOLOGICAL STRESS

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**Introduction/Aim:**The gene pathways that regulate ectomycorrhizal symbiosis may vary considerably among diverse plant-fungal combinations, and have been elucidated in only a small number of fungal species. Understanding these pathways will facilitate further investigation of how these symbioses evolved, how they develop, and how they adapt to an ever-changing environment. Here, we examine gene expression in the ectomycorrhizae of *Pseudotsuga menziesii* and *Truncocolumella citrina*, focusing on how expression varies through developmental time and in response to drought stress.

**Materials and Methods:**We conducted two growth chamber experiments with Douglas fir seedlings (*Pseudotsuga menziesii*). In the first, *Suillus lakei*, *Truncocolumella citrina*, and *Rhizopogon evadens* were used for inoculation. To produce drought stress, we stopped watering five days before harvesting tissue. In the second, seedlings were inoculated with *Truncocolumella citrina*, and trees were harvested at crucial time points during the development of mycorrhizae: 1, 2, 4, and 6 months after inoculation.

**Results:**In the drought experiment, the different fungal species altered plant water use, with *R. evadens*-inoculated seedlings generally maintaining the most consistent transpiration rates with water reduction. Differential gene expression analyses are in progress for *T. citrina*, and will reveal how plant and fungal gene expression respond to these environmental and developmental conditions.

**Discussion:**We expect upregulation of symbiosis-specific genes, with additional drought response modulating symbiotic and non-symbiotic expression.

**Conclusions:**These experiments will inform our understanding of plant-fungal cooperation at the molecular level, including how this cooperation allows for adaptation to environmental stressors. Insights of this kind will facilitate broader ecological and evolutionary comprehension of the mycorrhizal symbiosis.

**Keywords:** gene expression, drought stress, developmental biology, ectomycorrhizal symbiosis, ecology, physiology

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## Molecular Biology & Physiology

### SMALL BUT POWERFUL: EXPLORING THE ROLE OF TREES' SMALL SECRETED PEPTIDES IN REGULATING ECTOMYCORRHIZAL ASSOCIATIONS AND FUNCTIONS

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**Introduction/Aim:**In boreal and temperate forests, most of the symbiotic associations formed by trees to improve their mineral nutrition are with ectomycorrhizal (ECM) fungi. Providing mineral nutrients in exchange for organic carbon, they have an energetic cost for the tree. To use their resources efficiently, trees perceive and integrate environmental nutritional cues to regulate adequately their ECM interactions but the signaling pathways involved are unknown.

In herbaceous, Small Secreted Peptides (SSPs) transduce local and long distance signals regulating symbiotic associations with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi in response to abiotic and biotic nutrient cues.

More than 4,000 SSP families exist in herbaceous, most remain to be functionally characterized. Several of these families were found in trees.

**Materials and Methods:**To investigate their role in the regulation of ECM symbiosis, we used an iterative method of HMM and blast to identify in the poplar and oak genomes the members of 21 SSP families known to regulate plant-microorganism interactions or to be induced during nutrient stresses in herbaceous. Amongst, we selected a set of SSPs transcriptionally regulated by ECM associations and/or nitrate stresses in poplar and tested their effects on ECM symbiosis through exogenous application of synthetic peptides.

**Results:**We found that several CLEs peptides enhance the ECM association between poplar and the fungus *Laccaria bicolor*.

**Discussion:**This suggests their participation to the formation or regulation of ECM symbiosis in poplar.

**Conclusions:**Further functional characterization of these peptides is required to understand their functions.

**Keywords:** Ectomycorrhizae, Small Secreted Peptide, Nutrient signaling, Nitrate

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**Molecular Biology & Physiology**

**THE MARCHANTIA PALEACEA MYC-FACTOR RECEPTOR LYR IS NOT REQUIRED FOR AM ESTABLISHMENT**

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**Introduction/Aim:**Most land plants form a symbiosis with arbuscular mycorrhizal fungi (AM) to acquire water and phosphorus, while nitrogen-fixing symbiosis with rhizobia (NFS) is restricted to legumes (Oldroyd, 2013).

**Materials and Methods:**Genetic studies have revealed the crucial roles of plasma membrane LysM Receptor-Like Kinases (LysM-RLK) in perceiving bacterial NOD-factors essential for establishing NFS (Buendia et al., 2018). In contrast, single loss-of-function mutants of LysM-RLKs in angiosperms only have quantitative effects on AM establishment, raising the question of whether AM formation strictly requires perception by LysM-RLKs of Myc-factors. To avoid potential functional redundancy in angiosperms, we conducted a systematic study using *Marchantia paleacea*, an AM forming liverwort which has only four LysM-RLKs in its genome. We generated all single loss-of-function mutants and examined their ability to host AM.

**Results:**In parallel, we generated similar mutants in a background expressing aequorin to assess their ability to respond to microbial compounds such as chitin oligomers (CO) and/or lipochitooligosaccharidic (LCOs) signals.

**Discussion:**Mutants of the CERK1 orthologue (MpaLYKa) completely lost the ability to establish AM while mutants of the single *Marchantia* LYR gene retained their ability to form AM. Interestingly, both MpaLYKa and MpaLYR mutants lost responsiveness to COs and MycLCOs as measured by cytosolic calcium variations.

**Conclusions:**Furthermore, binding experiments demonstrated that MpaLYR is a bona fide receptor for this class of molecules. Overall, our findings question the crucial role of Myc-factors in AM establishment in *Marchantia Paleacea*.

**Keywords:** *Marchantia*, LysM-RLK, Calcium, Perception

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## Molecular Biology & Physiology

### ESTABLISHING TRANSFORMATION SYSTEMS FOR THE ECTOMYCORRHIZAL FUNGUS *PAXILLUS INVOLUTUS*

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**Introduction/Aim:** Ectomycorrhiza are important as partners for trees of temperate and boreal forests. The fungal partners are ascomycetes or basidiomycetes that can be generalists or specialists. Although many aspects of ectomycorrhizal fungi have been studied in detail, molecular genetic work remains scarce. Here, we aim to establish transformation methods for *Paxillus involutus*, a basidiomycete that undergoes symbiosis with different tree species, including the genetically tractable poplar.

**Materials and Methods:** To this end, we test markers for selection of transformants, including different antibiotics and carboxin, and clone the corresponding genes into suitable vectors. For transformation, we work on both, protoplast-mediated and *Agrobacterium*-mediated transformation.

**Results:** *Paxillus involutus* showed different growth on the diverse selection media we tested. While we were able to generate protoplasts, regeneration of these protoplasts did not work so far.

**Discussion:** Protoplast-mediated transformation has been reported to be difficult in ectomycorrhizal fungi and we are pursuing different approaches to overcome this difficulty. For instance, basidiomycetes have a different nuclear content than ascomycetes, which could result in protoplasts without any nuclei. Protoplasting of vegetative spores should in general be more efficient in this regard than protoplasting mycelium.

**Conclusions:** However, with a robust transformation system for *Paxillus involutus*, we will be able to study gene functions and to learn more about the molecular mechanisms underlying ectomycorrhizal symbiosis, which is of major importance for temperate and boreal forests.

**Keywords:** ectomycorrhiza, *Paxillus involutus*, genetic transformation, selection marker, protoplast, *Agrobacterium*

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**Molecular Biology & Physiology**

**ULTRASTRUCTURE OF ARBUSCULAR MYCORRHIZAL FUNGAL TRADING NETWORK**

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**Introduction/Aim:**Arbuscular mycorrhizal fungi (AMF) interact closely with their host plant and enable a cross-species trade system between different plant species in which they act as intermediaries. While the importance of AMF to agriculture and ecosystems is becoming increasingly evident, even fundamental questions such as how the fungi can control the speed and direction of transport of traded goods are largely unknown.

**Materials and Methods:**Here, we combined state-of-the-art compression-free thinning methods with cryo-electron microscopy (cryoEM) techniques. This approach enabled visualization of the internal fungal structure at the nanoscale with unprecedented detail. In addition, cryogenic fixation preserved the delicate ultrastructure and cellular organization of AMF hyphae, providing insights into their near-natural state.

**Results:**This approach provided new insights into the architecture of the fungal cell wall and the structural organization of the trading network.

**Discussion:**This combination of methods opens new avenues in the study of AMF-host plant interaction at the structural level and allows a better understanding of the fungal control mechanisms of the trading system. In addition, the application of this method to other AMF strains could reveal differences in their ultrastructures that provide insight into the underlying control mechanisms of the trafficking network. Further research in this area is essential to realize the benefits of AMF in addressing global challenges such as food security and environmental sustainability.

**Conclusions:**In conclusion, the synergy between compression-free thinning techniques and cryo-electron microscopy offers new perspectives on the structural organization of AMF networks and their interaction with the host plant at the ultrastructural level.

**Keywords:** cryoEM, ultrastructure, AMF, trading-system, hyphae

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## Molecular Biology & Physiology

### THE FUNCTIONAL SIGNIFICANCE OF MUCOROMYCOTINA 'FINE ROOT ENDOPHYTES' IN FLOWERING PLANTS

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**Introduction/Aim:** Mucoromycotina 'fine root endophytes' (MFRE) are a globally distributed group of soil fungi that form symbiotic associations with plants spanning the land plant phylogeny, often co-colonising alongside arbuscular mycorrhizal fungi (AMF). To date, little is known about the functional significance of MFRE associations in vascular plants, particularly when AMF are also present. To address this lacuna and to assess the physiological plasticity of both symbionts, we compared symbiotic function of MFRE and AMF in single and dual colonisations across a range of flowering plants, under both modern and simulated future atmospheres.

**Materials and Methods:** Using a combination of radio- (<sup>33</sup>P, <sup>14</sup>C) and stable (<sup>15</sup>N) isotope tracers in soil-based microcosms, we tracked the movement of plant-fixed carbon for fungal-acquired nutrients in host plants colonised by either MFRE or AMF or both simultaneously. We performed the experiments under modern (440 ppm [CO<sub>2</sub>]) and simulated future (800 ppm [CO<sub>2</sub>]) atmospheric conditions.

**Results:** We found considerable diversity in function of MFRE compared to AMF symbioses under both modern and high atmospheric CO<sub>2</sub>, with dual colonisation offering the most benefits to host plants in terms of fungal-acquired nutrient for plant-fixed carbon exchange.

**Discussion:** Our results underline the ecological and physiological importance of mycorrhizal MFRE associations in improving host plant access to soil nutrients in exchange for plant-fixed carbon, today and in the future.

**Conclusions:** Our findings highlight an important niche for AMF and MFRE mycorrhizal associations in single and dual colonisations, providing a physiological mechanism underpinning their persistence across plant groups and varied habitats.

**Keywords:** Mucoromycotina, fine root endophytes, flowering plants, nutrients, exchange, function

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**Molecular Biology & Physiology**

**LOW TEMPERATURE TOLERANCE OF ARBUSCULAR MYCORRHIZAL FUNGI PRODUCED BY ASYMBIOTIC CULTURE**

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**Introduction/Aim:**Arbuscular mycorrhizal (AM) fungi are obligate symbionts and depend on the host for lipid nutrition. Recently, it has been shown that myristate (C14:0) can initiate AM fungal growth under asymbiotic conditions. Although AM fungi can grow with myristate alone, a mixture of fatty acids with myristate and palmitate (C16:0) increases biomass production more. Depending on externally supplied fatty acids, the lipid composition in AM fungi alerted. The ratio of saturated to unsaturated fatty acids in phospholipids is known to affect low-temperature tolerance in a variety of organisms. In this study, we evaluated the germination ability of spores grown in asymbiotic culture after exposure to low-temperatures to determine the effect of the combination of fatty acids added on the low-temperature tolerance.

**Materials and Methods:***Rhizophagus irregularis* was grown on a medium containing fatty acids for 4 months. The generating spores were stored at 4°C for one month and then germinated.

**Results:**Myristate-induced spores completely lost their germination ability. On the other hand, spores generated by asymbiotic culture supplemented with myristate and palmitate were able to germinate. Furthermore, when AM fungi were cultured on a medium containing myristate, palmitate, and palmitoleate, germination rate of their next generation spores reached 96%.

**Discussion:**Saturated fatty acids accounted for 91% of the acyl groups in the phospholipid fraction of AM fungi supplemented with myristate. However, half of the acyl groups in the phospholipid of fungal materials supplemented with mixed fatty acids were unsaturated fatty acids.

**Conclusions:**Fatty acid mixture in asymbiotic culture increased the low-temperature tolerance of AM fungi.

**Keywords:** asymbiotic culture, fatty acid, low temperature, spore germination

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**Molecular Biology & Physiology**

**DIFFERENTIAL EFFECT OF FERTILISER APPLICATION ON ARBUSCULAR MYCORRHIZAL SYMBIOSIS AT THE SUB-CELLULAR LEVEL**

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**Introduction/Aim:**Arbuscular mycorrhizal symbiosis (AMS) is a widespread mutualism between land plants and fungi. In this interaction, the plant controls the accommodation of the fungus through a complex signalling cascade and restricts the development of the interaction in well-fertilized conditions. Previously, it was shown that overall colonisation and arbuscule density are impacted, but we know little about how fertilisation impacts the nutrient exchange structure of the symbiosis – the arbuscule. The nutrient transfer depends on proper arbuscule development, lifespan and transporter composition and these are the aspects we investigated in our rice-Rhizophagus irregularis system.

**Materials and Methods:**In variable levels of phosphate and nitrate we studied (1) arbuscule morphology and lifespan via staining the structures with WGA-Alexafluor488 and (2) symbiotic transporter abundance on the peri-arbuscular membrane (PAM) using translational-fusion fluorescent reporters.

**Results:**The results show that both high P and high N conditions lead to premature arbuscule collapse and fewer transporters localise to the PAM in response to high fertilisation, with high P treatment depressing the presence of phosphate importer OsPT11 and high N treatment depressing the presence of ammonium importer OsAMT3;1.

**Discussion:**Together, these results suggest that the functionality of arbuscules is decreased in high nutrient conditions. Further experiments also showed that transporter abundance is affected long before overall colonisation, and narrowed down the timepoint of these arbuscular changes, paving the way for examining the associated signalling.

**Conclusions:**This study demonstrates a specific impact of plant's nutrient status on the symbiosis at the arbuscular level as well as expands our knowledge of arbuscule plasticity and individuality.

**Keywords:** arbuscular mycorrhizal symbiosis, arbuscule, fertilisation, P regulation of symbiosis, arbuscule development



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**Molecular Biology & Physiology**

**THE ARBUSCULAR MYCORRHIZAL FUNGAL CELL ATLAS**

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**Introduction/Aim:**Arbuscular mycorrhizal (AM) fungi form extensive underground networks of filamentous hyphae that connect plant roots with the soil matrix. Interestingly, the AM fungal mycelium is coenocytic, resembling an 'open pipe' system with no cross barriers. This means all cellular contents flow through the mycelium in a common cytoplasm. This unique cellular organisation raises questions about how cellular components are organised, and how internal flows influence this organisation. Image analyses of the hyphal cell components can be challenging due to their fast movements, and very little is known about the structure of AM fungal cells and the underlying mechanisms regulating cytoplasmic flow.

**Materials and Methods:**We built a custom-designed fluorescence microscope which, combined with fluorescent probes, enabled us to image the extraradical mycelial cellular components spatiotemporally without disrupting the network.

**Results:**Together with other microscopy techniques in live and fixed cells, these methods allowed us to explore the organisation, relative abundance and volume of nuclei and lipids, the structure of the microtubule cytoskeleton and their relation to nuclei.

**Discussion:**While previous research has provided some insights into the organisation of such cellular contents, the objective of our work is to fully resolve the AM fungal cell. Here, we demonstrate the relative distribution of important organelles and cellular components across mycorrhizal networks. The next step is to expand our fluorescence techniques to further visualise additional components, such as actin filaments.

**Conclusions:**These results begin to uncover fundamental aspects of AM hyphal structure and function, and provide a foundation for exploring the mechanisms controlling cytoplasmic flow.

**Keywords:** Arbuscular mycorrhizal fungi, extraradical hyphae, fluorescence microscopy, nuclei, cytoskeleton

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## Molecular Biology & Physiology

### AMF COLONIZATION ALTERS THE PLANT SECONDARY METABOLISM RESPONSE TO DROUGHT

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**Introduction/Aim:** Climate change is a significant challenge to global food security. While the association between plants and arbuscular mycorrhizal fungi (AMF) have been shown to enhance plant drought resilience, the extent to which this advantage holds under additional biotic stresses remains largely unexplored.

**Materials and Methods:** In this study, we investigated the impact of a maize-AMF association on the plant secondary metabolism under drought conditions. Maize plants were grown in presence of the AMF *Rhizoglyphus irregularis* (Swiss Agricultural Foundation SAF22) under ambient or drought conditions (two levels corresponding to the predicted levels in the RCP2.6 and RCP 8.5 scenarios).

**Results:** We observed a decline in colonization rate of maize plants with *R. irregularis* under increasing drought conditions. Intriguingly, the association of maize with AMF resulted in a lower photosynthesis rate and a nutrient deficiency. Additionally, drought induced the production of benzoxazinoids, namely DIMBOA-Glc, DIMBOA-Glc-Glc, and DIM2BOA-Glc, in the roots, but the presence of AMF limited the drought-induced BX increase.

**Discussion:** Drought-mediated BX induction was previously observed in maize and suggested to be involved in plant resilience to abiotic stress. It is thus tempting to speculate that, if AMF-colonization enhances plant tolerance to drought, the need for the plant to utilize additional tolerance strategies, such as the production of benzoxazinoids, was reduced. Alternatively, AMF colonization could result in trade-offs and resource limitations preventing the plants to induce BXs.

**Conclusions:** Understanding how AMF colonization affects the plant secondary metabolism is crucial to better comprehend the impact of climate on plant-environment interactions.

**Keywords:** Maize, Climate Change, Arbuscular Mycorrhizal Fungi (AMF), *Rhizoglyphus irregularis* (SAF22), Benzoxazinoids, Physiology

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**Molecular Biology & Physiology**

**A METHOD ASSESSING ECTOMYCORRHIZAL FUNGUS COLONIZATION IN ROOTS OF TREES BY USING RELATIVE QUANTITATIVE PCR**

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**Introduction/Aim:** Ectomycorrhiza significantly influences establishment and stability of forest ecosystems. While evaluating ectomycorrhizal colonization is important for ectomycorrhizal symbiosis research, conventional methods using microscopes prove both time-intensive and subjective. To overcome this, we developed a high throughput and objective method for evaluating ectomycorrhizal colonization in target trees by using quantitative PCR (qPCR).

**Materials and Methods:** Primers specific for *Populus tomentosa*, *Cenococcum geophilum* (Cg) and *Laccaria japonica* (Lj) were designed and validated.

After separately inoculating Cg and Lj, as well as co-inoculating Cg and Lj to *P. tomentosa*, the root samples were harvested at three different time points; 10, 20, and 40 days post-inoculation. Ectomycorrhizal colonization was assessed by conventional method and qPCR with the aforementioned primers.

**Results:** The designed primers showed a high specificity and quantitativity to each species. Although both ectomycorrhizal species showed a positive correlation between conventional method and qPCR method, Cg (Spearman correlation = 0.917,  $p < 0.001$ ) showed a higher correlation compared to Lj (Spearman correlation = 0.690,  $p < 0.01$ ).

**Discussion:** The high correlation between the two methods indicates the practicality of the qPCR method in ectomycorrhizal assessment. Comparatively lower correlation in Lj is assumed to be caused by the fast turnover of Lj which can lead to the discrepancy between DNA amount and the colonization rate. Therefore, the qPCR method might be a better option in assessing functional ectomycorrhiza.

**Conclusions:** By using highly species-specific primers, we established a method to evaluate the ectomycorrhizal colonization by qPCR, which can be an alternative and solution to the conventional method.

**Keywords:** Ectomycorrhiza, Relative qPCR, colonization evaluation, *Cenococcum geophilum*, *Laccaria japonica*

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**Molecular Biology & Physiology**

**STARVATION FOR SYMBIOSIS: PRODUCING THE PLANT HORMONE KL TO PROMOTE THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS**

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**Introduction/Aim:**D14L/KAI2 is essential for symbiosis in higher plants, perceiving the unidentified plant hormone KAI2-ligand (KL) and triggering the degradation of SMAX1, a negative regulator which blocks plant-fungal communication.

**Materials and Methods:**We use fluorescent biosensors to monitor the action of KL in planta and screen chemical libraries to identify D14L agonists that could promote beneficial symbiosis in agriculture.

**Results:**We produced protein biosensors to monitor the activation of D14L and the turnover of SMAX family proteins, tools to examine KL signaling on a cellular scale, as well as its interplay with other plant hormones. We identify agonists of D14L in vitro and examine the impact of such chemical treatment on plant growth and mycorrhizal associations.

**Discussion:**We show that KL signaling is induced by N starvation as well as P starvation, and that SMAX1 displays complex stability dynamics during arbuscular mycorrhizal colonisation, suggesting regulation of the pathway is involved in arbuscule accommodation as well as predisposition for symbiosis. We present initial data on the impact of D14L agonist application on plant growth and the arbuscular mycorrhizal symbiosis.

**Conclusions:**Arbuscular mycorrhizal symbiosis offers many benefits to agriculture in terms of yield stability in the face of drought and other stressors, but is suppressed in medium - high input systems. We want to modulate or enable continuous arbuscular mycorrhizal colonization to maximize benefit from the interaction without the pleiotropic or regularly hurdles of genetic modification.

**Keywords:** D14L, SMAX1, KL, rice, biosensor, agonist

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## Molecular Biology & Physiology

### IS ARBUSCULAR MYCORRHIZAL FUNGI INTERNALIZING PHOTOSYNTHETIC CYANOBACTERIA?

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**Introduction/Aim:**The symbiogenesis theory describes the birth of eukaryotic life as a result of endosymbiosis, where single cell organisms turn into the organelles of eukaryotic cells. Half a century later, questions like “how the endosymbionts were acquired”, and “how they became permanent organelles” remain unanswered. Naturally occurring endosymbiotic systems can help explore these long-lasting questions but are hard to encounter. Despite their rarity, here we provide the first evidence of an uncharted endosymbiotic interaction between cyanobacteria and soil filamentous fungi.

**Materials and Methods:**Upon co-culturing the cyanobacterium *Nostoc* together with the model arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis*, we show internalization of – or parts of – *Nostoc* cells in the fungal hyphae. To further explore this interaction, we use novel in vitro culturing techniques alongside confocal and electron microscopy as well as molecular analysis.

**Results:**Initial evidence suggest that the fungus incorporates large amounts of chlorophyll in its mycelia and spores. Furthermore, we observe intriguing cell wall structural alterations at the interface of the two organisms, possibly to facilitate internalization of *Nostoc* cell or cell components.

**Discussion:**We now aim to understand how stable and widespread this interaction is and what function it serves. Whether this is a mutualistic or predatory interaction remains to be determined.

**Conclusions:**This novel system provides an exciting opportunity to address long-lasting evolutionary questions regarding the evolution of AMF and endosymbiotic processes in general.

**Keywords:** Arbuscular mycorrhizal fungi, Cyanobacteria, endosymbiosis, *Nostoc*

**Regulation  
and  
Transport  
in  
Mycorrhizal Networks**

LT-066

## Regulation and Transport in Mycorrhizal Networks

### EPIGENETICS: A NEW LEVEL OF REGULATION IN ECTOMYCORRHIZA DEVELOPMENT AND ABIOTIC CUE SIGNALING?

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**Introduction/Aim:** Ectomycorrhizas (ECM) are intrinsic component of tree nutrition and responses to environmental variations. We studied how ectomycorrhizas help trees cope with environmental changes and how epigenetic mechanisms regulate these mutualistic interactions

**Materials and Methods:** Ectomycorrhizal or non-ectomycorrhizal poplar cuttings were submitted to short-term cessation of watering or ozone fumigation and poplar gene expression, metabolite levels, and hormone levels were measured in roots, leaves, mycorrhizas. We analysed the ability of hypo or hyper-methylated transgenic poplar lines to form ectomycorrhizas with *Laccaria bicolor* together with their methylomes and transcriptomes

**Results:** ECM buffered hormonal changes in response to short-term environmental variations with part of the root ABA-signaling alleviated by their presence. ECM systemically prepared the root system for further fungal colonization and modified the leaf ozone responses.

The hypomethylated poplar lines display lower mycorrhization rate compared to wild-type. We identified poplar and fungal genes and transposable elements differentially methylated between those lines, including poplar genes involved in root initiation, ethylene and jasmonate-mediated pathways, immune response but also terpenoid metabolism.

**Discussion:** Our data suggest that ectomycorrhizas are less responsive than non-mycorrhizal roots to abiotic challenges, with poplar aromatic compounds and calcium signaling as integrators of ECM presence and abiotic cues. In addition, we provide the first investigation that host methylation status induces epigenetic remodeling of the fungal genome and vice-versa

**Conclusions:** *Populus-L. bicolor* symbiosis results in a systemic remodeling of the host's signaling networks in response to abiotic changes. In addition, host and fungal DNA methylation play a key role in their ability to form ECM.

**Keywords:** Poplar, {*Laccaria bicolor*}, multi-omics, ozone, soil water deficit, epigenetic

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## Regulation and Transport in Mycorrhizal Networks

### PHYTOPHAGY IMPACTS THE QUALITY AND QUANTITY OF PLANT CARBON RESOURCES ACQUIRED BY MUTUALISTIC ARBUSCULAR MYCORRHIZAL FUNGI

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**Introduction/Aim:** Plants interact with many biotrophic symbionts simultaneously, from mutualistic arbuscular mycorrhizal fungi (AMF) to parasitic potato cyst nematodes (PCN). As obligate biotrophs, AMF rely exclusively on their hosts for carbon in the form of carbohydrates and lipids and the impact of competing symbionts on this carbon flux is unknown.

**Materials and Methods:** We examined the movement of host-fixed carbon resources to AMF symbionts of potato with concurrent PCN infection, using a combination of radioisotope labelling, LC-MS and transcriptomics.

**Results:** We found that host resources were partitioned towards AMF-colonised, rather than PCN-infected roots of the same plant. Interestingly, resources were withheld by the roots rather than delivered to AMF, when PCN were distally infecting the root system. On PCN infected hosts, the delivery of monosaccharide-based carbon to AMF was dramatically reduced, whilst delivery of fatty acid was maintained. This reduction in monosaccharide but not fatty acid transfer to AMF was consistent on potato and Medicago hosts experiencing aphid herbivory, suggesting a potential general host response to phytophagy.

**Discussion:** This may have evolved as a mechanism for hosts to maintain beneficial symbiosis by supplying the required specific compounds to specific regions of the root system where benefit is perceived, whilst parasites are draining resources. Transcriptomic analysis indicates that differential delivery of specific carbon resources to AMF is potentially plant-led rather than AMF-regulated and may be regulated through expression of various sugar-transporters.

**Conclusions:** Revealing the molecular mechanisms underpinning the regulation of host resources to mycorrhiza in multi-symbiont scenarios is vital to effectively promote their efficacy as crop amendments.

**Keywords:** Resource transfer, parasitism, biotrophic interactions, competition



LT-068

## Regulation and Transport in Mycorrhizal Networks

### COMMON NON-MYCORRHIZAL NETWORKS: EXPLORING THE “DARK WEB”

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**Introduction/Aim:**The “Wood Wide Web” has captured pop-culture attention because of the fascinating fungal networks which the term describes, wherein mycorrhizal fungi may allow for the flow of resources among plants. Dark Septate Endophytes (DSE) are a type of non-mycorrhizal fungus and can also act as mutualists with plants. Despite the similarities between DSE and mycorrhizal fungi, there is little published about the possibility for non-mycorrhizal fungi to form common networks. The aim of this study is to assess the possibility that non-mycorrhizal fungi can connect plants in common networks and facilitate the flow of nitrogen and water among them.

**Materials and Methods:**We analyzed the transfer of isotopically enriched nitrogen between two sorghum (*Sorghum bicolor*) plants connected only by a DSE (*Alternaria alternata*) within custom sterile growth boxes in the laboratory. We also used light microscopy to assess dyed water movement between the plants and to confirm colonization.

**Results:**Microscopy revealed that the DSE crossed the barrier, colonized both plants, and brought dyed water to the unlabeled plant. Stable isotope data is forthcoming.

**Discussion:**The presence of the dyed water in the unlabeled plant indicates that a common DSE hyphal connection facilitated the transfer of water between the plants. The stable isotope data will be important in quantifying nitrogen movement among the plants.

**Conclusions:**Preliminary evidence suggests that more types of common fungal networks are possible than what was previously thought, including those which may connect plants of different mycorrhizal types.

**Keywords:** common mycorrhizal networks, CMNs, endophytes, DSE, dark septate endophytes, *alternaria alternata*

LT-069

## Regulation and Transport in Mycorrhizal Networks

### ECTOMYCORRHIZAL SYMBIOSIS IN SUBOPTIMAL ZN CONDITIONS, INCLUDING THE FUNCTIONAL CHARACTERIZATION OF TWO LACCARIA BICOLOR CDF-FAMILY TRANSPORTERS

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**Introduction/Aim:** Zinc is an essential micronutrient, though toxic when present in excess. Both, plant and fungal partners, in ectomycorrhizal (ECM) symbiosis require zinc for optimal cellular functioning but might show reduced fitness when exposed to high concentrations. Uptake of zinc and its cytoplasmic concentrations should therefore be tightly regulated. In this study, we investigate the impact of sub-optimal environmental zinc conditions (deficiency and toxicity) on establishment and maintenance of ECM symbiosis.

**Materials and Methods:** A co-culture experiment was set up between *Laccaria bicolor* and *Populus tremula x alba* and subjected to different concentrations of zinc: a depletion, an excess and a control condition. Colonization rate was calculated and impact on morphology of ECM was assessed by microscopy of root tip cuttings. In addition, gene expression of zinc transporters, reactive oxygen species decomposing enzymes and MiSSPs were studied in free-living mycelium, extraradical mycelium and ECM root tips. Lastly, two putative zinc transporters LbCDF-A and LbCDF-B were functionally characterized. After heterologous expression in *Saccharomyces cerevisiae* and its mutants these were subjected to drop-out assays and microscopy.

**Results:** This shows that CDF-A can transport zinc and is located on the endoplasmic reticulum whereas CDF-B is able to transport zinc across the vacuole.

**Discussion:** Altogether, the obtained results provide insights into how zinc pollution is impacting on ECM fungi and their symbiosis.

**Conclusions:** This can open pathways for the development of applications in the bioremediation of waste land.

**Keywords:** *Laccaria bicolor*, *Populus tremula x alba*, Zinc stress, CDF- family transporters, ECM symbiosis

LT-070

## Regulation and Transport in Mycorrhizal Networks

### DETERMINING THE NUTRITIONAL IMPORTANCE OF COMMON MYCELIAL NETWORKS IN A DESERT TRUFFLE MYCORRHIZAL SYMBIOSIS FOR SOIL NITROGEN REDISTRIBUTION

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**Introduction/Aim:**In semi-arid environments, maintaining plant biodiversity and mitigating desertification represent significant challenges. This study investigates the role of common mycelial networks (CMN) in redistributing nitrogen (N) among *Helianthemum almeriense* plants in symbiosis with the mycorrhizal fungus *Terfezia claveryi*. We hypothesized that CMN could facilitate N redistribution from nutrient-rich to nutrient-poor sites, potentially supporting plant growth and mitigating desertification effects.

**Materials and Methods:**A controlled experiment using compartmentalized mesocosms was designed to only allow mycelial connection between compartments. We investigated <sup>15</sup>N translocation from rich to poor N sites and assessed whether plant age affects the N redistribution. The tracer <sup>15</sup>N was applied to track the flow and absorption of N across different plant compartments and the associated mycelium.

**Results:**The study documented significant <sup>15</sup>N redistribution via CMN, with younger seedlings showing a higher percentage of the tracer in their N pool compared to adult plants. This indicates a more substantial role of CMN in the nutrition of seedlings.

**Discussion:**The findings suggest that CMN play a critical role in N redistribution in semi-arid ecosystems, potentially supporting the survival and establishment of new seedlings in nutrient-poor conditions. This mechanism may be fundamental for maintaining biodiversity and ecological balance in desertification-threatened areas.

**Conclusions:**The CMN have a great potential for N transfer to favour the survival of new mycorrhizal seedlings developing in desert truffle plantations or wild areas and, therefore, the maintenance of these ecosystems over time.

**Keywords:** common mycelial networks, nitrogen redistribution, semi-arid ecosystems, *Helianthemum almeriense*, desert truffle, *Terfezia claveryi*

**LT-071**

**Regulation and Transport in Mycorrhizal Networks**

**UNRAVELING THE FATE OF CARBON TRANSLOCATION BETWEEN EUROPEAN BEECH, DOUGLAS FIR AND THEIR SHARED ECTOMYCORRHIZAL FUNGI**

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**Introduction/Aim:** Trees allocate carbon (C) below-ground to their associated ectomycorrhizal (ECM) fungi. While it can be transported within the ECM network, it remains unclear whether C is transferred to other trees linked to the network. We investigated whether C is translocated from European beech to Douglas fir, allopatric tree species covering an increasing fraction of mixed forests in Central Europe.

**Materials and Methods:** We used pots with pairs of saplings (beech-beech or beech-Douglas fir) growing in close contact to each other. One sapling per pot was pulse-labeled with <sup>13</sup>C (donor, always beech) followed by complete harvest 5 days later. We then measured the isotopic signature in all above- and below-ground compartments of donor and recipient plants as well as in the fungus-colonized tissue and the transport plant tissue within the ectomycorrhizae. Additionally, we identified the ECM species colonizing the roots.

**Results:** In recipients, <sup>13</sup>C accumulated in the fungus-colonized tissue without being significantly channeled to the plant tissue. Moreover, Douglas fir recipients shared on average only 1 ECM species with beech donors and had a lower <sup>13</sup>C enrichment than beech recipients, which shared on average 3 out of 4 ECM species with donors.

**Discussion:** C was transferred from beech to the ectomycorrhizal fungi of conspecifics and of Douglas fir, confirming the distribution of C within the ECM network and between neighboring ECM fungi but not to neighboring trees.

**Conclusions:** We speculate that the ECM connections between European beech and Douglas fir might be reduced in mixed compared to monospecific beech stands, with consequences on C cycling.

**Keywords:** mycorrhizal networks, species interaction, resource exchange, pulse-labeling, introduced tree species

LT-072

## Mycorrhizas as Drivers of Interaction Networks

### STABLE ISOTOPE ANALYSIS INDICATES PARTIAL MYCOHETEROTROPHY IN ARBUSCULAR MYCORRHIZAL WOODY SEEDLINGS IN TROPICAL FORESTS

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**Introduction/Aim:** Chlorophyllous plants exhibiting partial mycoheterotrophy obtain carbon through both photosynthesis and mycorrhizal interactions. In arbuscular mycorrhizal plants, the *Paris*-morphotype (i.e. hyphal coils) of arbuscular mycorrhiza is considered a pre-requisite for mycoheterotrophic carbon gain. Numerous tree species in tropical lowland forests are in genera that form this arbuscular mycorrhiza morphotype, and under light- and nutrient-limitation in tropical forests, any additional carbon gain would be beneficial. However, if seedlings of woody species in the understory of tropical lowland forests exhibit partial mycoheterotrophy has not yet been explored.

**Materials and Methods:** Here we (a) examined the arbuscular mycorrhiza morphotype (*Paris*- or *Arum*-type) in seedlings of 42 tropical woody species, and (b) to determine if any of the *Paris*-type species are partially mycoheterotrophic, we compared their multi-element stable isotope natural abundance (<sup>13</sup>C, <sup>2</sup>H, <sup>18</sup>O, <sup>15</sup>N) to neighbouring autotrophic non *Paris*-type reference seedlings.

**Results:** 50 % of the investigated species (and 80 % of the genera) exhibited the *Paris*-type, expanding the number of tropical plant genera with *Paris*-type arbuscular mycorrhiza. Enrichment in <sup>13</sup>C, but not in <sup>18</sup>O indicated partial mycoheterotrophy in seedlings of 29 % of the investigated *Paris*-type arbuscular mycorrhizal woody species (6 out of 21).

**Discussion:** Our results provide the first evidence that carbon gain through mycoheterotrophy occurs in seedlings of arbuscular mycorrhizal tropical tree species.

**Conclusions:** In tropical forests, partial mycoheterotrophy during seedling establishment may provide ecological advantages with possibly far-reaching implications.

**Keywords:** endomycorrhiza, C gain, *Paris*-type, stable isotope natural abundance, saplings, lowland forests

LT-073

## Regulation and Transport in Mycorrhizal Networks

### PLANT PESTS INFLUENCE THE MOVEMENT OF PLANT-FIXED CARBON AND FUNGAL-ACQUIRED NUTRIENTS THROUGH ARBUSCULAR MYCORRHIZAL NETWORKS

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**Introduction/Aim:** Plants typically interact with multiple, co-occurring symbionts, including arbuscular mycorrhizal (AM) fungi which can form networks, connecting neighbouring plants. A characteristic aspect of the mycorrhizal symbiosis is the bi-directional exchange of nutrients between host plants and fungal partners. Concurrent interactions with competing organisms such as aphids or plant-parasitic nematodes can disrupt the carbon-for-nutrient exchange between plants and AM fungi. However, the role of mycorrhizal networks in mediating these interactions remains unclear.

**Materials and Methods:** Using isotope tracing in multi-plant experimental systems, we investigated the movement of plant photosynthates and fungal-acquired soil phosphorus through mycorrhizal networks and the effect of plant nematode infection on this.

**Results:** We found evidence of preferential allocation of fungal-acquired phosphorus to plants that were not infected by nematodes compared to infected neighbours. Contrary to previous findings using single plants, we detected no nematode-induced reduction in the amounts of plant carbon delivered to AM fungi in multi-plant systems. However, the mycorrhizal network(s) moved more plant-fixed carbon away from the nematode-infected host plants when these were present.

**Discussion:** Our work highlights the responsiveness of mycorrhizal networks to interactions with below-ground organisms. It also strengthens the argument for a more myco-centric view of AM-plant symbioses.

**Conclusions:** Experimental designs of increasing ecological complexity are needed for a more comprehensive understanding of the carbon-for-nutrient dynamics in AM fungi-plant networks. This will, in turn, elucidate the role of AM fungi in terrestrial carbon cycling and their function in agricultural systems.

**Keywords:** arbuscular mycorrhizal networks, biological markets, carbon-for-nutrient exchange, competition, mutualism, soil carbon

LT-074

## Regulation and Transport in Mycorrhizal Networks

### DUAL COLONIZATION OF LOBLOLLY PINE BY THE ECTOMYCORRHIZAL FUNGI PAXILLUS AMMONIAVIRESCENS AND AMANITA PERSICINA IMPROVE PLANT PHOSPHORUS UPTAKE

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**Introduction/Aim:**Loblolly pine (*Pinus taeda* L.) is an economically significant species, long established in the southeastern United States, where soils with limited phosphorus (P) availability are predominant. Ectomycorrhizal (ECM) fungi improve nutrient uptake by plant roots through the production of extraradical hyphae. In forests, trees are colonized simultaneously by multiple ECM symbionts.

**Materials and Methods:**To determine the effect that multiple ECM fungi may have on increasing P uptake, two ECM fungi were grown separately and collectively with *P. taeda* seedlings in sufficient (SP, 0.5 mM) and limited (LP, 0.05 mM) P treatments. *Paxillus ammoniavirescens* Pou09.2 was chosen as a fast growing, well studied species of fungi. An isolate of *Amanita persicina* was selected for its locality, being isolated from a coastal plain pine forest in the southeastern United States. Eight weeks after inoculation, seedlings were harvested and shoot P concentrations were determined.

**Results:**Both fungi significantly increased shoot P concentration in LP, but only *P. ammoniavirescens* increased P significantly at SP. When both fungi were grown on the same seedlings, however, shoot P concentration significantly increased in both conditions, compared to the control plants as well as plants inoculated with a single fungus.

**Discussion:**While both fungi were able to increase P concentration individually, our results indicate that there may be a synergistic effect in P uptake when multiple species are grown together on the same plant.

**Conclusions:**Studying the effects and interactions of multiple ECM fungi may be necessary to understand their involvement in plant nutrition.

**Keywords:** *Amanita persicina*, Ectomycorrhizal symbiosis, *Paxillus ammoniavirescens*, Phosphorus, *Pinus taeda*

LT-075

## Regulation and Transport in Mycorrhizal Networks

### PLANT MYCORRHIZAL STATUS INDICATES PARTNER SELECTIVITY IN ARBUSCULAR MYCORRHIZAL NETWORKS

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**Introduction/Aim:** Mycorrhizal symbiosis, specifically arbuscular mycorrhiza (AM), is one of Earth's oldest and most widespread symbioses. Plant species differ in the frequency they associate with mycorrhizal partners as some plant species have been reported to be always (obligately mycorrhizal, OM), some sometimes (facultatively mycorrhizal, FM), and some never (non-mycorrhizal, NM) associating with AM fungi. Here, we examine if plant species' frequency of associating with AM fungi, named plant mycorrhizal status, provides information about the variation in symbiotic mycorrhizal traits such as AM fungal colonization intensity and network indices of the AM fungal networks.

**Materials and Methods:** We sampled the roots and rhizosphere soil of 19 plant species, each from three habitats in Estonia. We determined AM fungal colonization in the roots and AM fungal community composition in the roots and soil using molecular methods.

**Results:** Arbuscular mycorrhizal hyphal colonization and coefficient of variation of AM fungal colonization were significantly lower in NM plants than in FM plants but did not differ between FM and OM plants. Arbuscular mycorrhizal fungal networks in the roots of OM plant species had a higher normalized degree and lower resource range than networks in the roots of FM plant species.

**Discussion:** Our results suggest that FM and OM plant species do not differ significantly in terms of hyphal colonization intensity in their roots but have distinctive partner selection, with OM plant species being more general.

**Conclusions:** Plant mycorrhizal status can give valuable information for some but not all symbiotic mycorrhizal traits.

**Keywords:** facultatively mycorrhizal, obligately mycorrhizal, AM fungal colonization, AM fungal community, network analysis



**P-187**

## **Regulation and Transport in Mycorrhizal Networks**

### **MOLECULAR TOOLS TO DESCRIBE AMF-WHEAT INTERACTIONS: INCREASE IN SOIL PHOSPHATE AVAILABILITY INDUCES SHIFT OF AMF COMMUNITY AND MYCORRHIZAL NITROGEN UP**

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**Introduction/Aim:**In order to be able to promote arbuscular mycorrhizal fungi (AMF) in agriculture we need to develop tools to better understand the dynamic of the nutritional interactions between AMF and crops.

**Materials and Methods:**We combined AMF characterisation in wheat roots on a 50-year phosphorus (P) fertilisation trial during two years, with targeted experiments in controlled conditions using nitrogen (N) and P gradients to (1) evaluate the community shift with increased P and to (2) assess the potential of mycorrhizal marker genes to characterise symbiotic status and function.

**Results:**Our results show a shift of the AMF community from low to high-P levels, decreasing diversity and notably increasing the dominance of species *Funneliformis mosseae*. In controlled conditions, we also observed an increase of the relative abundance of *F. mosseae* with high-P, when co-inoculated with *Rhizophagus irregularis*. The selected marker genes were specifically expressed during mycorrhization. Expression of AM3, an early colonisation marker gene, proved to be a good proxy for colonisation. Field results showed contrasting effect of the P-levels on AM3 expression across years, with inhibition of mycorrhizal colonisation in high-P in 2022 but not in 2019. A complementary experiment in controlled conditions revealed that this might be explained by distinct N availability..

**Discussion:**Expression genes (coding for the ammonium, nitrate and phosphate transporters localized in the peri-arbuscular membrane) revealed that high-P level increases mycorrhizal N nutrition when N is limiting

**Conclusions:**Our results indicate that these markers may allow to explore potential functional variability among AMF and their dependence on the abiotic context.

**Keywords:** AMF, wheat, marker-genes, nutrition, community analysis

## Regulation and Transport in Mycorrhizal Networks

### THE UNDERGROUND ECONOMY: REASSESSING THE ECOLOGICAL IMPACT OF TRADE IMBALANCES IN COMMON MYCORRHIZAL NETWORKS

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**Introduction/Aim:** Common Mycorrhizal Network (CMN) can connect neighboring plant roots, promoting species co-existence and enhancing intercropping productivity by facilitation. Trade imbalance in CMNs is suggested as a critical component, where one plant invests more carbon (C) into the network but doesn't receive proportional nutrient benefits. Despite increasing research on trade imbalances within CMNs, the underlying mechanisms and their ecological significance remain unclear.

**Materials and Methods:** We hypothesize that trade imbalances within CMNs affect plant productivity, favoring plants with a positive trade imbalance, enhancing mycorrhiza-driven increase in productivity, a concept termed- mycorrhizal dependency (MD). Additionally, we investigated how various CO<sub>2</sub> levels and altered nutrient demands impact trade and MD. C<sub>3</sub> and C<sub>4</sub> plants were grown in monoculture on a monotypic mycorrhizal network (MMN), and mixed, connected to a CMN. We assessed the C contribution of each plant to the CMN by analyzing the C isotopic signature of fungal lipids.

**Results:** At low CO<sub>2</sub>, a notable trade imbalance existed between intercropped plants, where C<sub>4</sub> dominates the carbon supply to CMN, while C<sub>3</sub> gained in productivity and nutrients. Interestingly, the trade imbalance didn't affect MD in both plants, consistent across MMN and CMN conditions.

**Discussion:** Rising CO<sub>2</sub> triggered strong productivity effects in C<sub>3</sub>-plants reflecting their strong dependency on AMF due to increasing nutrient demand. Consequently, trade imbalances decrease in C<sub>3</sub>, indicating significant environmental influence on CMN dynamics, unlike C<sub>4</sub> plants.

**Conclusions:** However, changes in carbon contribution and trade don't affect a plant's MD in MMN/CMN suggesting that trade imbalances have no discernible effect on growth and nutrient uptake within CMNs.

**Keywords:** mycorrhizal dependency, CMN, AMF, trade imbalance, intercropping, mycorrhiza

## Regulation and Transport in Mycorrhizal Networks

### THE ECTOMYCORRHIZAL FUNGUS *PAXILLUS AMMONIAVIRESCENS* INFLUENCES THE EFFECTS OF SALINITY ON LOBLOLLY PINE IN RESPONSE TO POTASSIUM AVAILABILITY

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**Introduction/Aim:**Salinity is an increasing problem in coastal areas affected by saltwater intrusion, with deleterious effects on tree health and forest growth. Ectomycorrhizal (ECM) fungi may improve salinity tolerance of host trees, but the impact of external potassium (K<sup>+</sup>) availability on these effects is still unclear.

**Materials and Methods:**Here, we performed several experiments with the ECM fungus *Paxillus ammoniavirescens* and loblolly pine (*Pinus taeda* L.) in axenic and symbiotic conditions at limited or sufficient K<sup>+</sup> and increasing sodium (Na<sup>+</sup>) concentrations. Growth rate, biomass, and nutrient contents were recorded for the fungus, and the colonization rate, root development parameters, and shoot nutrient accumulation were determined for mycorrhizal and non-mycorrhizal plants.

**Results:***P. ammoniavirescens* was tolerant to high salinity, although growth and nutrient concentrations varied with K<sup>+</sup> availability and increasing Na<sup>+</sup> exposure. While loblolly pine root growth and development decreased with increasing salinity, ECM colonization was unaffected by pine response to salinity.

**Discussion:**The mycorrhizal influence on loblolly pine salinity response was strongly dependent on external K<sup>+</sup> availability.

**Conclusions:**This study reveals that *P. ammoniavirescens* can reduce Na<sup>+</sup> accumulation of salt-exposed loblolly pine, but this effect depends on external K<sup>+</sup> availability.

**Keywords:** Ectomycorrhizal symbiosis, *Paxillus ammoniavirescens*, *Pinus taeda*, Potassium, Salinity, Sodium

## Regulation and Transport in Mycorrhizal Networks

### THE POPLAR SWEET1C GLUCOSE TRANSPORTER PLAYS A KEY ROLE IN THE ECTOMYCORRHIZAL SYMBIOSIS

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**Introduction/Aim:**The mutualistic interaction between ectomycorrhizal fungi and trees is characterized by the coordinated exchange of soil nutrients with soluble sugars. Despite the importance of this process, the precise mechanism by which sugars are transported from the roots to hyphae remains unclear. This study aimed to identify the specific membrane transporters responsible for the unloading of sugars at the symbiotic interface, with a focus on the role of the root Sugars Will Eventually Be Exported Transporter (SWEET) uniporters.

**Materials and Methods:**

**Results:**Our study employed RNA sequencing and quantitative PCR to identify *PtaSWEET1* genes in *Populus tremula* × *alba* during its symbiotic relationship with *Laccaria bicolor*. Our results suggest that PtaSWEET1 is primarily responsible for transporting glucose and sucrose, as demonstrated by the yeast assays. Moreover, we used a promoter-YFP reporter to localize *PtaSWEET1c* expression in the cortical cells surrounding the Hartig net hyphae, supporting its major role in supplying glucose at the symbiotic interface. Furthermore, our observations confirmed the localization of PtaSWEET1c-GFP in the plasma membrane. Inactivation of *PtaSWEET1c* reduced ectomycorrhizal root formation and 13C in the extraradical mycelium.

**Discussion:**

**Conclusions:**Our findings highlight the crucial role of PtaSWEET1 in facilitating glucose and sucrose transport at the symbiotic interface of *Populus-L. bicolor* symbiosis.

**Keywords:** ectomycorrhizal fungi, poplar, SWEET1, sugar transporter, *Laccaria bicolor*, glucose

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## Regulation and Transport in Mycorrhizal Networks

### THE UNDERAPPRECIATED VALUE OF HYDROGEN ISOTOPES IN ELUCIDATING HETEROTROPHIC CARBON GAINS BY PLANTS

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**Introduction/Aim:** Green plants are conventionally considered as photoautotrophic organisms. With the discovery of simultaneous carbon gains from photosynthesis and from fungi by some chlorophyllous forest orchids this simplistic view on plant carbon nutrition was seriously called into question. Stable isotope natural abundance analysis turned out as a core tool to separate heterotrophic fungus-to-plant carbon fluxes from carbon gains through photosynthesis. In the beginning, natural <sup>13</sup>C and <sup>15</sup>N enrichment in tissues of fungi simultaneously forming ectomycorrhizas with forest trees was used to elucidate fungus-to-plant carbon fluxes. Later on it turned out that not all fungal groups are necessarily enriched in <sup>13</sup>C. Based on <sup>15</sup>N enrichments without simultaneous <sup>13</sup>C enrichment found for green orchids mycorrhizal with fungi of the saprotrophic rhizoctonia group a “cryptic mycoheterotrophy” was postulated for this important group among the Orchidaceae. This puzzle of cryptic carbon gain from a fungal source was solved by adding hydrogen isotope abundance.

**Materials and Methods:** Here we present a comparison of carbon and hydrogen isotope natural abundances in twelve ectomycorrhiza-associated temperate forest orchid species in order to test their suitability as tools to quantify mycoheterotrophic carbon gains based on linear two-source mixing models.

**Results:** Both variables turned out as closely correlated ( $R^2 = 0.845$ ), and hydrogen isotope abundance was even better suited for quantification purposes of heterotrophic carbon gains due to smaller within-species standard deviations.

**Discussion:** The value of hydrogen isotopes in elucidating heterotrophic carbon gains by plants has obviously been underappreciated in the past.

**Conclusions:** Multi-element stable isotope applications are recommended to deepen our insight into mycoheterotrophic plant nutrition.

**Keywords:** Carbon isotopes, hydrogen isotopes, mycorrhiza, mycoheterotrophy, photosynthesis

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## **Regulation and Transport in Mycorrhizal Networks**

### **EVOLUTION OF TRANSPORTER GENE FAMILIES IN THE GENUS SUILLUS IN RELATION TO MINERAL WEATHERING CAPABILITY**

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**Introduction/Aim:** A number of ectomycorrhizal fungi are reported to biologically weather minerals, a process suggested to play a role in atmospheric carbon dioxide sequestration, biogeochemical cycles and plant acquisition of key nutrient like base cations, however the significance of its contributions is debated. The ectomycorrhizal genus *Suillus* is of particular focus, frequently showing heightened base cation content in mycelia when grown with minerals, but the mechanistic understanding of nutrient mobilisation associated with weathering remains largely unknown. We hypothesise that mineral weathering species have higher copy-numbers of base cation transporter genes to enable rapid uptake and transfer of recently mobilised nutrients and that *Suillus* species will exhibit greater base cation uptake than other species when grown with minerals.

**Materials and Methods:** We inferred the evolutionary expansions and contractions of base cation transporter genes on a phylogenomic tree that included 108 Agaricomycotina species. We also quantified mycelial base cation uptake by the ectomycorrhizal genera *Suillus* and *Piloderma*, and two saprotrophic fungi, when grown in pure culture with and without minerals.

**Results:** We show that 22 transporter gene families are significantly expanding and contracting in the genus *Suillus*, ten corresponding to base cation transporter families and two to base cation transporter accessories.

**Discussion:** The expansions and contractions of base cation transporter families in the genus *Suillus* indicate base cation transport is a key factor in their adaptation to the environment.

**Conclusions:** These results indicate the importance of base cation uptake and transport in the life strategy of *Suillus* species, and highlight the role of base cation transporters in mineral weathering.

**Keywords:** Biological mineral weathering, base cation transport, gene family evolution, CAFE5

## Regulation and Transport in Mycorrhizal Networks

### STR2 – A PLANT HALF-SIZED ABCG TRANSPORTER WITH A MULTIFACETED ROLE IN AM SYMBIOSIS

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**Introduction/Aim:** Investigation of a *Zea mays* mutant generated in a forward genetic screening<sup>1</sup> led us to discover an ‘early’ symbiosis phenotype, with the colonisation of arbuscular mycorrhizal (AM) fungi blocked at an early stage. The mutant was named *independent of AM symbiosis (ina)*.

**Materials and Methods:** The colonisation of *ina* plants by *Rhizophagus irregularis* was further tested through a nurse plant system and supplementation of wild-type root exudates. Meanwhile, positional cloning and CRISPR/Cas9 gene editing was used to identify the which gene causes the AM symbiosis phenotype.

**Results:** Growth with both wild-type nurse plants or root exudates partially recovered the colonisation of *ina* plants, but surprisingly the individual plants that were colonised in these conditions only developed stunted arbuscules. Positional cloning and CRISPR/Cas9 gene editing revealed the gene responsible was *STUNTED ARBUSCULE 2 (STR2)* – encoding a half-sized ABCG transporter.

**Discussion:** Heterodimers of STR2 and STR1 are believed to mediate lipid delivery to the fungus at the periarbuscular membrane<sup>2</sup>, with the stunted arbuscule phenotype already reported in Medicago and rice<sup>3-4</sup>. However, this new early colonisation phenotype identified in *ina* indicates STR2 has a second function before symbiosis starts, perhaps through interaction with another ABCG partner. Considering this, here we also present results from our efforts to identify potential binding partners of STR2 that act during the early colonisation role.

**Conclusions:** Since colonisation of *ina* can be complemented with exudates, this suggests that an unknown molecular component (potentially lipidic) is needed for AM fungus to initiate symbiosis. This work re-contextualizes our understanding of the STR2 protein's role.

**Keywords:** Presymbiotic phenotype, Root exudate, Lipid, ABCG transporter, {*Zea mays*}

## Regulation and Transport in Mycorrhizal Networks

### CYTOPLASMIC STREAMING IN ASYMBIOTIC ARBUSCULAR MYCORRHIZAL FUNGI

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) engage in mutualistic relationships with most plants where they exchange phosphorus and other resources acquired from the soil for plant-derived carbon. AMF demonstrate complex patterns of cytoplasmic streaming, involving fast, bi-directional flows within their large coenocytic networks. It is still unknown whether these fluid dynamics reflect symbiotic interactions such as nutrient exchange with the host, or physiological fungal functions independent of the symbiosis.

**Materials and Methods:** We explored a novel technique to grow AMF asymbiotically without a host. Suigura et al. (2020, PNAS) discovered that AMF can use myristate, a fatty acid, as a direct carbon source. We imaged the model species *Rhizophagus irregularis* grown on myristate-enriched MSR medium to study multiple traits related to network growth and cytoplasmic streaming. We extracted network topology from high-resolution images, and streaming velocities from videos by using kymographs.

**Results:** Initial results indicate that early-stage asymbiotic networks form more complex networks than germ tubes, but less complex than symbiotic mycelium. Cytoplasmic streaming in early-stage asymbiotic AMF differ strikingly from symbiotic flows. In symbiotic networks, flow speeds can reach  $>30 \mu\text{m/s}$ , while in early-stage asymbiotic hyphae the flows are limited to around  $5 \mu\text{m/s}$ . These asymbiotic velocities are comparable to those observed in germ tubes ( $5 \mu\text{m/s}$ ) in the absence of added carbon.

**Discussion:** Our data to date therefore suggest that symbiotic context strongly impacts cytoplasmic streaming dynamics within AMF hyphae.

**Conclusions:** How these differences in flow behaviors are related to altered network topology and resource exchange with the host requires further investigation.

**Keywords:** Arbuscular Mycorrhizal Fungi, cytoplasmic streaming, fluid dynamics, symbiosis, network topology, myristate



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## Regulation and Transport in Mycorrhizal Networks

### SYNTHETIC SYMBIOLOGY: RECONSTRUCTION OF A MUTUALISTIC SYMBIOSIS BETWEEN PLANT AND FUNGUS

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**Introduction/Aim:** Arbuscular mycorrhizal (AM) symbiosis is a 500-million-year-old partnership between most terrestrial plants and fungi of the Glomeromycotina that helps plants adapt to terrestrial environments by facilitating nutrient exchange, particularly in phosphate-deficient soils. Plants exchange photosynthetic carbon for phosphate by exploiting the lifestyle of the fungi.

Studying AM fungi is challenging due to their obligate biotrophic nature, resistance to genetic manipulation and complex structures. However, *Arabidopsis thaliana*, which is typically resistant to AM fungi, forms a mutualistic relationship with the ascomycete *Colletotrichum tofieldiae*, which is easier to cultivate and genetically modify. This provides a unique opportunity to study root endophytism and mutualism in *A. thaliana*.

**Materials and Methods:** In our project, we are engineering a synthetic mycorrhiza-like system between genetically engineered *A. thaliana* and *C. tofieldiae* to study the mutual exchange of nutrients between plant and fungal partners, along with their functional adaptation to metabolic interaction.

**Results:** We are generating and studying genetically modified *A. thaliana* lines and *C. tofieldiae* strains that either lack or overexpress genes involved in AM symbiosis. This is achieved using the model system *Lotus japonicus*, or by studying their orthologs from the two interacting partners.

**Discussion:** Our functional genomics approach aims to understand the mechanisms underlying mutualistic symbioses by dissecting the regulatory processes of both plant and fungal participants in this symbiotic relationship.

**Conclusions:** This reductionist strategy, termed 'Synthetic Symbiology,' seeks to unravel the genetic and molecular foundations of symbiotic interactions, paving the way for advancements in ecological and agricultural biotechnology by reconstructing and examining these biological systems in a controlled environment.

**Keywords:** mutualistic symbioses, *Colletotrichum tofieldiae*, *Arabidopsis thaliana*, metabolic interaction, synthetic symbiology

## Regulation and Transport in Mycorrhizal Networks

### THE INFLUENCE OF ARBUSCULAR MYCORRHIZAE ON PLANT DECISION MAKING PROVIDES INSIGHT INTO THE FUNCTION OF COMMON MYCELIAL NETWORKS

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**Introduction/Aim:** Plant-mycorrhizal relationships are a globally significant biotic interaction, determining community structure at all ecosystem levels, and fundamental to worldwide agriculture. There are established models which detail this interaction at the molecular and chemical interface, but little has been done to understand these interactions from a behavioural perspective

**Materials and Methods:** This study tests the hypothesis that mycorrhizae can influence plant decision making as a form of parasitism, examining whether such influences are mediated by the common mycelial network, as a channel for communication.

**Results:** To achieve this, morphological and physiological responses of *Nicotiana tabacum* subjected to three different Arbuscular Mycorrhizal (AM) treatments were measured in response to light competition scenarios. These were created via an experimental set up simulating taller and shorter neighbouring plants.

**Discussion:** Variance in responses between isolated and interconnected plants, either colonised or uncolonized by mycorrhizae was examined. It was shown that isolated AM colonised plants exhibited parasitic traits, negatively affecting plant performance, with no discernible effects on plant decision making. By contrast, plants that were AM colonised and allowed to form a network showed significantly reduced variation in response to light competition.

**Conclusions:** The results of this study demonstrate that networks of AM fungi can act parasitically in their influence of plant behaviour for their own gain. This has the significant implications, appearing to contradict the mutual network theory of plant communication via common mycelial networks.

**Keywords:** Network, Parasitism, Competition, Decision-making, Mutualism

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## **Regulation and Transport in Mycorrhizal Networks**

### **FROM FOREST HABITAT STANDS TO POT EXPERIMENTAL CONDITIONS: MYCOBIOME OF ULMUS LAEVIS**

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**Introduction/Aim:**The soil and root mycobiome is important part of each tree that links plant and soil ecological processes. Identifying the mycobiome community structure of elms suffered Dutch Elm Disease is essential due to fungi's multifaceted involvement in plant health, and development under diverse environmental conditions. To our study, *Ulmus laevis*, as one of Poland's native elm species and widespread in central Europe has been selected.

**Materials and Methods:**To reveal the mycobiome of *U. laevis*, eighteen locations representing various habitats including forest (riparian and oak-hornbeam forests) and non-forest habitats (forest nurseries, urban, rural, and post-industrial areas) were chosen. A pot experiment was also conducted to uncover the structure of mycorrhizal symbiosis of elm seedlings in the neighborhoods of tree species representing different types of mycorrhiza. Microscopic and metagenomics analyses were applied.

**Results:**Arbuscular colonization of elm roots was higher in forest habitats compared to non-forest ones, particularly evident in urban and post-industrial sites where non-mycorrhizal roots comprised over 50% of the total.

**Discussion:**Metagenomic analysis of soil samples showed significant differences in both mean species richness and the Shannon diversity of all fungi across different habitat types, with the evident prevalence of symbiotic fungi in forest habitats. A pot experiment's metagenomic results of soil samples showed the proximity of the roots of other tree species, remaining in arbuscular or ectomycorrhizal symbiosis, significantly affects the structure of soil fungal communities.

**Conclusions:**The lowest share of symbiotic fungi was found in the variants where only elm and maple seedlings were grown. This research was funded partly by the National Science Centre, (2020/37/N/NZ9/01915) and the Institute of Dendrology Polish Academy of Sciences.

**Keywords:** arbuscular mycorrhiza colonization, European white elm, soil, and roots fungal community

## Regulation and Transport in Mycorrhizal Networks

### ENHANCEMENT OF PLANT NITROGEN METABOLISM BY AM FUNGI UNDER GLOBAL CHANGE CONDITIONS

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**Introduction/Aim:** Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with most terrestrial plants, historically believed to increase plant nitrogen uptake. However, the impact of AM fungi on the internal nitrogen metabolism processes in plants is less understood.

**Materials and Methods:** To address this, a pot experiment was designed to investigate changes in plant nitrogen metabolism following AM fungal inoculation and their response to global changes, specifically warming and nitrogen addition. After growing *Leymus chinensis* for 120 days with AM fungi inoculation, we assessed metabolic substances and nitrogen metabolism enzyme levels in plants.

**Results:** Results showed an increase in metabolites such as arginine, proline, and lysine, predominantly enriching the amino acid metabolism pathway. AM fungi enhanced the levels of glutamine synthetase (GS) and glutamate synthase (GOGAT) without significantly affecting nitrate reductase (NR) and nitrite reductase (NiR). Notably, the beneficial effects of AM fungi on amino acid metabolism were further intensified under warming conditions, while nitrogen addition appeared to negate these benefits.

**Discussion:** The highest plant nitrogen content under warming conditions in the AM fungi inoculation treatment supports this perspective. Enzyme activity was a less effective predictor of plant nitrogen content, whereas metabolic substances better predicted changes in plant nitrogen content.

**Conclusions:** Overall, inoculation with AM fungi elevated nitrogen metabolism enzyme levels and intensified amino acid metabolism processes in plants. The relationship between AM fungi and plant nitrogen metabolism is regulated by global changes, indicating the need for further research to understand their interaction in a changing climate environment.

**Keywords:** Arbuscular mycorrhizal (AM) fungi, Nitrogen metabolism, amino acid metabolism, global change

## Regulation and Transport in Mycorrhizal Networks

### NITROGEN SOURCE PREFERENCE AND TRANSFER TO HOST PLANTS BY SYMBIOTIC MUCOROMYCOTINA FUNGI

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**Introduction/Aim:** Mucoromycotina ‘fine root endophyte’ (MFRE) fungi form nutritional mutualisms with plants but are understudied, despite their role in plant nitrogen acquisition. They co-occur with arbuscular mycorrhizal fungi but may utilise different nutrient sources thanks to their facultative saprotrophic capabilities.

**Materials and Methods:** We used four different <sup>15</sup>N-labelled compounds to track nitrogen transfer between MFRE and *Plantago lanceolata*. Nitrogen sources were applied singly and in mixtures to discern MFRE nitrogen source preference. We used <sup>15</sup>N/<sup>13</sup>C-glycine to track organic carbon assimilation by MFRE from a complex nitrogen source across a concentration gradient of nitrogen in the substrate. We used <sup>14</sup>CO<sub>2</sub> to determine plant-to-MFRE carbon transfer for each nutrient source tested.

**Results:** MFRE preferentially transferred glycine-N and ammonium-N to plants, over nitrate-N and urea-N. MFRE supplied with glycine and ammonium contained more plant carbon than those supplied with other nitrogen sources. Glycine-derived carbon was used by MFRE to increase nutrient transfer to plants under nitrogen limitation.

**Discussion:** MFRE play a significant role in plant nitrogen acquisition via organic nitrogen sources. Nitrogen transfer occurred in the absence of other microbes. Glycine derived-C appears to be used by MFRE to offset the ‘costs’ of degrading larger, organic molecules so that nitrogen transfer to host plants is maintained.

**Conclusions:** Our findings support the hypothesis that MFRE occupies a distinct symbiotic niche. They highlight the need for further research on MFRE nitrogen acquisition mechanisms and to explore potential competition or collaboration between MFRE and other root symbionts and soil microorganisms in plant nitrogen nutrition.

**Keywords:** nitrogen, Mucoromycotina, mycorrhiza, fine root endophyte, symbiosis, fungi

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## **Regulation and Transport in Mycorrhizal Networks**

### **GLOBAL CLIMATE CHANGE AND MYCORRHIZAL BIOMES DYNAMICS**

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**Introduction/Aim:** This study will explore global carbon flux variations in the carbon cycle linked to three mycorrhizal fungi types across tropical, temperate, and boreal zones. It will analyze the colonization strategies of Arbuscular mycorrhizal fungi mycelium in roots and soil globally. Furthermore, it will investigate the influence of climate change (rising temperatures and elevated CO<sub>2</sub> levels) on these colonization strategies and carbon flux related mycorrhizal from plants to soil. Additionally, it aims to evaluate the potential impact of reforestation on colonization strategies and carbon fluxes related to mycorrhizal carbon pools.

**Materials and Methods:** The related data will be searched and collected with keywords like "mycorrhizal fungi", "climate", "carbon flux", "carbon cycling", "Afforestation", "mycorrhizal community" ... and related dataset like FungalRoot et al. will also be used in this research. Related data analysis and modeling building will be done with R, Python, MATLAB et al. software.

**Results:** The results may show the global carbon flux distribution pattern of three types of mycorrhizal carbon pools and the influences on mycorrhizal fungi communities species composition at a global scale. Besides, it may provide an insight on how global climate change affects the colonization strategies and C cycling pattern of mycorrhizal fungi, telling us the potential influence of afforestation on mycorrhizal biomes.

**Discussion:** In this part, I will discuss the potential relationship between mycorrhizal biome and global climate change, the future dynamic trend of mycorrhizal community composition and how these changes of mycorrhizal could affect forest ecosystem and agricultural ecosystem.

**Conclusions:** The conclusions will be drawn based on above results.

**Keywords:** mycorrhizal, carbon flux, reforestation, climate change, forest

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## Regulation and Transport in Mycorrhizal Networks

### DIFFERENCES IN SECRETED METABOLITES DURING EARLY INTERACTION BETWEEN THE ECTOMYCORRHIZAL FUNGUS *PISOLITHUS MICROCARPUS* AND ITS HOST *EUCALYPTUS GRANDIS*

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**Introduction/Aim:** Tree root colonisation by ectomycorrhizal (ECM) fungi is the result of numerous signalling exchanges between the organisms, many of which occur before physical contact. However, information is lacking about these exchanges and the compounds that are secreted by each organism before contact. This is partly due to a lack of low disturbance sampling methods with sufficient temporal and spatial resolution to capture these exchanges.

**Materials and Methods:** Using a novel in situ microdialysis approach, we sampled metabolites released from *Eucalyptus grandis* and *Pisolithus microcarpus* independently and during indirect contact over a 48-h time-course using UPLC-MS.

**Results:** Our findings revealed that indirect contact between organisms altered the secretion of metabolites to produce a distinct metabolomic profile compared to either organism independently. We were also able to detect differences in the metabolite profiles between two fungal isolates with different colonisation efficiencies, highlighting the intricate signalling involved in early symbiotic interactions. Many of the metabolites were secreted within the first hour of contact and included several phenylpropanoids, fatty acids and organic acids.

**Discussion:** These findings show that the secreted metabolome, particularly of the ECM fungus, can rapidly shift during the early stages of pre-symbiotic contact and highlight the importance of observing these early interactions in greater detail.

**Conclusions:** We present microdialysis as a useful tool for examining plant–fungal signalling with high temporal resolution and with minimal experimental disturbance.

**Keywords:** ectomycorrhizal fungi, pre-symbiotic signalling, eucalyptus, metabolomics, microdialysis

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## Regulation and Transport in Mycorrhizal Networks

### COMMON MYCORRHIZAL NETWORKS MEDIATE CADMIUM ACCUMULATION, GLOMALIN PRODUCTION AND SOIL ENZYME ACTIVITY IN CO-CULTURES OF POPLARS AND LEEKS

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**Introduction/Aim:** Cadmium (Cd) is a mobile metal that can be toxic to plants and microorganisms and is little explored in terms of common mycorrhizal networks. Arbuscular mycorrhizal fungi (AMF) can improve Cd tolerance in plants by sequestration into fungal structures and in glomalin. We examined how CMN would affect Cd accumulation, glomalin production and microbial enzyme activity.

**Materials and Methods:** A glasshouse experiment with *Populus trichocarpa* and *Allium porrum* was carried out in factorial design, with three main factors: Cd contamination (Control vs Cd, 27 mg kg<sup>-1</sup>), mycorrhization (NM vs AM) and culture type (single vs co-culture, 1 poplar + 10 leek seedlings). We assessed biomass, Cd uptake, glomalin and activity of soil carbon cycling enzymes.

**Results:** Poplar biomass was unaffected by Cd exposure while mycorrhization decreased Cd in leaves by 34%. Cd accumulation was highest in NM poplars when co-cultured with leeks (414 µg plant<sup>-1</sup>), which decreased to 114 µg plant<sup>-1</sup> under CMN. Cd increased glomalin by 10-fold compared to controls, while enzyme activities were enhanced in the presence of mycorrhizal leeks, but not mycorrhizal poplars.

**Discussion:** Elevated Cd accumulation in NM co-cultured poplars indicates increased mobility due to root exudation and rhizosphere acidification. Cd uptake decreased in CMN co-cultures, possibly due to glomalin, hyphal binding or metal transference.

**Conclusions:** Cadmium promotes glomalin production and, despite resource competition and higher Cd mobilisation, co-culture between poplars and leeks under CMN is beneficial, stimulating microbial enzyme activity, whilst restricting Cd accumulation in poplars.

**Keywords:** arbuscular mycorrhizal fungi, GRSP, heavy metal, soil contamination



## Regulation and Transport in Mycorrhizal Networks

### THE SELF-REGULATORY MECHANISMS OF ARBUSCULAR MYCORRHIZAL FUNGI

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**Introduction/Aim:** Arbuscular mycorrhizal (AM) fungi can form endosymbiosis with 72% of land plants for obtaining carbon (C) sources, in return, AM fungi promote plant nutrients acquisition to enhance plant fitness. However, how AM fungi orchestrate its own signaling components in response to nutrient availabilities remains elusive.

**Materials and Methods:** To clarify the regulatory and transport genes, we combined heterologous expression in yeasts, and biochemical and molecular studies with reverse genetics approaches during AM symbiosis.

**Results:** Here, some lines of evidence show a regulatory role for the Pho4-CACGTK cis-acting element module in *Rhizophagus irregularis*. Under Pi starvation, the nucleus-localized transcription factor (TF) Pho4 binds to the CACGTK elements in its target genes. Arbuscule development in RiPho4-RNA interference lines was significantly inhibited under low-Pi conditions, and RiPho4 was shown to positively regulate Pi transporter genes of the PHO pathway from *R. irregularis*. Genome-wide analysis revealed CACGTK elements in the promoters of 106 genes from *R. irregularis* with predicted functions in the transport and metabolism of Pi, sugars, and lipids, such as genes that might be related to  $\beta$ -MAGs and monosaccharide transport, including RiMST2 and RiABCGs.

**Discussion:** In silico analysis predicted that 67% of AMF TF-encoding genes in *R. irregularis* could be directly regulated by RiPho4. These implicate that Pho4 may serve as a regulatory hub for integrating C and nutrient exchange during symbiosis in AM fungus under Pi starvation.

**Conclusions:** These recent findings reveal a possible scenario in which AM fungi perceive the environmental Pi availability through integrated dual-regulatory systems that enable the success of the AM symbiosis.

**Keywords:** arbuscular mycorrhizal fungi, *Rhizophagus irregularis*, phosphate signaling, hub transcription factor, RiPho4, self-regulatory mechanisms

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## **Regulation and Transport in Mycorrhizal Networks**

### **A NEW APPROACH TO DISSECTING ARBUSCULAR MYCORRHIZAL FUNGAL NITROGEN FORAGING BEHAVIORS**

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**Introduction/Aim:** AM fungi forage like animals and plants. Studying AM fungal nutrient foraging will improve our understanding of nutrient cycling, plant community assembly, and ecosystem functioning. However, there are many gaps in our understanding of AM fungal foraging. Here we identify AM fungal foraging behaviors at different nitrogen availabilities (concentrations and forms).

**Materials and Methods:** To address gaps in AM fungal foraging knowledge we set up three in vitro experiments using carrot (*Daucus carota* L.) hairy roots inoculated with *Rhizophagus irregularis* in bi-plates with different nitrogen availabilities. We imaged plates, and measured the length of branched absorbing structures (BAS) (responsible for nutrient uptake), and runner hyphae (RH) (responsible for soil exploration), then analyzed a new metric (BAS length/RH length) to evaluate their foraging behaviors. We tested (1) how nitrogen availability affects foraging behaviors, (2) how foraging behaviors change before, during and after encountering a patch, and (3) if encountering a new patch affects the foraging behavior in an old patch.

**Results:** BAS/RH increased with nitrogen concentration, but dropped at a high nitrogen level. Before, during and after an encounter with a nitrogen patch, BAS/RH changed over time. Also, when encountering a patch with higher relative ammonium concentration, BAS/RH in the old patch increased.

**Discussion:** Higher nitrogen availability drives AM fungal to produce more BAS over RH, potentially to absorb more nitrogen, and foraging in occupied patches influences foraging in additional patches.

**Conclusions:** We show AM fungal foraging behavior shifts with nitrogen availabilities, and provides a foundation for future studies.

**Keywords:** foraging behavior, patch use choice, branched absorbing structures, runner hyphae, nitrogen

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## Regulation and Transport in Mycorrhizal Networks

### COMMERCIAL INOCULUM OF ARBUSCULAR MYCORRHIZAL FUNGI IMPACTS ON RICE PRODUCTION, ZINC TRANSPORTATION ROUTES AND GRAIN CONTENT

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**Introduction/Aim:** Zinc (Zn) malnutrition is pervasive and detrimental to human health, particularly in regions relying on staple foods like rice. Biofortification of these crops holds promise as a solution, yet challenges remain, as excess Zn can be phytotoxic. Arbuscular mycorrhizal fungi (AMF) offer the potential to enhance crop resilience, yet their precise impact on rice Zn regulation remains elusive.

**Materials and Methods:** We present a greenhouse experiment investigating the influence of AMF on rice production and grain Zn content across varying Zn application rates (0, 20, and 120 mg Zn kg<sup>-1</sup>). Assessments were conducted at three growth stages, encompassing vegetative, reproductive, and ripening phases. Gene expression analyses via quantitative real-time PCR elucidated the involvement of key Zn transporters (Cation Diffusion Facilitator, ZIP family, and heavy metal P1B-ATPases, HMAs) in root uptake, translocation, and grain loading processes.

**Results:** Compared to non-AMF treatments, AMF-inoculated rice exhibited significant improvements in production, particularly under high Zn conditions. Colonization rates were influenced by Zn supply, with the presence of AMF enhancing both grain yield and quality. qRT-PCR results revealed differential Zn transport routes in AM-colonized rice under varying environmental Zn levels.

**Discussion:** This study represents a pioneering effort to investigate the impact of arbuscular mycorrhizal fungi colonization on the expression of 16 genes associated with rice. Our findings showed that rice plants with mycorrhizal symbiosis exhibit heightened efficacy in zinc accumulation compared to their non-mycorrhizal counterparts.

**Conclusions:** Our study contributes to the theoretical foundation for integrating plant-mycorrhizae bioremediation and biofortification strategies, offering insights for sustainable agricultural practices in addressing global nutritional challenges.

**Keywords:** Arbuscular mycorrhizal fungi, Zinc, Rice, Zn transporters, Gene expression.

# **Evolution & Taxonomy**

O-052

## Evolution & Taxonomy

### EARLY FUNGI IN THE FOSSIL RECORD: STATE OF THE ART AND NEW INPUTS

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**Introduction/Aim:**The earliest direct evidence of Fungi associated with plants comes from the Devonian and is based on organisms fossilized in situ in the 407 million year old Rhynie cherts. Fossilization occurred through inundation of the biota with fluids from a nearby hot-spring system, and the resulting silicification preserved organisms in exquisite detail.

**Materials and Methods:**Material examined includes historic petrographic thin sections housed in museums and newly prepared materials. We used brightfield and Confocal Laser Scanning Microscopy (CLSM) to document the microfossils. Recently, we have been experimenting with Fluorescence Lifetime Imaging (FLIM) to distinguish among certain classes of organism.

**Results:**CLSM outperforms brightfield microscopy by improving image clarity and enables digital reconstruction of the organisms in three-dimensions. Initial results from FLIM indicate that it can discriminate among fabrics of different organic chemistry.

**Discussion:**We provide an overview of the state of the art of early fossil fungi focussing on our recent findings. The earliest fossil fungi described with confidence are from the Rhynie cherts, and they are associated with plants. Direct fossil evidence of fungi during the Proterozoic (2.5 Ga - 538 Ma) is questionable and limited to rare examples from deep-sea basalts or shallow marine sediments. Fossil hyphae and spores are frequently cited as the earliest evidence of Glomeromycotina at ~460 Myrs. However, this record is not widely accepted.

**Conclusions:**CLSM and FLIM in combination with other methods (e.g., Fourier-transform infrared spectroscopy) are enabling us to establish the affinities of fossil fungi and their interactions with other organisms, shedding new light on their early evolution.

**Keywords:** Fossil, Fungi, Evolution, Confocal microscopy

O-053

## Evolution & Taxonomy

### PHYLOGENOMIC ANALYSIS OF PEZIZOMYCETE FUNGI PROVIDES NEW INSIGHTS INTO THE EVOLUTION OF THE ECTOMYCORRHIZAL TROPHIC MODE

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**Introduction/Aim:**The fungal class Pezizomycetes includes an estimated 2000 fungal species in 23 families representing a broad array of trophic strategies from saprobic fungi such as *Ascobolus* and *Morchella*, pyrophilic fungi such as *Pyronema* and *Geopyxis*, and many ectomycorrhizal (ECM) fungi such as *Tuber*, *Helvella*, and *Terfezia*. The ECM trophic mode appears to have evolved several times among Pezizomycetes, but uncertainty about the phylogenetic relationships between different ECM lineages and their sister groups has limited our understanding of patterns of ECM trophism in Pezizomycete fungi. This issue is further complicated by taxonomic issues resulting from the application of older genera names such as *Peziza* to diverse taxa across the Pezizomycetes. Here, we present the first broadly sampled phylogenomic analysis of the Pezizomycete fungi.

**Materials and Methods:**Draft genome assemblies of over 350 specimens were generated and assembled *de novo* using a custom genome assembly pipeline. Additional assemblies were obtained from JGI. Phylogenomic analysis was conducted with Maximum Likelihood and coalescent methods.

**Results:**A more fully resolved phylogenomic tree of Pezizomycete fungi provides new insights about the diversity of ECM fungi and the multiple origins of ECM trophism.

**Discussion:**Careful study of individual groups is required to validate ecological hypotheses and accurately characterize Pezizomycetes diversity in a taxonomic framework. We discuss specific examples within Morchellaceae and Pezizaceae where intense sampling efforts and careful study have shed light on independent evolution of ECM trophism.

**Conclusions:**We highlight how these results might be used across other ECM fungal lineages.

**Keywords:** ectomycorrhizal fungi, Pezizomycetes, phylogenomics, taxonomy

O-054

## Evolution & Taxonomy

### PHYLO- AND PAN-GENOMICS OF THE LACCARIA BICOLOR SPECIES COMPLEX

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**Introduction/Aim:** *Laccaria bicolor* (Maire) Orton is the preeminent model system for understanding the genetic underpinnings of the ectomycorrhizal-host mutualism, and is comprised of several phylogenetic lineages that are widely distributed across the Northern Hemisphere. Across its substantial distribution *L. bicolor* establishes in a wide array of environments and likely associates with both angiosperm and gymnosperm hosts. Despite this geographic and phylogenetic diversity, most experimental studies of ectomycorrhizal colonization within *L. bicolor* have utilized a single isolate (S238N). It is unknown whether the genetic pathways necessary for host colonization within S238N are strain/lineage specific, or are widely distributed across *L. bicolor*. Here, we generated substantial genomic resources within the *L. bicolor* species complex to (i) resolve species boundaries within this diverse clade, and (ii) identify whether previously characterized mycorrhizal pathways are part of the core pan-genome.

**Materials and Methods:** Specifically, we conducted whole-genome sequencing of 50 *L. bicolor* museum collections, including type specimens of closely related taxa, and created a summary coalescent phylogeny of the complex to resolve species boundaries. We then re-sequenced several geographically distinct *L. bicolor* specimens with Oxford Nanopore sequencing and generated highly-contiguous “reference” quality genomes assemblies using a hybrid-assembly approach.

**Results:** With these new reference quality assemblies, we constructed a preliminary pan-genome of *L. bicolor* and determined the diversity of necessary mycorrhizal pathways across the species complex.

**Discussion:** We find that *L. bicolor* exhibits a diverse global pan-genome and is comprised of geographically distinct phylogenetic lineages.

**Conclusions:** This work provides a novel insight into the evolution of the ectomycorrhizal symbiosis on a global scale.

**Keywords:** pan-genome, *Laccaria bicolor*, ectomycorrhizae, whole genome sequencing

O-055

## Evolution & Taxonomy

### EVOLUTIONARY HISTORY OF ARBUSCULAR MYCORRHIZAL FUNGI AND GENOMIC SIGNATURES OF OBLIGATE SYMBIOSIS

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**Introduction/Aim:**The colonization of land is intimately linked to the evolutionary history of their symbiotic fungal partners. Extant representatives of these fungal lineages include mutualistic plant symbionts, the arbuscular mycorrhizal (AM) fungi in Glomeromycota and fine root endophytes in Endogonales (Mucoromycota). These fungal groups separate into three monophyletic lineages but their evolutionary relationships remain enigmatic confounding ancestral reconstructions.

**Materials and Methods:**In this study, we recognize three monophyletic lineages as phyla, and use a balanced taxon sampling and broad taxonomic representation for phylogenomic analysis.

**Results:**We reject a hard polytomy and resolves Glomeromycota as sister to a clade with Mucoromycota and Mortierellomycota. Low copy numbers of genes associated with plant cell wall degradation could not be assigned to the transition to a plant symbiotic lifestyle but appears to be an ancestral phylogenetic signal. Both plant symbiotic lineages, Glomeromycota and Endogonales, lack numerous thiamine metabolism genes. The lack of fatty acid synthesis genes is specific to AM fungi. Many genes previously thought to be missing specifically in Glomeromycota are either missing in all analyzed phyla, or in some cases, are actually present in some of the analyzed AM fungal lineages.

**Discussion:**Based on a broad taxon sampling of fungal genomes we present a well-supported phylogeny for AM fungi and their sister lineages. We show that among these lineages, two independent evolutionary transitions to mutualistic plant symbiosis happened in a genomic background profoundly different from that known from the emergence of ectomycorrhizal fungi in Dikarya.

**Conclusions:**These results call for further reevaluation of genomic signatures associated with plant symbiosis.

**Keywords:** Glomeromycota, Evolution, Phylogeny, Endogonales, plant-fungal symbiosis



O-056

## Evolution & Taxonomy

### LONG- AND SHORT-READ METABARCODING OF ARCHAEORHIZOMYCETES REVEALS HIGH PHYLOGENETIC DIVERSITY STRUCTURED BY VEGETATION AND CLIMATE, NOT SUBSTRATE

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**Introduction/Aim:** Archaeorhizomycetes is a newly described class of soil-dwelling fungi with a globally widespread distribution. They were originally proposed to be associated with plant-roots, but their ecology and nutritional mode are still not clearly defined. To increase the knowledge about Archaeorhizomycetes' ecology, we here investigate how they are distributed along major environmental gradients, as well as different soil compartments (litter, soil, and plant roots).

**Materials and Methods:** We used both short- and long-read metabarcoding, with newly developed group-specific primers for the long reads, to investigate the abundance and phylogenetic richness of Archaeorhizomycetes in environmental samples collected in different soil types and climate zones throughout Norway.

**Results:** The long-read sequences revealed a high phylogenetic diversity of Archaeorhizomycetes lineages, grouping into five main clades with different ecologies and biogeographic distributions. The short-read sequences showed that Archaeorhizomycetes is a dominant fungal class in alpine vegetation types dominated by ericaceous plants, as well as in boreal spruce and pine forests. Archaeorhizomycetes are primarily structured by vegetation and climate, while the different substrate types - litter, soil and plant roots – have limited influence on their distribution.

**Discussion:** Archaeorhizomycetes are markedly more abundant in soil and plant roots as compared to litter, supporting the idea that Archaeorhizomycetes are at least partly dependent on the presence of plant roots, in particular of ericaceous plants.

**Conclusions:** Our study illustrates that targeted group-specific amplification coupled to long-read sequencing is a powerful approach to explore the diversity and ecology of poorly known organismal groups such as the Archaeorhizomycetes.

**Keywords:** Root-associated fungi, soil fungi, Taphrinomycotina, long-read sequencing, Norway

O-057

## Evolution & Taxonomy

### TAXONOMY AND BIOGEOGRAPHY OF YELLOW CHANTERELLES

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**Introduction/Aim:**The genus *Cantharellus* in the Basidiomycota is the ancestral lineage of the ectomycorrhizal symbiosis for woody plants. The fleshy fruiting bodies of *Cantharellus* are known as chanterelles. In particular, yellow chanterelles are one of the most famous edible mycorrhizal mushroom groups in the world. The yellow chanterelles are widely distributed throughout the world and have high economic, ecological, and scientific research value.

**Materials and Methods:**We collected over 100 *Cantharellus* specimens from Japan, and conducted macroscopic and microscopic observations. Then molecular phylogenetic analyses were performed on the selected specimens targeting the LSU, ITS1, and ITS2 of rRNA gene and transcription elongation factor gene (*tef1*). The concatenation method was used to construct a supergene matrix for the construction of the phylogenetic tree.

**Results:**The 4 distinct yellow chanterelle species, i.e., *C. anzutake*, *C. cibarius*, *Cantharellus* sp. 2, *Cantharellus* sp. 3, were compared with the North American *C. roseocanus* and *C. formosus*.

**Discussion:***Cantharellus* sp. 2 was phylogenetically related to *C. formosus*, but morphologically distinct. *Cantharellus* sp. 3 was phylogenetically and morphologically independent.

**Conclusions:**Based on the divergence time estimation analysis, we discuss possible evolutionary patterns and migration routes of these yellow chanterelles across continents.

**Keywords:** Bioresource, Ectomycorrhizal fungi, Edible mushrooms, Forest ecology, Molecular phylogeny

O-058

## Evolution & Taxonomy

### UPDATES ON THE DIVERSITY OF HYPOGEOUS FUNGI FROM GENUS BALSAMIA IN EUROPE

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**Introduction/Aim:**The study aimed at analysing the hypogeous genus *Balsamia* in Europe, with a focus on clarifying taxonomy and phylogenetic position of populations found in the Western Carpathians (Poland and Slovakia).

**Materials and Methods:**We analysed the morphology and phylogeny of the Carpathian *Balsamia* samples and comparative collections of *Balsamia oregonensis* (USA), and *Barssia peyroneli* (Italy). Maximum likelihood phylogeny, including a wider taxonomic context, was based on ITS and LSU sequences.

**Results:**Analyses revealed an isolated Carpathian clade, introduced here as *Balsamia microspora* sp. nov. *Barssia peyroneli* positioned phylogenetically within *Balsamia* and was transferred to this genus. European collections previously identified as *Barssia oregonensis* represent the phylogenetically close *B. peyroneli*.

**Discussion:***Balsamia peyroneli* was hitherto known only from its type locality in the Alps; our study extends its known geographical range to the Carpathians. Earlier misidentification of *B. peyroneli* as *B. oregonensis* was caused by their morphological similarity paired with the lack of phylogenetic tools at that time. These sister species restricted to different continents (Europe and North America, respectively) support a scenario of speciation following long-range dispersal.

**Conclusions:**Based on this study and historical records, current list of European *Balsamia* species includes 10 of 29 known taxa: *B. aestivalis*, *B. ambigua*, *B. fragiformis*, *B. fuispora*, *B. hellenica*, *B. microspora*, *B. peyroneli*, *B. platyspora*, *B. polysperma*, *B. vulgaris*. While *B. oregonensis* was removed from the list, *B. microspora* and *B. peyroneli* were added. Our study extends the knowledge on *Balsamia* in Europe, and shows that phylogenetic relationships within this genus are more complex than previously thought.

**Keywords:** Ascomycota, Helvellaceae, Hypogeous fungi, Western Carpathians, Taxonomic revision

O-059

## Evolution & Taxonomy

### DNA BARCODING OF AM FUNGI: WHICH LOCUS?

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**Introduction/Aim:** Arbuscular mycorrhizal (AM) fungi pose a persistent challenge to accurate identification. Sequencing of nuclear ribosomal loci is the most popular choice, but there is no consensus on which loci to target, and intra- and interspecific differences of these loci, either individually or in combination, have never been thoroughly investigated, nor have their comparisons with protein-coding genes such as H<sup>+</sup>-ATPase, glomalin, or RPB1. The aim of this study was to evaluate the performance of nuclear ribosomal loci together with protein-coding genes for the identification of AM fungal species.

**Materials and Methods:** We performed PacBio sequencing of nuclear ribosomal loci on 300 cultures, representing all known families of AM fungi. In addition, a subset of these cultures were subjected to Sanger sequencing for H<sup>+</sup>-ATPase and glomalin.

**Results:** Preliminary results show a high level of intra- and interspecific variation characterizing the nuclear ribosomal loci, mainly the ITS and LSU due to INDELS. Glomalin and RPB1 genes are promising alternatives for accurate identification of AM fungi. Paralogs of H<sup>+</sup>-ATPase were detected in some AM fungal groups, disqualifying this gene as a reliable DNA barcode for AM fungi.

**Discussion:** Characterization and inventory of the most common variants of rRNA genes, as well as reference sequences of protein-coding genes from well-characterized cultures, are required to facilitate the identification of AM fungi at both species and community levels.

**Conclusions:** Accurate identification of AM fungi, whether as a community or as individual cultures, is complex and requires careful consideration of target loci and distance thresholds used to discriminate species.

**Keywords:** Arbuscular mycorrhizal fungi, DNA barcoding, Nuclear ribosomal loci, Species Identification, Protein-coding genes

O-060

## Evolution & Taxonomy

### HIGH RATE OF GENE FAMILY EVOLUTION IN PROXIMITY TO THE ORIGIN OF ECTOMYCORRHIZAL SYMBIOSIS IN INOCYBACEAE

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**Introduction/Aim:**The genomes of Ectomycorrhizal (ECM) fungi have a reduced number of genes encoding CAZymes (Carbohydrate Active EnZyme), expansions in Transposable Elements (TE) and genes for mycorrhiza-induced Small Secreted Proteins (SSPs) compared to saprotrophs. There are also reports of fewer genes for specific peptidases and lipases in ECM. It is unclear yet if these changes occur at the shift to the ECM habit or are more gradual throughout the evolution of ECM lineages.

**Materials and Methods:**We sequenced the genomes of 20 species in the ECM lineage Inocybaceae and compared them with six saprotrophic species.

**Results:**Inocybaceae genomes have fewer CAZymes, peptidases, lipases, secondary metabolite clusters and SSPs and higher TE content than their saprotrophic relatives. There is an increase in the rate of gene family evolution at the transition to the ECM lifestyle. We found that the rate of evolution in CAZymes was very high at the branch with the transition to ECM, and this branch had the largest number of contractions. Other significant changes along this branch included expansions in transporter genes, transposons-related genes and communication genes like fungal kinases.

**Discussion:**Our study suggests that loss of specific genes, which could have possible roles in triggering the host plant immune system, may be key for the transition to the ECM lifestyle and that the loss or otherwise tight control of those genes is important for this lifestyle.

**Conclusions:**There is a concentration of ECM lifestyle linked genetic changes on the transition branch, which correspond to changes identified as key for the gain of this lifestyle.

**Keywords:** Mycorrhiza, Symbiosis, Phylogenomics, Comparative Genomics, Evolution, Ectomycorrhiza

O-061

## Evolution & Taxonomy

### GENETIC STRUCTURE AND LONG-TERM GENETIC DIVERSITY ASSESSMENT OF BURGUNDY TRUFFLE POPULATIONS IN CENTRAL EUROPE

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**Introduction/Aim:**The Burgundy truffle is an abundant ectomycorrhizal symbiont that occurs in a wide range of temperate climates. Despite its prized underground ascocarps, its complex lifecycle and possible response to anthropogenic climate change are largely unknown.

**Materials and Methods:**We ran a citizen science monitoring of Burgundy truffle ascocarp production with a 3-week resolution in 23 natural populations at the center of its European distribution over up to 11 years. We genotyped more than 3000 truffles using microsatellite markers to assess the genetic structure and diversity of these populations in space and time.

**Results:**Preliminary results support well-differentiated genetic groups on a small spatial scale and throughout the study area, with often low levels of individual admixture. Within populations, genotypes from different genetic groups co-occurred, while only a few perennial genotypes dominated, producing most of the ascocarps. Further analysis indicate that genetic diversity was generally low in most populations and fluctuated over time with unpredictable trend.

**Discussion:**Our data suggest that among-population gene flow in Burgundy truffles is limited, even if they grow only a few kilometers apart. This restricted dispersal ability could prevent the northward migration of advantageous alleles from more heat- and drought-tolerant southern populations.

**Conclusions:**Given that ascocarp production in truffle populations is highly sensitive to dry conditions, our data call for genomic studies on local adaptation of this valuable ectomycorrhizal fungus, which could guide the use of well-adapted provenances for truffle cultivation and restoration.

**Keywords:** *Tuber aestivum*, genetic diversity, monitoring, climate change

O-062

**Evolution & Taxonomy**

**VARIATION IN PLANT AND FUNGAL TRAITS INDICATES COEVOLUTIONARY SELECTION FOR  
ECTOMYCORRHIZAL FUNGAL FITNESS**

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**Introduction/Aim:** Much progress has been made in analyzing the dynamics and consequences of coevolution, but little is understood about how coevolutionary selection operates within multispecies interactions. We investigated natural selection on ectomycorrhizal fungi (ECM) fungal and pine seedling traits through a common garden experiment.

**Materials and Methods:** We planted 1178 Monterey pine (*Pinus radiata*) seedlings from all five native populations of Monterey Pine, as well as crosses to represent intermediate phenotypes/genotypes, for a total of 47 unique genetic families. We then measured pine seedling traits, ECM fungal traits, and ECM species composition.

**Results:** Our previous work in this system found evidence that certain fungal operational taxonomic units (OTUs), families and exploration types have the potential to exert selection on plant traits; however, their corresponding effect on fungal fitness is unclear. Here we related pine seedling traits, ECM fungal traits, and ECM species composition to relativized measures of fungal fitness using standard procedures for calculating selection gradients.

**Discussion:** We found similar patterns such that certain OTUs, families and exploration types have the potential to exert selection on plant traits to improve fungal fitness.

**Conclusions:** This work suggests the potential for multispecific coevolutionary selection to drive trait evolution in a multispecies nutritional symbiosis.

**Keywords:** common garden, ectomycorrhizal fungi, *Pinus radiata*, interspecific selection

O-063

### Evolution & Taxonomy

#### MULTI-LOCUS BASED ANALYSIS AND A NEW 'FINE ROOT ENDOPHYTE' GENOME RESOLVE PHYLOGENETIC UNCERTAINTY BETWEEN MUCOROMYCOTINA AND GLOMEROMYCOTINA FUNGI

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**Introduction/Aim:** Within the Mucoromycota subphyla, Glomeromycotina and Mucoromycotina clades include notable symbiotic fungi such as arbuscular mycorrhiza (AM) and Mucoromycotina fine root endophyte (MFRE) that are both endomycorrhizal and engage in nutritionally mutualistic relationships with plant hosts. However, the phylogenetic relationship between the clades remains unresolved and it is unclear as to how these fungal lifestyles arose through adaptation and evolution.

**Materials and Methods:** We performed a comprehensive phylogenomics pipeline by incorporating 182 genomes and extracting 535 common Benchmarking Universal Single-Copy Orthologs (BUSCO) genes to construct concatenation and coalescent phylogenies.

**Results:** Increased taxon sampling of the Mucoromycota clade and the inclusion of a new MFRE genome (Hoysted et al 2023) – *Lycopodiella inundata* isolate 1 – helped resolve the phylogenetic uncertainty between Mucoromycotina and Glomeromycotina fungi. Our resolved concatenation and coalescent phylogenies converge on the same tree topology, presenting Glomeromycotina and Mucoromycotina as direct sister clades.

**Discussion:** This finding indicates that there is a likely shared common origin of mycorrhizal lifestyle between AM and MFRE fungi.

**Conclusions:** In conclusion, increasing the number of culturable species as well as sequenced genomes within the Mucoromycotina will be crucial to understand the evolutionary history of these fungi and their lifestyles.

**Keywords:** Arbuscular mycorrhiza, Mucoromycotina fine root endophyte, phylogenetics, genome, BUSCO



O-064

## Evolution & Taxonomy

### PROTEOMIC-BASED INTRA-SPECIES BIOTYPING OF ARCHAESPORACEAE USING MALDI-TOF-MS

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) are ubiquitous microorganisms belonging to the phylum Glomeromycota that lives as symbionts with the majority of land plants (Öpik et al., 2013). Approximately 347 species of Glomeromycota are currently documented, and many isolates belonging to the same species have been described. However, catch AMF intra-species diversity is a difficult task in less-known lineages (e.g. Archaeosporaceae), due to poor morphological information and low coverage of DNA sequences (Marrassini et al., 2024). Matrix-Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS) proteomic-based biotyping has been demonstrated successful in solving AMF taxonomy (Crossay et al., 2017), but it has not yet been applied to study AMF intra-species diversity.

**Materials and Methods:** Sixteen isolates belonging to three species of the family Archaosporaceae have been identified by MALDI-TOF-MS and by a molecular approach. Isolates of *Funneliformis mosseae* were used as control.

**Results:** The proteomic-based biotyping reveal a high intra-species diversity in Archaosporaceae. Isolates of AMF belonging to the same species and collected in close-related geographical sites shared similar proteomic spectra, while geographical far-related isolates display distinct spectra. By contrast, the diversity within isolates of *F. mosseae* was lower.

**Discussion:** This study indicates that AMF identification by MALDI-TOF-MS could be a reliable alternative to DNA sequencing for intra-species discrimination of AMF. Moreover, the proteomic-based AMF intra-species diversity highlighted in the isolates of Archaosporaceae supports a large biodiversity within this family.

**Conclusions:** MALDI-TOF-MS biotyping represents a novel technique for a rapid identification at intra-species level in AMF. This might also be applied to describe novel AMF species.

**Keywords:** Archaeosporaceae, MALDI-TOF-MS, biotyping, intra-species diversity

O-065

## Evolution & Taxonomy

### CONVERGENT EVOLUTION AMONG PHYLOGENETICALLY DISTANT MYCOHETEROTROPHS

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**Introduction/Aim:**Initial mycoheterotrophs (IM) are parasites that rely on fungal carbon during early ontogenesis and could be found in phylogenetically distant plant groups. For long time, the only cultivable IM were orchids.

**Materials and Methods:**We compared pyroloids and orchids as representatives of phylogenetically distant IM plants. With our novel protocol for pyroloid in vitro cultivation and using various histochemical methods we studied their early ontogenetic development from seeds to adults with the aim to find convergent traits.

**Results:**Pyroloid and orchid seeds share a globular, undifferentiated embryo surrounded by dead testa. Germination begins with enlargement of the embryo without organ differentiation. Enlargement is isodiametric in orchids and polar in pyroloids. Later, the shoot meristem is formed in orchids and the root meristem in pyroloids. In orchids, the structure formed after germination is called protocorm and in pyroloids, a functionally similar but smaller structure is formed as well, which precedes the formation of the true body as orchid protocorm. In both groups these structures can utilise trehalose and have similar storage compounds.

**Discussion:**The ontogenetic development of orchids and pyroloids share similarities – undifferentiated embryo, post-germination protocorm development which forms only one meristem pole, similar storage compounds or trehalose utilisation. This indicates convergent evolution between both groups and preliminary results suggests that much many convergent traits are shared also among tropical arbuscular mycorrhizal mycoheterotrophs.

**Conclusions:**Various mycoheterotrophs share multiple convergently evolved traits. We hypothesise that germination into protocorm, trehalose utilisation or unipolar organ development could be a general feature of mycoheterotrophic plants having dust seeds.

**Keywords:** in vitro, germination, protocorm, initial mycoheterotrophy, convergent evolution

O-066

## Evolution & Taxonomy

### FIVE *MYCENA* SPECIES INDUCED THE SEED GERMINATION OF MYCOHETEROTROPHIC ORCHIDS, *GASTRODIA* SPECIES

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**Introduction/Aim:**The genus *Mycena* is known as a saprotrophic genus, but several lineages form orchid mycorrhizae and ectomycorrhizal-like structures with tree roots, one of the important genera for understanding the switch of fungal nutritional mode from saprotrophic to biotrophic. Here, we showed that five *Mycena* species promoted seed germination and seedling growth of mycoheterotrophic orchids, *Gastrodia* species.

**Materials and Methods:**The fungal isolates of five *Mycena* species (*M. epipterygia*, *M. haematopus*, *M. polygramma*, *M. pura* and *M. aff. pura*) from four sections (*Calodontes*, *Fragilipedes*, *Hygrocyboideae* and *Lactipedes*) were obtained from basidiospores and cultured with the *Gastrodia* seeds of *G. confusa*, *G. elata*, *G. nipponica* and *G. pubilabiata*.

**Results:***M. haematopus* and *M. polygramma* had a high affinity for *Gastrodia* and induced protocorm formation in all four *Gastrodia* species and tuberization of seedlings in two *Gastrodia* species. *M. epipterygia* also induced protocorm formation in all four *Gastrodia* species and root formation of seedlings in *G. pubilabiata*. Other two *Mycena* species induced protocorm formation in two to three *Gastrodia* species.

**Discussion:**Our results suggest that phylogenetically diverse *Mycena* species can associate with *Gastrodia* species as orchid mycorrhizal fungi.

**Conclusions:**This study contributes to the understanding of the diversity of fungal trophic mode and mycorrhizal evolution.

**Keywords:** Mycenaceae, mycoheterotrophy, orchid mycorrhizae, saprophytes

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## Evolution & Taxonomy

### A TAXONOMIC INVESTIGATION OF IRISH ARBUSCULAR MYCORRHIZAL FUNGI FROM VEGETATIVE BUFFER STRIPS

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**Introduction/Aim:**Arbuscular mycorrhizal fungi (AMF) exhibit a cosmopolitan distribution, colonising all major lineages of land plants. Despite this, a dearth of taxonomic research has been conducted into these microorganisms in Ireland, with few studies documenting their occurrence. Agricultural runoff is the primary cause of eutrophication in Ireland, with the current mitigation strategy utilising vegetative buffer strips (VBSs). This research will identify species of AMF present in Irish VBSs that, once characterised, will be incorporated into a myco-phytoremediation plan for control of nutrient runoff.

#### Materials and Methods:1. Cultures

VBS soil is used as inoculum to initiate trap cultures. AMF colonise the host plant over the course of 3-4 months. AMF spores from completed cultures are isolated *via* sucrose-density centrifugation. Individual spores are used to initiate pure pot cultures and *in vitro* monoxenic cultures.

#### 2. Identification

Morphological characterisation: spore staining with Melzer's reagent, root clearing and staining.  
Molecular characterisation: DNA extraction, sanger sequencing of glomalin gene.

**Results:**Morphological and molecular identification ongoing with species from 10 established pure cultures. Email author for images of preliminary results.

**Discussion:**The suite of novel culturing, morphological and molecular techniques employed in this investigation of Irish AMF is crucial for advancing taxonomic research of the Glomeromycota. The ecological data extends its significance to wider applications, having the potential to bridge the gap between taxonomy and sustainable agricultural practices.

**Conclusions:**The incorporation of AMF into VBSs is emerging as a high impact management strategy for mitigating agricultural runoff, yielding innovative solutions at the forefront of mycorrhizal research.

**Keywords:** AMF, Taxonomy, VBS, Agriculture, Runoff, Myco-Phytoremediation.

P-208

### Evolution & Taxonomy

#### DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI FROM CITRUS ORCHARDS OF THE CANARY ISLANDS UNDER TWO SOIL TREATMENTS

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**Introduction/Aim:** Citrus plants are highly dependent on arbuscular mycorrhizal fungi (AMF) symbiosis, due to better nutrient uptake, water absorption and defense system. The implementation of sustainable soil managements in agrosystems aims, apart from reducing the rate of water evaporation, erosion and pest pressure, to protect rhizosphere community composition.

**Materials and Methods:** In this work, soils from a citrus orchard located in Tenerife (Canary Islands, Spain) were sampled with two managements, cover crop (*Festuca elatior* L.) and bare soil. The soils were analysed physicochemically. The number of infectious AMF propagules in the soils and the percentage of AMF colonization in trap plants were determined. DNA was extracted from AMF spores and colonized roots, amplified by PCR, and sequenced.

**Results:** In soil samples under crop cover, the percentage of organic matter and the concentration of phosphorus, sodium and magnesium were higher than in bare soil. In addition, a greater number of AMF spores was found, obtaining higher percentages of colonization in roots grown in these soils. Sequence analysis revealed the presence of several AMF species under both soil managements.

**Discussion:** The application of plant covers must be done taking into account the effect on the physical, chemical and microbiological state of the soil. Knowledge of the native diversity of AMF associated with citrus plants is the first step towards the implementation of techniques that promote the richness of specific taxa, or even their use as biofertilizer.

**Conclusions:** Cover crops, as a soil management technique, influence the AMF microbial community associated with citrus plants in Tenerife.

**Keywords:** AMF, cover crops, colonization, DNA, sequencing

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## Evolution & Taxonomy

### CLASSIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGAL ISOLATES USING ILLUMINA MISEQ SEQUENCE

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**Introduction/Aim:** Sequences of rRNA region are generally used for identification of organisms. In case of arbuscular mycorrhizal fungi however, situation is not simple because of their high polymorphism in their rRNA sequences. In the current study, I examined whether MiSeq sequencing is available to identify AM fungal isolates.

**Materials and Methods:** Thirty-one strains belonging to NARO culture collection were used. Template DNAs were prepared from isolated spores of each strain using Kaneka Easy DNA Extraction Kit ver. 2. Five primer sets were used for MiSeq analysis. After denoising, merging, and removing chimera through QIIME2 pipeline, minor sequences whose ratio was smaller than 1% of sum of the reads were removed. Arbuscular mycorrhizal fungi classification pipeline provided by Kazusa DNA Research Institute was also employed for processing amplicon data obtained using FLd3-FLR2 primer. Cluster analysis and PCoA plot based on weighted UniFrac distances were performed using “cluster” and “phyloseq” packages on R.

**Results:** All primers could generally separate the sequences according to the genus level and AMV4.5NF-AMVR gave best resolution. As for species level separation, primer sets of ITS1-ITS2, gITS7-ITS4 and FLd3-FLR2 successfully distinguished *Claroideoglossum etunicatum* and *Claroideoglossum claroideum*. *Gigaspora rosea* belonged to the same clade with *Gigaspora margarita* when 1422-1642 or gITS7-ITS4 were used, but it was closely plotted with *Dentiscutata* with AMV4.5NF-AMVR, FLd3-FLR2 primer sets.

**Discussion:** Single primer set was difficult to use for AM fungal identification.

**Conclusions:** Combinative use of AMV4.5NF-AMVR or FLd3-FLR2 for genus level classification and ITS1-ITS2 for species determination would be a good option to identify AM fungi in species level.

**Keywords:** Arbuscular mycorrhizal fungi, MiSeq, rRNA, Culture collection

P-210

## Evolution & Taxonomy

### GLOBAL DISTRIBUTION AND PREDICTION OF PLANT MYCORRHIZAL TRAITS

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**Introduction/Aim:** Mycorrhizal associations are widespread and known to strongly influence plant performance, structure plant communities, and ultimately shape ecosystem dynamics. Plant mycorrhizal traits (PMTs), which describe the characteristics of different mycorrhizal associations and the capacity of plants to form them, are among the most promising for capturing plant belowground functionality. This study aims to map global variations in PMTs, including plant mycorrhizal types and statuses, analyze their phylogenetic and environmental correlates, and enhance the prediction of PMTs by integrating environmental data, plant taxonomy, phylogeny, and other functional traits.

**Materials and Methods:** We compiled empirical mycorrhizal trait information and global occurrence data for 11,770 vascular plant species. This includes a comprehensive analysis of global PMT distribution, the influence of plant phylogeny and environmental variables, and predictive modelling based on plant phylogenetic position, functional traits, and environmental niche.

**Results:** Our analysis reveals that plant mycorrhizal type exhibits greater phylogenetic conservation than status. Environmental variables explain a significant variation in plant mycorrhizal status. Predictions based on phylogeny or genus are more effective for arbuscular and ectomycorrhizal types and non-mycorrhizal status, whereas environmental and plant trait data better predict facultative and obligatory mycorrhizal statuses.

**Discussion:** Environmental modulation of PMTs has implications for soil processes, including nutrient cycling, carbon capture and retention, and further consequences for plant dynamics and productivity.

**Conclusions:** In addition to phylogenetic and taxonomic dependencies, PMTs are influenced by a range of climatic and edaphic variables. Integrating plant phylogeny, functional traits, and environmental factors provides a more comprehensive understanding of PMT distributions, reflecting both evolutionary and environmental influences.

**Keywords:** symbiotic mycorrhizal traits, plant mycorrhizal statuses, plant phylogeny, environmental factors, plant above and below ground traits

## Evolution & Taxonomy

### EXPLORING EFFECTS OF RADIATION ON MUTATION ACCUMULATION AND PHENOTYPE IN THE MODEL ARBUSCULAR MYCORRHIZAL FUNGUS *RHIZOPHAGUS IRREGULARIS*

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF, Glomeromycotina) are obligate root symbionts that associate with a majority of plant species across terrestrial ecosystems. AMF exist in soils as coenocytic mycelial networks, in which tens of thousands of nuclei can be found both within the continuous hyphal cytoplasm and individual spores. In the model species *Rhizophagus irregularis*, strains can be homokaryotic, in which all nuclei are genetically similar, or heterokaryotic, containing nuclei of two genetically divergent nucleotypes. Stability and lack of genetic variation within haplotypes suggest that *R. irregularis* may possess mechanisms to minimise the accumulation of mutations in the nuclear genome.

**Materials and Methods:** To explore this hypothesis, we plan to compare mutation accumulation rates and phenotypic profiles of *R. irregularis* homokaryons cultured across a range of mutagenic conditions at NASA Space Environmental Effects test facilities in Huntsville, Alabama. Multiple generations of *R. irregularis* homokaryon strains will be grown under exposure to sub-lethal doses of ionising, ultraviolet (200-400 nm) and vacuum ultraviolet (115-200 nm) radiation and compared with control lines grown under standard conditions.

**Results:** Phenotypic profiling will include measurements of hyphal growth, spore production and viability, spore and network nuclear content and morphology.

**Discussion:** The selection of loci for sequencing to extrapolate genomic mutation rate estimates will consider known compartmentalisation of the *R. irregularis* genome, where substitution rates differ widely between structural compartments.

**Conclusions:** Increasing understanding of low mutation rates will shed light on AMF cell biology and evolution, and may generate insights relevant to the transformation and generation of novel AMF strains for agricultural research and applications.

**Keywords:** Arbuscular mycorrhizal fungi, radiation, mutagenesis, evolution



**P-248**

**Evolution & Taxonomy**

**A NEW SPECIES OF *MORUS* IDENTIFIED AND CONFIRMED BY USING SSU, ITS, LSU COMBINED PHYLOGENY**

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**Introduction/Aim:**Morus is a multipurpose plant genus. It is playing a significant role in improving the economy, used for the formation of many important medicines.

**Materials and Methods:**For the correct and appropriate use of its medicinal benefits, it is indispensable to understand its taxonomic and phylogenetic position. Its taxonomic status always remains complex and disputed.

**Results:**A very little research work has been done on its taxonomic and phylogenetic identification. Many species are still wrongly identified create problems in any new or existing species identification.

**Discussion:**Up till now, merely four species have been documented on the basis of their morphological features. In this research work, one new species of Morus have been reported based on morphological and phylogenetic approaches.

**Conclusions:**It is first detailed study on the phylogenetic analysis of Morus lahorensis from Pakistan. Complete morphological and phylogenetic analysis based on SSU, ITS & LSU region has been illustrated and described.

**Keywords:** flora, mulberry, variety, phylogenetic study, taxonomic study, authentic identification

# **Management & Applications**

O-067

## Management & Applications

### BIOCHAR AS AN EFFECTIVE CARRIER OF THE AMF AND PGPR INOCULUM

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**Introduction/Aim:**In addressing global food security, intensive agriculture accelerated soil organic carbon mineralisation, leading to compromised soil structure, disrupted nutrient cycling, and degradation of soil health. To investigate possible solutions, our research evaluated the efficacy of biochar as a potential carrier of beneficial microorganisms. This was done in a frame the project BIOCIKRL TN02000044 aimed on development of integrated biofertilizer for sustainable agriculture.

**Materials and Methods:**Our study involved pot and field experiments investigating the effects of microgranular biochar biotized with arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria (PGPB) on various crops. Maize, wheat, onion, pea or tomatoes were supplied either with biochar microgranules or microgranules biotized with beneficial microbes to evaluate the growth response and mycorrhiza development.

**Results:**Although adding biochar did not directly affect crop yield, a significant enhancement in mycorrhization of crop roots was observed with biochar supplementation. Biochar microgranules, when enriched with the AMF, demonstrated efficiency in introducing mycorrhizal inocula as well as other beneficial microbes.

**Discussion:**The study has shown possible synergies of combining biochar and microorganisms like the AMF or PGPB. Thus the utilisation of biochar microgranules, enriched with microbes seem to be an efficient vector for introducing microbial inocula. However, the benefits to plants were not universally consistent, warranting further research to determine the optimal composition and dosage for various crop species.

**Conclusions:**This study has highlighted the substantial potential of biotized biochar in enhancing soil fertility, even when applied in minimal doses, while concurrently elevating the nutritional quality, growth and yield of tested crops.

**Keywords:** microgranulated biochar, biotization, crop response, AMF, PGPB

O-068

## Management & Applications

### MULTIPLE CROP ASSESSMENT OF NATIVE VS. COMMERCIAL MYCORRHIZAL APPLICATIONS IN ORGANIC AGRICULTURE

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**Introduction/Aim:** Native arbuscular mycorrhizal (AM) fungi have shown superior benefits in ecological restoration compared to those from commercial sources. However, their impact in agricultural systems remains largely unexplored. Here, we investigated the efficacy of native inoculum to commercial alternatives in field and greenhouse trials at organic farms in Lawrence, KS, USA.

**Materials and Methods:** Basil (*Ocimum basilicum*), chard (*Beta vulgaris*), Echinacea pallida, and hemp (*Cannabis sativa*) were inoculated with five native AM fungi individually, combined, or non-inoculated. Commercial inoculum was also assessed the hemp field trial. We evaluated fungal effects on plant growth, nutrient concentrations (basil and chard), and secondary compounds (cannbigerol in hemp and caffeic acid Echinacea). Three crops were evaluated after inoculation with native fungi or 14 commercial inoculants.

**Results:** Plant biomass and nutrient concentrations varied among native AM fungi. Species promoting plant growth enhanced micronutrient uptake, with biomass correlated with various leaf tissue elements (e.g. total carbon, nitrogen, phosphorus, copper, and manganese). Native fungal mixtures outperformed the commercial mixtures and improved secondary compound production in field soils. Overall, commercial inoculants exhibited low to non-viability and hindered crop growth by -7%, while native fungi improved growth by +37%.

**Discussion:** Like what has been shown in native ecosystem restoration, we found the superior performance of native mycorrhizal applications over commercial alternatives in agroecosystems for multiple crop performance metrics. Moreover, we confirmed the vital role of species diversity in mycorrhizal application.

**Conclusions:** Based on our results, we advocate for the integration of native mycorrhizal inoculants in farming practices, offering a promising avenue for enhancing crop productivity.

**Keywords:** Arbuscular Mycorrhizal Fungi, Agriculture, Native Inoculum, Crop Growth, Plant Secondary Compounds, Commercial inoculum

O-069

## Management & Applications

### FIELD INOCULATIONS WITH ARBUSCULAR MYCORRHIZAL FUNGI TO PROMOTE AGRICULTURAL SUSTAINABILITY

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**Introduction/Aim:** Global demand for food continues to rise as a result of population growth. However, higher crop yields often come at the expense of the environment. Alternative solutions to mineral fertilisers and pesticides that reduce the environmental impact of agricultural production are urgently needed. Arbuscular mycorrhizal fungi (AMF) play a crucial role in this context, as they can enhance plant nutrient uptake and reduce plant stress. However, large-scale field inoculation trials with AMF are lacking, and to date the success of AMF inoculation in agricultural fields remains unpredictable due to high context dependency.

**Materials and Methods:** We conducted on-farm experiments in 54 fields in Switzerland and quantified the effects on maize growth. In order to assess the factors explaining inoculation success, a total of 52 physico-chemical soil parameters was analysed in combination with PacBio sequencing of the soil and root microbiome.

**Results:** The growth response to AMF inoculation was highly variable, ranging from -12% to +40%. Using only a few soil parameters and mainly indicators of the soil microbiome, we were able to successfully predict 86% of the variation in plant growth response to inoculation. The abundance of pathogenic fungi, rather than nutrient availability, was the best predictor of AMF inoculation success (33%).

**Discussion:** Our results indicate that soil microbiome indicators offer a sustainable perspective to predict inoculation success at the beginning of the growing season.

**Conclusions:** As a result, AMF inoculation can become a powerful management option and thus an integral part of agricultural sustainability.

**Keywords:** arbuscular mycorrhizal fungi, field inoculations, sustainable agriculture, soil and root microbiome, pathogens, mycorrhizal growth response

O-071

## Management & Applications

### COINOCULATION WITH ARBUSCULAR MYCORRHIZAL FUNGI AND A RAPID ROOT COLONIZING FUNGUS PROVIDES SYNERGISTIC DISEASE CONTROL EFFECTS AGAINST FUSARIUM WILT

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**Introduction/Aim:** Soil-borne pathogens pose a particular challenge to crop protection and agricultural productivity. AMF (arbuscular mycorrhizal fungi) are known for their bioprotective effects, but require the establishment of a symbiosis prior to pathogen attack. Therefore, the combination of a beneficial rapid root colonizing fungus with AMF may result in a more successful bioprotective effect, allowing protection even with simultaneous inoculation. In this study, we investigated the synergistic potential of combining the root endophytic fungus *Serendipita herbamans* (Sh) with AMF to control *Fusarium oxysporum* f. sp. *lycopersici* (Fol) in tomato plants.

**Materials and Methods:** Our experiments, conducted in both greenhouse and growth cabinet settings, evaluated the efficacy of single inoculations versus coinoculations of these beneficial fungi. In addition, we performed targeted metabolomics to reveal the involvement of protective plant metabolites in root and shoot parts at an early stage of the interaction.

**Results:** Over an eight-week cultivation period, a reduction in disease incidence was observed when Sh was inoculated alone (67%) or in combination with AMF (53%). This reduction persisted at 16 weeks, with disease incidence reduced by 25% and 42% for single and combined inoculations respectively. Plant metabolite analysis revealed distinct metabolite patterns for root and shoot at 21 dpi.

**Discussion:** Synergistic effects of AMF and Sh were evident in the modulation of defence-related plant metabolites, suggesting enhanced plant resistance to Fol infection.

**Conclusions:** These findings highlight the potential of integrated fungal biocontrol strategies to reduce the threat of soil-borne pathogens in agriculture, while offering promising avenues for sustainable disease management and crop protection.

**Keywords:** biological control, *Serendipita*, plant defence, plant metabolites, root endophytes, *Fusarium* wilt

**O-072**

**Management & Applications**

**MYCORRHIZAS AS GAME-CHANGER CONCERNING C-SEQUESTRATION? IMPACT OF AM INOCULATION ON C SEQUESTRATION & YIELD ON >10.000 ACRES IN PRACTICE**

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Introduction/Aim: Mycorrhizas as game-changer concerning C-sequestration? impact of AM inoculation on C sequestration & yield on >10.000 acres in practice.

Materials and Methods: Although the results vary often an increase in yield and nutrient uptake is found, though large scale field studies are scarce. Moreover, co-benefits are seldom studied in the field. One potential co-benefit that has not been studied at a large scale is the potential of enhanced C-sequestration.

Results: Although the results vary often an increase in yield and nutrient uptake is found, though large scale field studies are scarce. Moreover, co-benefits are seldom studied in the field.

Discussion: One potential co-benefit that has not been studied at a large scale is the potential of enhanced C-sequestration.

Conclusions: Mechanisms concerning the stability of this newly formed soil C in MAOM, as well as implications and the way forward concerning C accreditation and the involvement of farmers are discussed.

**Keywords:** AMF-inoculation, Biomass, SOC, Yield, Field study

O-073

## Management & Applications

### AM FUNGAL TRAIT AND COMMUNITY DYNAMICS VARY WITH DISTURBANCE HISTORY AND FREQUENCY

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**Introduction/Aim:**Arbuscular mycorrhizal (AM) fungi associate with over 80% of plants, but our understanding of aboveground effects on AM fungi is lacking. Trait-based approaches can provide insight into AM fungal responses to aboveground processes; and demonstrate how resilience, plasticity, and environmental filtering, influence AM fungal communities. Here we evaluated how disturbance effects on AM fungal spore traits influence community composition and mutualisms.

**Materials and Methods:**We assessed variation in AM fungal spore responses to fire regimes by comparing traits (e.g., pigmentation and sporulation), community composition, and mutualistic outcomes (*Rudbeckia hirta* L. growth) between pots that burned 0-3 times over two generations of *R. hirta*. We hypothesized that fire driven changes in community composition and *R. hirta* growth would correlate with fire effects on spore traits (e.g., darker pigment and smaller spores), and that this effect would increase with fire frequency.

**Results:**Fire altered AM fungal community composition and reduced *R. hirta* growth, and these effects were mediated by filtering for darker pigmentation and decreases in sporulation. Fire's initial effect on sporulation and communities persisted after the first round of fires (even in Gen. 2 no burn pots) and this effect increased with fire frequency. Data collection is ongoing.

**Discussion:**AM fungal spore trait and community responses to fires were immediate, persisted without repeated fires, and this effect increased with fire frequency. We predict that trait and community responses to fire will continue to increase with fire frequency.

**Conclusions:**This demonstrates that trait-based approaches can help improve our understanding of AM fungal community responses to aboveground processes.

**Keywords:** traits, disturbance, fire, community assembly, fire history



O-074

### Management & Applications

#### ECOLOGICAL SUPPORTING SYSTEM FOR ENSURING THE FUNCTIONALITY OF ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITY IN ECOLOGICAL RESTORATION PRACTICES

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**Introduction/Aim:** Harnessing plant-beneficial microbiomes, such as arbuscular mycorrhizal (AM) fungi, holds great potential as a nature-based solution for ecological restoration. Despite of efforts to stimulate, enrich, or engineer exogenous or indigenous AM fungal communities to maximize their ecological services in restoration practices, the efficacy of these practices is not guaranteed.

**Materials and Methods:** (As this is a viewpoint, but not a research paper, there is no materials and methods to be described)

**Results:** To tackle this challenge, we propose an Ecological Supporting System (ESS) that includes selecting mycotrophic plants, managing the biotic interactions of AM fungi with indigenous soil microbiomes, and improving the soil environment to ensure the survival and functionality of AM fungi.

**Discussion:** To promote ecosystem restoration, we prioritize the study of biotic interactions in ESS. This involves identifying and managing the facilitative and antagonistic microbes associated with AM fungi and fostering directed coevolution of the plant-AM fungi-soil microbiota tripartite system. Furthermore, guidelines should be developed based on field practices to ensure the applicability and sustainability of AM fungal technologies within the ESS framework. Finally, we advocate conserving soil microbial biodiversity and integrating the core microbiota/microbiome rather than solely relying on AM fungi for ecological restoration.

**Conclusions:** The EES framework is expected to guide the application of AM fungi in ecological restoration practices.

**Keywords:** arbuscular mycorrhizal fungi, ecological restoration, ecological supporting system, biotic interaction, microbial inoculants

O-075

## Management & Applications

### DIVERSIFICATION OF CROPS AND INTENSIFICATION OF CANOLA IMPACT THE AMF DIVERSITY, COMMUNITY STRUCTURE, AND PRODUCTIVITY OF SUCCESSIVE CROPS IN ROTATION

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**Introduction/Aim:** We aim assess the influence of different rotation systems, incorporating canola, on AMF diversity and community structure in the roots and the rhizosphere, and on the productivity of each crop present within the rotation.

**Materials and Methods:** We conducted a field experiment with three cropping systems grown in 4-year rotation cycles, based on wheat, pea, oat, and canola, which were seeded in different sequences over the 4-year cycle. A complete randomized block design with 48 plots in four blocks, each comprising all combinations of three rotation systems and four rotation phases cultivated in parallel.

**Results:** Doubling the frequency of canola occurrence in a 4-year rotation did not reduce the productivity of the other crops in the rotation, nor did it result in a decrease in AMF biodiversity in the roots or rhizosphere of these crops, except for canola itself. Interestingly, the diversity of AMF was higher in canola roots compared to wheat roots, indicating that canola might serve as a rudimentary host of AMF.

**Discussion:** The rotation systems did, however, alter the AMF community structure in both roots and rhizosphere environments, exhibiting positive or negative correlations with crop productivity. This suggests that a simple modification of the cropping system could be employed to manipulate root or rhizosphere microbiomes, potentially enhancing overall crop productivity.

**Conclusions:** In conclusion, increasing the intensity of canola in a 4-year rotation did not reduce the productivity of the other crops in the rotation, nor did it result in reduced biodiversity of AMF in crop roots and rhizosphere, except in canola itself.

**Keywords:** microbiome, canola, arbuscular mycorrhizal fungi, rhizosphere, endosphere, crop rotation

O-076

## Management & Applications

### MYCORRHIZAL FUNGI AND ASSOCIATED MICROORGANISMS IN GRASSLAND SOILS IN AN AGROFORESTRY SYSTEM

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**Introduction/Aim:** Agroforestry has been reported to increase carbon sequestration and water- and nutrient-use efficiency, to improve soil health, and to provide a microclimate around trees. Nonetheless, its efficiency depends on many interacting factors that need to be characterised.

**Materials and Methods:** We compared an agroforestry system in four grasslands in Doubs (France) with multilayer hedgerows including both endomycorrhizal and ectomycorrhizal trees. Analyses were carried out from 2021 to 2024 at 2, 5 and 20 meters from each hedgerow to determine plant growth and nutrient content, and soil physicochemical properties. Roots and soil were collected to determine root arbuscular mycorrhizal (AM) colonisation levels and to analyse the soil microbiome through metagenomics.

**Results:** AM levels in plant roots varied greatly between grassland sites. There were also large spatiotemporal variations in mycorrhizal levels within each plot. Lowest mycorrhizal root levels were linked to high soil phosphorus and nitrate content. Conversely, highest root AM levels were correlated with higher soil porosity, soil organic matter and ammonium content, together with higher relative abundance of AM fungal DNA sequences. Relative abundance of bacterial and saprophytic fungal DNA sequences was influenced by the soil physicochemical properties. Hedges shaped soil microbial diversity, with bacteria involved in N and P cycling and ectomycorrhizal fungi more abundant at 2 meters than at 20 meters from the hedgerow.

**Discussion:** The results indicate that hedgerows positively influence soil health and functioning but the extent of this impact is dependent on agricultural practices.

**Conclusions:** In conclusion, in order to maximise hedgerow effects, it is important to adopt compatible cultivation practices.

**Keywords:** Agroforestry, grasslands, agricultural practices, soil microbial diversity, nutrient cycling

O-077

## Management & Applications

### MYCORRHIZAL INOCULANT AND PHOSPHATE DOSES AFFECT CORN YIELD IN A WIDE RANGE OF TROPICAL AND SUB-TROPICAL CONDITIONS

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**Introduction/Aim:** Mycorrhizal inoculants have been available in Brazil for a few years, grain crops being the main potential markets. Aiming to assess inoculant efficiency in different soil and climate conditions, we evaluated the yield of corn inoculated with a commercial product using "*Rhizophagus intraradices*" (167 10<sup>3</sup> propagules g<sup>-1</sup>) in tropical and sub-tropical conditions.

**Materials and Methods:** Field trials were conducted in five locations in a 2 x 3 factorial scheme with two inoculation conditions (-FMA / + FMA) and three phosphate fertilizer levels (0, 50, and 100% of the recommended P dose). Three sites were in tropical conditions, and two sites were in regions with milder climates.

**Results:** In the sites in subtropical conditions (Santa Catarina, Lat. 29.6 S and Rio Grande do Sul, Lat 27.2 S), inoculation led to 10.2 to 14.4% increases in yield, with the highest effects in areas with no P application. In tropical conditions, different inoculation responses occurred. In two sites (Piauí, Lat. 8.4S and Brasília, Lat. 15.9S), there were 12.4 to 21.9% yield increases, regardless of the P dose. On the other hand, there was no inoculation effect in a site in tropical conditions (Bahia, Lat. 12.6), with irrigation and high available P.

**Discussion:** Our data support previous findings that plants in soil with high nutrient availability do not respond to mycorrhizas.

**Conclusions:** In conclusion, arbuscular mycorrhizal fungal inoculants are promising for corn production in tropical and subtropical conditions. However, management aspects must be considered to maximize the benefits of this technology in the country's widely varying soil and climate conditions.

**Keywords:** commercial, inoculant, maize, *Zea mays*,

O-078

## Management & Applications

### IMPACT OF FOREST MANAGEMENT ON SOIL MICROBIAL COMMUNITIES IN CALCAREOUS AND SILICATE BEDROCK FORESTS

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**Introduction/Aim:** Forest management significantly impacts soil microbial communities, essential for nutrient cycling and tree health, potentially reducing ectomycorrhizal fungi diversity and abundance, and affecting ecosystem functions. This is critical for sustainable forestry and climate change mitigation, given the role of forests as carbon sinks.

**Materials and Methods:** Our study investigated how different management practices affect the soil microbiome in beech-dominated forests, considering variations between calcareous and silicate bedrock. We analyzed soil from nine European plots (in Slovenia, Croatia, and Italy), categorized by management type (close-to-nature, medium combined objective, and undisturbed) and bedrock (calcareous or silicate), using Illumina MiSeq sequencing.

**Results:** The results showed significant differences in bacterial and fungal diversity based on management and bedrock types, particularly affecting fungal communities. The highest fungal richness was observed in undisturbed forests, while tree removal significantly reduced ectomycorrhizal fungi relative abundance.

**Discussion:** Notably, bedrock type was a major driver of ectomycorrhizal community composition, underlining the importance of geological factors in such studies. Subsequently, soil pH emerged as an important driver of both fungal and bacterial communities.

**Conclusions:** These findings emphasize the need for integrated forest management approaches that consider microbial communities, as well as soil conditions, and bedrock types. Addressing these elements is crucial for more informed decisions on forest management practices in times of climate change.

**Keywords:** forest management, bedrock, fungi, ectomycorrhizal fungi, bacteria

**LT-076**

## **Management & Applications**

### **ARBUSCULAR MYCORRHIZAL FUNGI AS AN APPLE REPLANT DISEASE CONTROL STRATEGY**

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**Introduction/Aim:**Apple Replant Disease (ARD) is a soil-based disease that affects economically nurseries and orchards worldwide. To date, the only way to control the disease is by the use of chemical fumigants that are harmful for the environment. The main aim of our research Consortium (ORDIAMur.de, BONAres) is the development of more environmentally friendly solutions to control ARD.

**Materials and Methods:**To address this aim, several experiments were conducted to test the efficacy of different ARD control strategies including: commercial and non-commercial Arbuscular Mycorrhizal Fungi (AMF), commercial and non-commercial Biostimulant mixtures, Catch crops and Tagetes amongst others. At the end of the experiments, plant growth was measured and root samples were collected for the evaluation of apple root mycorrhization via microscopy, qPCR (Rhizophagus irregularis or Ri) and amplicon sequencing.

**Results:**First results of a greenhouse trial showed that none of the tested control strategies could counteract ARD induced growth depression. However, some of the AMF and Tagetes treatments slightly enhanced apple plant growth in ARD soils. We found that ARD affected plants have no Ri or low abundance of Ri in roots. Meanwhile, Ri abundance was the highest for those plants growing in ARD soils amended with Tagetes or with both Tagetes and AMF fungi, corroborating the ability of Tagetes to attract AMF fungi and facilitate its propagation.

**Discussion:**In conclusion, AMF and Tagetes amendments may be a potential strategy to increase infective potential of AMF and to control ARD.

**Conclusions:**Application of AMF fungi together with mycorrhization promoters (here Tagetes) may be key in treatment successfulness.

**Keywords:** Apple Replant Disease, Rhizoglosum irregularis, Arbuscular mycorrhizal fungi, qPCR, Tagetes

LT-077

## Management & Applications

### ARBUSCULAR MYCORRHIZAE MITIGATE NEGATIVE IMPACTS OF SOIL BIODIVERSITY LOSS ON GRASSLAND PRODUCTIVITY

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**Introduction/Aim:** Grassland degradation leads to decreased ecosystem productivity and reduced soil biodiversity. The interactions between plants and soil microorganisms are crucial for grassland restoration. However, it remains unclear how different functional groups of plants and soil microbial diversity interact to influence ecosystem function.

**Materials and Methods:** A greenhouse experiment was conducted using three plant functional diversity gradients (one, two, and three functional plants) and four soil biodiversity gradients (high, low biodiversity, bacteria only, arbuscular mycorrhizal fungi only) to investigate the mechanisms of plant-microbe diversity on grassland productivity.

**Results:** Both above- and below-ground biodiversity drive plant community productivity and composition. Regardless of soil biodiversity, mixtures of three functional plants increased grass biomass but decreased legume and forb biomass under high and low soil biodiversity, as well as bacteria-only soils. This is likely due to the higher competitiveness of grasses compared to legumes and forbs. In low biodiversity soil, biomass of plant communities was greater in mixtures of three functional plants compared to monocultures of one functional plant.

**Discussion:** Loss of soil biodiversity reduced plant community and legume biomass but did not affect grass and forb biomass. Inoculation with either bacteria or AMF significantly increased plant community biomass compared to low soil biodiversity.

**Conclusions:** Diverse plant functional groups can enhance the productivity of degraded grasslands through ecological niche complementarity. High soil biodiversity and beneficial microorganisms can mitigate the negative effects of soil degradation and biodiversity loss on plant productivity, underscoring the importance of above- and belowground biodiversity in grassland restoration.

**Keywords:** Plant functional diversity, soil biodiversity, dilution-to-extinction approach, arbuscular mycorrhizal fungi, bacteria, vegetation productivity

LT-078

## Management & Applications

### KAMÂALULA: MANAGING NATURAL TRUFFLE GROVES FOR THE PRODUCTION OF DESERT TRUFFLES IN AN HYPER ARID DESERT

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**Introduction/Aim:**With an aridity index of 0.018, the AlUla region in northwest Saudi Arabia is a hyper-arid desert area. At the heart of these extreme conditions, natural populations of *Helianthemum lippii*, a Cistaceae host of the White Desert Truffle, *Tirmania nivea*, were identified.

**Materials and Methods:**These natural truffle orchards are located in the immediate vicinity of drainage axes upstream of sandstone massifs on sandy soils with a relatively high proportion of clay and silt. In order to improve our understanding of the ecology of this symbiosis and the factors favourable to truffle production, the populations of *Helianthemum lippii* were characterized: density, health status, browsing, companion plants, associated microbiota.

**Results:**The description of the optimal environmental conditions for the production of these truffles will enable to develop tools for the detection of new truffles, as well as the preservation and improvement of the production of Desert Truffles in the protected areas of AlUla.

**Discussion:**The valorization of desert ecosystems is an important tool for their ecological restoration, their preservation and ultimately, their sustainable management.

**Conclusions:**Optimized management of natural truffle orchards will allow their rational exploitation and ensure the protection of these fragile ecosystems.

**Keywords:** Desert truffles, Hyper-arid ecosystems, *Helianthemum*, Saudi Arabia



LT-079

## Management & Applications

### IMPORTANCE OF WINTER SOIL MYCELIUM IN DESERT TRUFFLE PLANTATIONS AND WILD AREAS

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**Introduction/Aim:** Shrubs play a crucial role in Mediterranean forests, contributing not only to ecosystem restoration but also to soil protection, biodiversity, and carbon sequestration. In these arid regions, mycorrhizal woody plants like *Helianthemum* spp. form symbiotic relationships with edible desert truffles. Its cultivation represents an emerging and sustainable crop for these areas. Our aim was to study the mycelium dynamics of the desert truffles *Terfezia claveryi* in plantations and wild areas over time.

**Materials and Methods:** Fungal biomass was monitored in *Helianthemum* rhizospheres by real-time quantitative PCR, in each season for 4 years, referring to different areas of the Region of Murcia (Spain). The relationship between this biomass and environmental parameters was also investigated.

**Results:** The highest fungal biomass was found in half of the plantations studied, while no differences were observed between wild areas. The seasonal dynamics of *T. claveryi* extraradical mycelium did not follow an annual or seasonal pattern. However, winter fungal biomass presented a strong correlation with the autumn agroclimatic variables.

**Discussion:** We have previously shown that winter is a key season for host plant photosynthetic rate and bacterial diversity in desert truffle plantations. Furthermore, the importance of autumn agroclimatic parameters on *Helianthemum* bud sprouting, as well as on later fruit body production, is well established. Our data provide an additional input that will contribute to build models for desert truffle production and plantation management.

**Conclusions:** This research underscores the importance of winter soil mycelium in desert truffle ecosystems and suggests a potential connection between weather conditions and the growth of this valuable fungus.

**Keywords:** Desert truffles, {*Terfezia claveryi*}, soil mycelium, {*Helianthemum*} spp., shrubland management

LT-080

## Management & Applications

### IMPACTS OF ROOT-ASSOCIATED MICROORGANISMS ON PLANT SOIL FEEDBACK UNDER DIFFERENT GRASSLAND MANAGEMENT

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**Introduction/Aim:** This study explored the impact of root-associated microorganisms from different plant species under contrasting grassland management regimes (intensive versus extensive management) on plant-soil feedback (PSF) processes within the Yorkshire Dales.

**Materials and Methods:** Utilizing a classic PSF experimental design, which included both conditioning and feedback phases, this research aimed to explore the effects of plant species richness and identity on community biomass and soil properties in varied management regimes. Additionally, it investigated the influence of root-associated microorganisms associated with plant monocultures and mixtures on PSF in soils derived from both intensively and extensively managed grasslands.

**Results:** Our results suggested that (1) Plant species richness and grassland management could promote plant growth and significantly affect soil properties. (2) Roots and root-associated organisms contribute to positive PSF, enhancing plant growth and soil health, which is essential for effective ecosystem management, agriculture, and conservation efforts. (3) PSF from mixtures was not predicted based on the response from single species alone.

**Discussion:** These results emphasized the need to incorporate microbial dynamics into grassland management and conservation strategies to maintain productive ecosystems.

**Conclusions:** This study highlighted the key roles played by root-associated microorganisms in driving PSF.

**Keywords:** Plant soil feedback, grassland management, root-associated microorganisms, plant species richness

**LT-080**

## **Management & Applications**

### **FUNGI FOR FOREST RESTORATION (FUNGI4RESTOR)**

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**Introduction/Aim:** Ectomycorrhizal fungi (EMF) are important for tree health and thought to improve the establishment success of newly planted trees. Currently, there is uncertainty over the relative benefits of planting trees or encouraging natural colonisation when creating new woodlands on former agricultural land. These methods represent two different starting points in terms of the potential for root colonisation by EMF. Whilst naturally colonising trees may experience a lack of suitable EMF inoculum, planted trees are likely to already be colonised from tree nursery settings.

**Materials and Methods:** There is interest in the practice of adding amendments, such as woodland soil, as inoculum in order to introduce more tree-species specific EMF. However, the success of these amendments may depend on the initial EMF community as well as edaphic properties at planting locations. There is also interest in the role of 'nurse' plants as facilitators in the colonisation of woodland EMF.

**Results:** In the Fungi for Forest Restoration Project (Fungi4Restor), we are seeking to gain a better understanding of the typical composition of nursery EMF communities and factors shaping EMF communities in new woodland establishment schemes on former agricultural land in order to better advise on the potential success of amendments.

**Discussion:** Further, we quantify the effect of amendments, both on tree health/growth and on the EMF community colonising newly planted trees over time.

**Conclusions:** First results will be presented of the composition of EMF communities present on select tree species in a number of major and smaller UK tree nurseries and at subsequent time intervals after planting.

**Keywords:** Tree, nursery, tree establishment, inoculum

LT-081

## Management & Applications

### IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI AND PLANT GROWTH-PROMOTING RHIZOBACTERIA ON GROWTH ENHANCEMENT OF COMMERCIAL TREE CROPS IN INDIA

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**Introduction/Aim:** This study was conducted to evaluate the impact of arbuscular mycorrhizal (AM) fungi and plant growth-promoting rhizobacteria (PGPR) individually and in combination on the growth of commercial tree crops such as *Melia dubia*, *Gmelina arborea*, *Santalum album* and *Pterocarpus santalinus*.

**Materials and Methods:** The selected tree species were raised and nurtured under controlled nursery conditions. They were treated with PGPR strains (*Bacillus*, *Azospirillum*, *Azotobacter* and *Rhizobium*), AM fungi species (*Glomus*, *Acaulospora* and *Scutellospora*) and combination of both PGPR and AM fungi. Untreated seedlings were kept as control for comparison. After a period of 3 months, plant growth parameters were evaluated.

**Results:** The results demonstrated a significant improvement in plant growth among the seedlings that were inoculated with both AM fungi and PGPR, as compared to the control. Notably, plants treated with combination of PGPR and AM fungi exhibited enhanced growth, surpassing the growth achieved by the application of PGPR alone.

**Discussion:** The results of this investigation highlighted the potential of utilizing AM fungi in tandem with PGPR to augment plant growth and development, thereby maximizing its effectiveness.

**Conclusions:** Overall, this study has revealed that AM fungi have the potential to complement the activities of PGPR by further enhancing the uptake of nutrients thereby promoting plant growth significantly. The synergistic effect of AM fungi and PGPR in improving the plant growth emphasizes the importance of incorporating multi-microbial strategies in commercial forestry to improve tree crop productivity and ensure its sustainability.

**Keywords:** AM fungi, PGPR, Plant-microbe interactions, Symbiosis, Tree productivity

## Management & Applications

### BIOFERTILIZERS OPTIMIZE LAND USE EFFICIENCY IN DRYLAND INTERCROPPING

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**Introduction/Aim:** Agroecology takes advantage of natural ecosystems services and uses local knowledge to plant a diversity of crops that boost the sustainability of the farming system. In the light of climate change, densely populated areas of the drylands and semiarid regions face cumulative challenges. Limited agricultural land, low-nutrient soils, and unpredictable rain patterns brought on by climate change are pressing challenges. Intercropping practices are rising as feasible means to address these challenges. In these systems two or more crop species are grown simultaneously on the same land. Farmers take advantage of the increased plant biodiversity and plant complementarity to increase resource-use efficiency as well as crop resilience and productivity. Biofertilizers centered around arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) are soil microorganism well-known for their far-reaching beneficial effects to plants. Improved grain yields, enhanced resistance to drought, and improved nutritional value are well known effects of plants inoculated with these soil microorganisms.

**Materials and Methods:** We tested for the establishment and effect of biofertilizers in both monocrops and intercrops of staple crops from southern India.

**Results:** Following biofertilizer inoculation, AMF root infection and spore production were significantly higher than control in both cropping systems. More significantly, biofertilizers had a positive effect on grain yields that was stable across seasons and sites.

**Discussion:** Specifically, our study demonstrates that the inoculation with biofertilizers improves the land use efficiency.

**Conclusions:** Our results suggest that biofertilizers in combination with intercropping are promising tools to stabilize and increase the yields of dryland agriculture.

**Keywords:** Agroecology, Intercropping, Biofertilizer, AMF

**LT-083**

## **Management & Applications**

### **CATTLE HARBORS AND PROPAGATES ARBUSCULAR MYCORRHIZAL FUNGI IN ITS INTESTINE**

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**Introduction/Aim:** Ishii and Horii first succeeded in the axenic culture of AMF (Patents 4979551 and 6030908). However, the AMF spore produced using axenic culture techniques is more expensive than spores produced using roots. Additionally, a dangerous mycorrhizal inoculum produced with genetically engineered hairy roots has recently been commercially available. Recently, we found arbuscular mycorrhizal fungus (AMF) in cattle feces. So, we conducted the following investigation.

**Materials and Methods:** AMF in several animal feces were observed using the mycorrhizal fungus-detection reagent developed by Ishii and Amanai (2020). Moreover, we developed the inoculum including AMF spores in cattle feces, and investigated its effect on the growth of green onions. Furthermore, we observed AMF in cattle gastrointestinal tract using the method described above, and also attempted to axenic culture of AMF using the cattle intestine contents (liquid).

**Results:** A lot of newly proliferated and active AMF spores were found in the cattle feces. In addition, this AMF inoculum using cattle feces promoted the growth of green onions. On the other hand, AMF spores were present in the contents of the abomasum and intestines (small and large) of the cattle, but many new spores were observed in the large intestine. By using the large intestine contents, several neospores were formed from one mother spore after 18 days-incubation at 25°C in dark in both autoclaved and non-autoclaved plots.

**Discussion:** Thus, cattle grow AMF and will play an important role in nature conservation and food production.

**Conclusions:** The discovery will greatly contribute to the development of mycorrhizal research.

**Keywords:** cattle, cattle intestine, AMF inoculum production, AMF axenic culture

LT-085

## Management & Applications

### IMPACT OF AM FUNGI-MEDIATED CADMIUM UPTAKE ON MAIZE GROWTH AND ANTIOXIDANT PHYSIOLOGY

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) establish symbiotic associations with crops, aiding in mitigating cadmium (Cd) stress. However, the impact of Cd absorption and transport by AMF on plant growth remains elusive.

**Materials and Methods:** AMF *Funneliformis mosseae* was used to inoculate maize cultivar Zhengdan 958 in a segmented rhizobox system in soil amended with or without Cd (50 mg·kg<sup>-1</sup> CdCl<sub>2</sub>). Plants were assessed 100 days after transplanting.

**Results:** AMF significantly enhanced the agronomic traits, photosynthesis, biomass, and antioxidant enzyme activities of maize. Cd content was higher in Cd + AMF treatment. Cd absorbed by AMF resulted in bioaccumulation, chelation, and membrane peroxidation in the leaves, consistent with the increased malondialdehyde content. Under Cd stress, AMF inoculation decreased the soluble protein content and increased the activities of catalase and superoxide dismutase in roots and leaves, while significantly enhancing the activity of ascorbate peroxidase and root growth.

**Discussion:** The observed change in the oxide bond amplitude suggests a potential link to the complexation reaction occurring during the binding of Cd ions to the cell wall. This could be attributed to the enhancement of glucose metabolism in photosynthesis by AMF. Conversely, Cd absorption through AMF hyphae inhibits this process, thereby promoting the formation of ascorbic acid in the metabolic pathway.

**Conclusions:** AMF significantly improved maize growth and antioxidant physiology, while mitigating Cd stress. AMF observed Cd assumes a pivotal role in the physiological metabolism of maize leaves, necessitating further exploration.

**Keywords:** extraradical hyphae, cadmium, antioxidant enzyme activity, ascorbate peroxidase

LT-086

## Management & Applications

### URBANIZATION EFFECTS ON PLANT-MYCORRHIZA ASSOCIATIONS ALONG GRASSLAND-WOODLAND ECOTONE

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**Introduction/Aim:** Environmental changes resulting from urbanization can profoundly alter the dynamics of plant-soil microbial interactions, with far-reaching implications for ecosystem function, biodiversity, and human wellbeing. However, our understanding of how urbanization influences symbiotic associations between plants and mycorrhizal fungi across diverse habitats remains largely unknown.

**Materials and Methods:** We examined arbuscular mycorrhizal (AM) colonization in *Plantago lanceolata* grown in soil collected from grass-woodland ecotones spanning grassland, transition, and woodland habitats along an urban-rural gradient. Additionally, we determined the identity and distribution of ectomycorrhizal (ECM) fungi associated with birch and pine. Plant and soil properties were also measured.

**Results:** Our preliminary results show that AM colonization in roots of *Plantago* was significantly reduced in semiurban and urban soils compared to rural soils. These decreases were associated with higher soil phosphorus, zinc, and lead concentrations, regardless of vegetation type. *Plantago* in urban soils exhibited higher biomass but lower AM colonization than those in semiurban and rural soils. Furthermore, ECM inoculum in both pine and birch was lower in grasslands compared to woodlands across all urbanization levels, with transition zones exhibiting contrasting patterns. Plant root-to-shoot ratios and C:N ratios were higher for birch and pine in grasslands, while urbanization shows less impact.

**Discussion:** Our findings suggest that urbanization-induced changes in soil nutrients and pollutants such as heavy metal may influence plant-mycorrhizal fungal symbiosis.

**Conclusions:** This study highlights the importance of understanding the complex interactions between urbanization, soil nutrients, and plant-mycorrhizal associations for urban planning.

**Keywords:** Urbanization, Arbuscular mycorrhizal fungi, ectomycorrhizal fungi, plant-mycorrhiza associations, Urban ecosystems, Habitat types



LT-087

## Management & Applications

### ENHANCING FIELD LIQUORICE GROWTH AND ACTIVE INGREDIENT ACCUMULATION: ROLE OF MYCORRHIZAL COLONIZATION INTENSITY OVER EXOGENOUS FUNGAL ABUNDANCE

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**Introduction/Aim:**Liquorice (*Glycyrrhiza uralensis*) is a crucial medicinal plant with significant market demand attributed to the accumulation of active ingredients, glycyrrhizin and liquiritin, within its roots. However, the development of the liquorice industry has been largely hampered by low yields and concentrations of these active compounds in cultivated liquorice plants.

**Materials and Methods:**This study conducted a field experiment to verify the mycorrhizal effects on liquorice growth and the accumulation of active ingredients. Additionally, exogenous arbuscular mycorrhizal (AM) fungus abundance in plant roots was tracked using Illumina high-throughput sequencing.

**Results:**The results demonstrate that AM inoculation improves plant growth and facilitates glycyrrhizin accumulation under field conditions, with a more pronounced effect observed under soil fumigation conditions. Furthermore, AM inoculation post-soil fumigation significantly impacts root fungi and AM fungal diversity, and enhances the abundance of exogenous AM fungus in roots.

**Discussion:**Our findings suggest that it is the intensity of mycorrhizal colonization, rather than the abundance of exogenous AM fungus in roots, that regulates plant root nutrient uptake, growth, and the accumulation of secondary metabolites.

**Conclusions:**This study provides a deeper understanding of the crucial role of AM fungi in regulating the secondary metabolism of liquorice plants, strongly supporting the potential utilization of AM fungi in the cultivation of medicinal plants.

**Keywords:** field condition, medicinal plant, secondary metabolism, exogenous fungal abundance

LT-088

## Management & Applications

### DIFFERENTIAL RECRUITMENT OF MYCORRHIZAL TAXA BY PRUNUS ROOTSTOCKS AND ITS IMPLICATIONS FOR AGRONOMIC EFFICIENCY IN STONE FRUIT CULTIVATION

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**Introduction/Aim:**The Prunus genus encompasses significant agricultural crops such as plum, peach, and almond, often cultivated as grafted varieties onto compatible rootstocks. Dwarfing or semi-dwarfing rootstocks, like those from the Rootpack® series, are used to limit tree size and vigor. This in turn facilitates their management and harvest, in contrast to trees grafted onto the conventional vigorous GF677, Garnem and Cadaman rootstocks. While all these have been extensively assessed for their agronomic characteristics, their interaction with arbuscular mycorrhizal fungi (AMF) remains unexplored. The study aimed to unveil the selective recruitment of AMF taxa by various Prunus rootstock genotypes to potentially improve nutrient and water use efficiency, as well as tolerance to abiotic and biotic stresses.

**Materials and Methods:**A targeted metagenomic approach was followed to assess soil, rhizosphere, and root mycorrhizal communities across six distinct Prunus rootstocks (GF677, Garnem, Cadamán, and the Rootpac series: RR20, R40, and RR) cultivated within an experimental almond orchard.

**Results:**A description of the core AMF community from each rootstock will be presented, as well as alpha and beta diversity metrics and key AMF taxa potentially correlated with agronomic traits such as fruit yield or canopy vigor.

**Discussion:**The observed variations in mycorrhizal community composition and diversity among Prunus rootstocks underscore the importance of rootstock selection in shaping microbial interactions within almond orchards.

**Conclusions:**These findings provide valuable insights into the intricate relationships between rootstocks and microbial communities, offering potential avenues for enhancing crop management practices and ultimately improving almond orchard productivity and resilience.

**Keywords:** Mycorrhizal symbiosis, almond tree, arbuscular mycorrhizal fungi, community networks, diversity.

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## Management & Applications

### EFFECT OF (NON)-ACCLIMATISED ARBUSCULAR MYCORRHIZAL FUNGI ON HYDRANGEA MACROPHYLLA

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) are one of the most ubiquitous plant-associated microorganisms, colonising about 80 percent of terrestrial plants worldwide. However, they are scarce among our crops due to the limited growth of these microbes under the chemical conditions of traditional farmland, which limits the use of their beneficial characteristics, such as access to water and limited nutrients, as well as enhanced tolerance to abiotic/biotic stresses. Nevertheless, these fungi are highly adaptable, being able to acclimatise to adverse conditions, such as heavy metals or high phosphate (P) concentrations. After only five generations of cultivation, the acclimatised *Rhizoglyphus irregulare* performed better than the non-acclimatised strain at high phosphate concentrations.

**Materials and Methods:** In our present project we apply the P-acclimatised and non-acclimatised AMF strains to improve crop performance in horticultural substrates. A greenhouse experiment was conducted, in which four different genotypes of *Hydrangea macrophylla* were inoculated with either P-acclimatised or non-acclimatised AMF, with the aim of studying the mycorrhizal capacity of *H. macrophylla* and in case of mycorrhization the effects on plant performance under low nutrient conditions.

**Results:** We found that *H. macrophylla* is a mycorrhizal plant, our 4 genotypes got mycorrhized in under 14 weeks.

**Discussion:** But interestingly, both AMF negatively influenced the growth (fresh/dry weight and height) of these plants under abiotic stress conditions, especially the acclimatised one.

**Conclusions:** Further experiments are needed to explain this behaviour, so we plan to infect mycorrhized plants with *Botrytis* sp. to test if there is a difference in behaviour under biotic stress tolerance than under abiotic stress.

**Keywords:** AMF, Acclimatised, Hydrangea, R. irregulare

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## Management & Applications

### THE VALUE OF METABARECODING STUDIES OF FOREST SOILS FOR NATIONAL RED-LIST ASSESSMENTS OF ECTOMYCORRHIZAL FUNGI - A SWEDISH CASE STUDY

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**Introduction/Aim:** Assessing the status and trends of fungi by red-listing is crucial for species to be considered in conservation and to facilitate the setting of conservation priorities.

**Materials and Methods:** The way to do the red-listing is to compile all available knowledge of species distribution, commonness and habitat requirements, together with habitat information. Largely, such information of fungi is based on sporocarp occurrences. These are to varying degrees uncertain and uncomplete due to the largely cryptic lifestyles of fungi and the formation of sporocarps being weather dependent. Hence, metabarcoding of environmental samples has been identified to complement traditional analyses based on sporocarp data.

**Results:** About 20 red-listed ectomycorrhizal fungal species were detected, mostly with few records.

**Discussion:** It is obvious that the probability to detect rare species by e-DNA is very small and that sporocarp records provides a much more comprehensive picture of less common species. However, although e-DNA analyses by methodological necessity mainly reveals the patterns of frequent species, it gives a more complete description of the fungal community. In addition, trends in common species' population sizes maybe followed this way.

**Conclusions:** Strengths and weaknesses with sporocarp recording and metabarcoding environmental samples to get information will be discussed.

**Keywords:** red-list, conservation, metabarcoding, sporocarps

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## Management & Applications

### THE ABUNDANCE OF *TUBER MELANOSPORUM* EXTRAMATRICAL MYCELIUM DECREASES WITH INCREASING SOIL MOISTURE IN A CANADIAN TRUFFIERE DURING A MODERATE DROUGHT

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**Introduction/Aim:**The French black truffle is a prized fruiting body of the ectomycorrhizal fungus *Tuber melanosporum*. Little is known about the optimal conditions for truffle production. Here, we investigated how three rates of irrigation influenced the abundance of extramatrical mycelia in a pre-production *T. melanosporum*-*Quercus robur* orchard during a heatwave.

**Materials and Methods:**Irrigation treatments were maintained at high (- 30 kPa), moderate (- 80 kPa), or low levels (non-irrigated) of soil moisture. Soil samples were collected once per month from June to September 2021 to determine the abundance of *T. melanosporum* extramatrical mycelia. The ectomycorrhizal fungal community was sampled in August from soil and mycorrhizal root tips. DNA of *T. melanosporum* was quantified using droplet digital PCR (ddPCR), whereas composition of the ectomycorrhizal communities was determined by PacBio sequencing.

**Results:**During the hottest and driest weeks *Tuber melanosporum*, DNA abundance was lowest in the high irrigation treatment as compared with the low and moderate treatments. Irrigation did not affect the fungal communities from roots or soil, but several dominant species were identified as potential competitors.

**Discussion:**This study represents the first North American trial involving *T. melanosporum*, where irrigation has been manipulated and DNA metabarcoding has been performed.

**Conclusions:**Our study suggests that limited irrigation may favour the growth of *T. melanosporum* mycelial abundance during elevated temperatures. Due to the expected rise in daily temperatures, our results suggest that orchards in coastal BC may be well-suited for *T. melanosporum* cultivation. In addition, we identified several species of *Scleroderma* as dominant ectomycorrhizal fungi on *Q. robur* roots.

**Keywords:** Tuber melanosporum, Irrigation, ddPCR, Extramatrical mycelium, Truffiere, Meta-barcoding

## Management & Applications

### ARBUSCULAR MYCORRHIZAS AS REMEDIATORS OF POLLUTANTS INTRODUCED TO AGRICULTURAL SOILS THROUGH INDUSTRIAL WASTES

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**Introduction/Aim:** Arbuscular mycorrhizal fungi (AMF) are frequently exposed to organic and inorganic pollutants released to soils. Here, we evaluated the ability of AMF in degrading complex organic pollutants in molasses distillery spent wash (MDSW); and removal and transfer of heavy metals from fly ash polluted soils to edible grains.

**Materials and Methods:** Sorghum (*Sorghum bicolor*) inoculated with a consortium of seven native AMF and barley (*H. vulgare*) with *Rhizophagus irregularis* MUCL 41833 were used as crop models. Sorghum was fertilised every 15 days with 10 mL MDSW. Leachates were collected in a beaker and removed to bottles in 30-day intervals. Barley received fly ash ranging in soil concentrations from 0 – 50%. After 16 weeks of growth; biomass, grain yield and mycorrhizal colonisation were measured. Concentrations of organic pollutants remaining in leachates of sorghum; and of N, P; and heavy metals Ni, Co, Pb and Cr in edible and vegetative barley tissue; and in the substrate were measured.

**Results:** Raw MDSW contained 65 complex-coloured organic pollutants. AMF accelerated the degradation of these pollutants. Leachates from mycorrhizal sorghum had only 26 simple colourless organic pollutants compared to the 42 of non-mycorrhizal sorghum. *R. irregularis* likewise removed heavy metals from the soil but amplified heavy metal concentrations in edible grains.

**Discussion:** AMF remediated polluted soils by removing pollutants. But long-term studies are needed to understand risks, if any, to human health due to AMF-mediated pollutant transfer to edible plant-tissues.

**Conclusions:** With their ability to clean polluted soils, AMF may have a keystone role in bioremediation of human introduced pollutants.

**Keywords:** Arbuscular mycorrhizas, organic pollutants, heavy metals, bioremediation, Agriculture

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## Management & Applications

### ARBUSCULAR MYCORRHIZAL FUNGI SPORE DENSITY OF NURSERY SOIL AND ROOT COLONIZATION LEVEL OF TREE SEEDLINGS IN TIGRAY, NORTH ETHIOPIA

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**Introduction/Aim:**In natural ecosystems, the root system of successful plants has microbial partnerships to survive and grow even in harsh conditions. Arbuscular mycorrhizal fungi (AMF) are among the mutual beneficial partners to plants in the drylands. Seedlings of trees prepared for the restoration of degraded drylands need to be inoculated and colonized in the nursery for successful survival and growth at planting site. The objective of the paper was to analyze the status of AMF spore density and root colonization in raised nursery tree seedlings and nursery soils in lowland, midland, and highland agroecology.

**Materials and Methods:**Both soil and root samples were collected from seven private, four community and nine state nurseries using stratified random sampling method. AMF Spore enumeration and root colonization analysis were done using 204 seedling containers having nine tree species from lowland, fourteen tree species from midland and six tree species from highland.

**Results:**All the 22 tree species found in the 20 nurseries were colonized by AMF. The highest average spore density and root colonization were enumerated in lowland ( $p < 0.001$ ) followed by mid land and highland. State nurseries had recorded higher average spore density (43.60 spores 100 g<sup>-1</sup> dry soil) and percentage of root colonization (39.51%) followed by community nursery and private nursery.

**Discussion:**The source of potting soils might come from degraded ecosystems with low AMF inoculum potential.

**Conclusions:**The spore density of the nursery soils and root colonization of seedlings was low. Tree seedlings raised in nurseries need to be inoculated with AMF spore from a non-disturbed soil source.

**Keywords:** agroecology, community nursery, private nursery, state nursery, nursery soil

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## Management & Applications

### THE OLIVE MYCORRHIZOSPHERE HARBOR DIVERSE PHOSPHATE SOLUBILIZING BACTERIA HAVING MULTIPLE GROWTH PROMOTING TRAITS

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**Introduction/Aim:** Phosphate chemical fertilizers are costly and raise concerns about environmental pollution through industrial production. The use of phosphate-solubilizing microorganisms (PSMs) as inoculants can be an option to increase P availability in soils. The aim of this work was to isolate PSMs from olive mycorrhizosphere, having multiple plant growth-promoting (PGP) traits.

**Materials and Methods:** Bacteria were isolated from the mycorrhizosphere of olive and tested for P-solubilization (Tricalcium phosphate (TCP), hydroxyapatite, and phosphate rock (PRs)) and PGP traits. Isolates were also identified via the Sanger sequencing of the 16S rRNA gene.

**Results:** Out of 105 isolates, 12 belonging to *Arthrobacter* sp., *Bacillus* sp., *Pantoea* sp., *Streptomyces* sp., and *Pseudomonas* sp. were found to be the best PSMs. All strains mobilized P from TCP phosphate, hydroxyapatite, and PRs. Additionally, most strains presented various PGP activities like IAA, Nitrogen fixation, Acc deaminase, and siderophore production.

**Discussion:** Results of this study indicated that the olive mycorrhizosphere contains diverse PSMs with multiple PGP traits. The contribution of PGPR in the P-acquisition strategies of the plant is complex and involves various mechanisms. As observed in results, by selecting the efficient PSMs-solubilizing and PGP abilities of different strains, the potential impact of each component on P nutrition could be increased.

**Conclusions:** The mycorrhizosphere of olive is a good source for the isolation of efficient PSMs-solubilizing with diverse PGP traits. Bacterial isolates obtained in this study has the potential to be used as inoculants for more sustainable agriculture in low-phosphorus soils such as calcareous soils.

**Keywords:** Phosphate-solubilizing microorganisms (PSMs), Plant growth-promoting (PGP) traits, Olive mycorrhizosphere, *Pseudomonas* sp. Bacterial inoculants.



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## Management & Applications

### EVALUATION OF THE USE OF ARBUSCULAR MYCORRHIZAL FUNGI IN TWO CULTIVARS OF *VITIS VINIFERA* L. DURING FOUR SEASONS

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**Introduction/Aim:**In this study, the use of two formulations with arbuscular mycorrhizal fungi (AMF) was evaluated in two cultivars of *Vitis vinifera* L.

**Materials and Methods:**A 28-year-old Syrah clone 01 cultivar with low production and a Malbec 598 clone planted in 2020 were selected. The trial took place in Lourdes, Maule Region, Chile. Four treatments were used: T0 (control), T1 (commercial product 1), T2 (commercial product 2) and T3 (combination of both products). For Syrah the product was injected into the soil, while for Malbec it was applied directly at the time of planting. Physiological and productive parameters were evaluated such as chlorophyll content, length of internodes, stem diameter, nutritional content of leaves and roots, pruning weight, weight of fruits per plant, number of clusters and berries, weight of clusters, grape quality, and mycorrhization (2021-2024) and the vivinification of the last season was also completed.

**Results:**The results showed varied responses in the cultivars both at a physiological and productive level. The productive results and quality of the grapes were especially important for viticulture. With treatment T2, a significant increase of 37%, 38% and 18% was recorded in the production of Syrah (2022, 2023, 2024) and an increase of 44% in the production of Malbec (2022).

**Discussion:**These results demonstrate a significant increase in production when using AMF. The study also highlighted the positive impact of commercially formulated products with HMA on production.

**Conclusions:**These findings emphasize the importance of using AMF in viticulture to improve crop productivity and achieve better results in the wine industry.

**Keywords:** Vitiviculture, Mycorrhiza, AMF, {*Vitis vinifera*} L.

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**Management & Applications**

**CANADIAN COLLECTION OF ARBUSCULAR MYCORRHIZAL FUNGI (CCAMF)**

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**Introduction/Aim:**The Ottawa Research and Development Centre (Agriculture and Agri-Food Canada) has housed the Canadian Collection of Arbuscular Mycorrhizal Fungi (CCAMF), an extensive in vivo and in vitro collection of AMF, since the 1980s. Here, we present how researchers can contribute to and benefit from the collection's ongoing initiatives to foster collaboration and knowledge exchange.

**Materials and Methods:**This living collection is maintained under in vivo conditions either in pot cultures or using superabsorbent polymer-based autotrophic systems, and under in vitro conditions in petri dishes using Ri T-DNA roots.

**Results:**The CCAMF maintains 400 pot cultures and over 100 strains growing in petri dishes. This represents a comprehensive collection of more than 70 species of AMF from all known taxonomic orders.

**Discussion:**The CCAMF is not only dedicated to biobanking, but also actively involved in the development of cultivation methods, including pioneering protocols for in vivo cultivation of AMF and in vitro cultivation of new roots transformed with Ri T-DNA.

**Conclusions:**From maintaining a diverse array of AMF species to providing state-of-the-art cultivation methods and biobanking, CCAMF serves as an important hub for advancing mycorrhizal research.

**Keywords:** Arbuscular Mycorrhizal Fungi, In Vivo Cultivation, In Vitro Cultivation, Biobanking, Collaboration, Mycorrhizal Research

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## Management & Applications

### A TRUFFLE (TUBER BORCHII) PLANTATION IN THE POST-MINING LANDSCAPE OF LUSATIA (GERMANY)

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**Introduction/Aim:** Truffle production in Europe is best known in countries with Mediterranean climate. In the last 100 years, however, there have been crop failures in naturally occurring truffle groves. As a result, truffle plantations are increasingly being established. Due to climate change, truffle production north of the Alps is shifting mainly to calcareous locations with a temperate climate. Lusatia is characterised by sandy soils and low rainfall. The naturally occurring and culinary bianchetto truffle (*Tuber borchii*) was discovered in this region. Lusatia has a long tradition of lignite mining. With the imminent exit from this industry, the region is facing new economic challenges. We want to know whether commercial truffle production on Lusatian soils is successful and can therefore contribute to the region's economy. As the first truffles are expected in 4 years at the earliest, we will genetically identify the mycorrhiza in order to investigate whether the bianchetto truffle is retained on the root system or replaced.

**Materials and Methods:** In October 2020, we planted a truffle plantation with 800 oak trees (*Quercus robur*, *Q. cerris*) inoculated with bianchetto truffle spores. Half of the plantation is irrigated.

**Results:** Over the 2.5-year sampling period, the mycorrhisation rate of the root decreased. Additionally, the bianchetto truffle seems to be displaced by other truffle species.

**Discussion:** The decrease of the mycorrhization rate is caused by plant shock due to water stress. Other truffle species have been introduced through the nursery.

**Conclusions:** As countermeasure, truffle traps were used in 2022 to increase the mycorrhisation rate of the bianchetto truffle.

**Keywords:** agroforestry system, ectomycorrhiza, ITS, *Quercus* sp., truffle plantation, *Tuber borchii*

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## Management & Applications

### APPLICATION OF CROPS IN ALPINE ARID DESERT MANAGEMENT

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**Introduction/Aim:** Mechanical sand barriers are one of the most important ways of desertification control in arid and semi-arid desert, which mainly based on the principles of wind-blown physics to prevent wind and fix sand. Under the premise of local water carrying capacity, vegetation restoration is a fundamental way once mechanical sand barriers done since it could utilize the function of soil-plant system to change the local environment, and then accelerate the process of ecosystem succession, which would shorten the ecological restoration cycle and promote the recovery of natural ecosystem.

**Materials and Methods:** Since 2014, my team have carried out a new model of desertification control using crops at the eastern shore of Qinghai Lake Basin. We adopted the method of planting living sand barriers of wheat, barley and oats to set up mechanical sand barriers, so that the cost was reduced by 90% compared with the 1m×1m wheat-board barriers, and 77% compared with the 1.5m×1.5m wheat-board barriers. Moreover, not only crops live aboveground part had obvious sand-blown effect, plant roots could also play an excellent function of sand fixation, which achieved win-win benefits by little investment with good results.

**Results:** In addition, we planted plateau excellent economic crop taro on the sand dunes where above crop living sand barriers set up. We chose four different varieties of taro (Qinghai taro No.1, Qinghai taro No.2, Qinghai taro No.3, Qinghai taro No.4) for planting test. It was found that the aboveground growth and the height of Qinghai taro No.1 was better. While Qinghai taro No.2 had a relatively longer growth cycle and higher survival rate, and Qinghai taro No.3 showed higher below-ground biomass and root-crown ratio. Qinghai taro No.4 showed lower survival rate, poorer adaptability to soil temperature and soil water content.

**Discussion:** Comprehensively, Qinghai taro No.1 and No. 2 were more suitable for planting in alpine semi-arid desert, which could be used as biological desertification controlling materials for the purpose of vegetation restoration and ecological management of desert.

**Conclusions:** They would not only support sand-blown effect with well-grown aboveground, but also provide a better ecological service value through the large underground root system. Above all, a mode of desertification control in alpine desert has been broadened, and a new perspective has been provided for the development of sand industry in arid and semi-arid areas as well.

**Keywords:** Crops, Application, Mechanical sand barrier, desertification

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**Management & Applications**

**PLANT-SOIL FEEDBACK DRIVES THE 'NURSING EFFECT' IN TREE MIXTURES**

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**Introduction/Aim:**The productivity of high-value focal trees in mixtures is often improved by inclusion of lower-value nursing species in mixtures, but the mechanisms underpinning such effects are poorly resolved. This gap in understanding also limits the capacity to develop efficient planting strategies to achieve net zero.

**Materials and Methods:**We established a plant-soil feedback experiment.

**Results:**We found that Sitka spruce, a focal species, grew better in mixture and in soil conditioned by nurse species compared to soil conditioned by conspecifics.

**Discussion:**It may be caused by colonization of ectomycorrhizal fungi on focal tree roots, that were positively related to plant biomass and plant growth strategy. Moreover, the positive associations among microbial biomass nitrogen, extractable ammonium in soil, and the activity of the nitrogen-degrading enzyme N-acetylglucosaminidase may influence plant growth in the longer-term by stimulating nitrogen cycling.

**Conclusions:**Overall, our findings show that the nursing effect is largely driven by the activity of both symbiotic and free-living microorganisms.

**Keywords:** Ectomycorrhizal fungi, Forest management, Nitrogen cycling, Nursing effect, Plant growth strategies



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