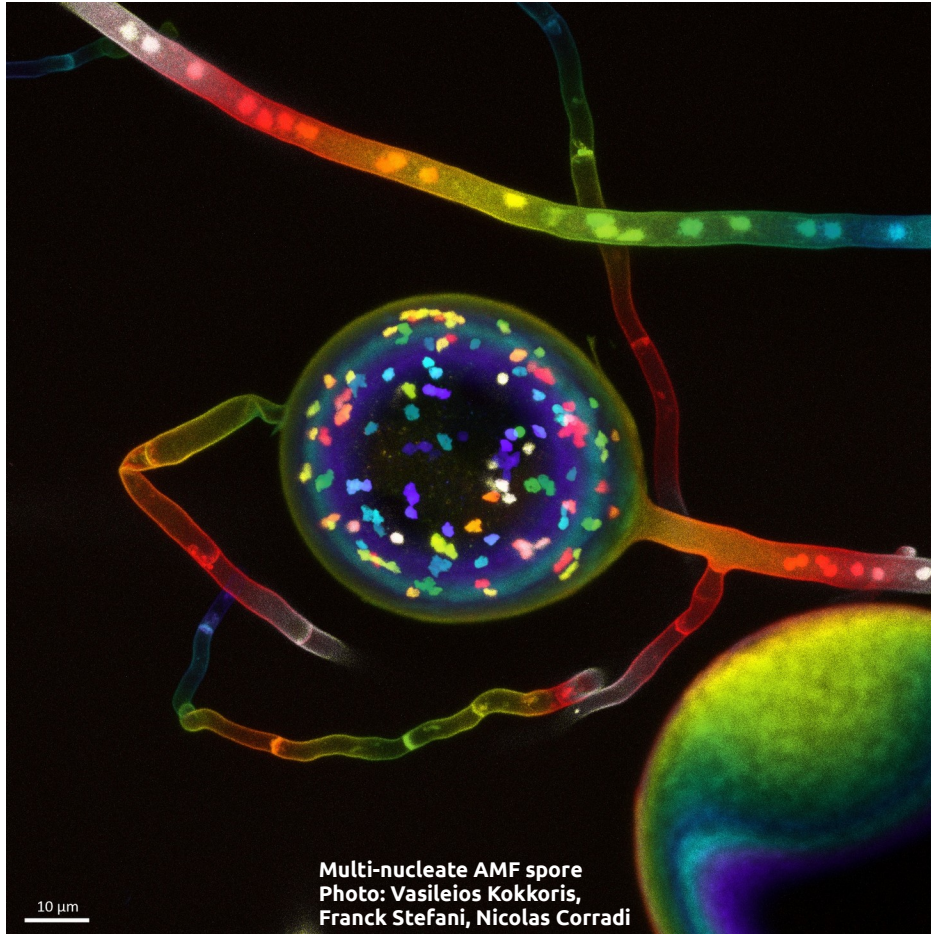# IMS Newsletter

The International Mycorrhiza Society quarterly e-newsletter

Multi-nucleate AMF spore
Photo: Vasileios Kokkoris,
Franck Stefani, Nicolas Corradi

10 µm

CONTENT:

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Evolution – Jason Hoeksema, University of Mississippi, US
Molecular biology – Jonathan Plett, Western Sydney University, Australia

Editorial

Marcel van der Heijden

César Marín

Francis Martin

We are very pleased to release the fourth Newsletter (Vol. 2, Issue 1) of the International Mycorrhiza Society (IMS). This is the second year birthday of the IMS Newsletter! Many thanks to all who contributed to this Newsletter, especially to César Marín, the Newsletter editor, and to the topic editors Justine Karst, Jason Hoeksema, and Jonathan Plett. Also, many thanks to a wide range of mycorrhizal researchers who have written nice highlights about their own ongoing mycorrhizal research, and/or to those that have appeared in our YouTube interviews. Please do contact us if you wish to being interviewed or to write a short article for an upcoming IMS Newsletter (cesar.marin@uoh.cl).

In this issue, we present our list of the Top 10 mycorrhizal papers for the last 4 months (September to December, 2020). This issue also contains four short research articles/commentaries providing new research insights on themes ranging from molecular ecology, plant invasion, to habitat restoration and signalling in the mycorrhizal symbiosis. We have also posted two YouTube interviews, one with Francis Martin on their latest article in *Nature Communications* reporting on the evolution of the mycorrhizal symbioses, and an interview with Nahuel Policelli on mycorrhizal invasions. The Tools section provides useful links to methods and databases for mycorrhizal research, and in the events section we highlight conferences and meetings linked to mycorrhizal research. Please do contact us if you have useful information that you wish to be mentioned in the "Tools" or "Events" sections.

We would like to congratulate Andrea Genre *et al.* (2020) for their nomination to the best mycorrhizal paper of the last 4 months. Their appealing article discusses recent phylogenomic, molecular, and cell biology studies, and presents the current state of knowledge of the origin of mycorrhizal fungi and the evolutionary history of their relationship with land plants (*Nat Rev Microbiol* 18: 649-660). Further highlights can be found in Sugiura *et al.* (2020), elucidating important steps to grow arbuscular mycorrhizal fungi without hosts plants on agar media (which was not possible until recently), and in Miyauchi *et al.* (2020) describing dozens of new mycorrhizal genomes and the evolutionary pathways leading to symbiosis in several fungal orders. See this newsletter for the complete list of the Top 10 papers. We are delighted to

see a diverse range of mycorrhizal research papers being published in top-tier scientific journals. We are also very happy to welcome four further members in the panel selecting the best mycorrhizal paper: Bala Chaudhary, Joseph Cooper, Annegret Kohler, and Junling Zhang.

This issue also contains four short research articles. One by Pierre-Marc Delaux from Toulouse University, France, who recently was awarded a prestigious European Research Council grant, and senior author of the best mycorrhizal paper of the May 2020 issue. Here, he discusses the origins and evolution of intracellular symbioses in plants. A second nice research article is written by Jan Clavel from Belgium on the role of arbuscular mycorrhizal fungi in non-native plant invasion along mountain roads. The third research article by Tanel Vahter from Estonia demonstrates very clearly and convincingly that the addition of native arbuscular mycorrhizal fungi together with plant seeds, can greatly enhance plant species richness in barren post-mining landscapes. The fourth article by Tania Ho-Plágaro and José Manuel García-Garrido dives into the molecular mechanisms explaining signalling between plants and arbuscular mycorrhizal fungi.

The world is still badly hit by the covid-19 pandemic and we wish you and your families all strength and perseverance, and sincerely hope vaccination in the mid-term will help to solve the pandemic. Please do register and schedule for the next meeting of the International Mycorrhiza Society (ICOM11), which is scheduled next year (2022) in Beijing, China.

Marcel G.A. van der Heijden, President
César Marín, Editor
Francis Martin, Vice & Past-President

Top 10 papers on mycorrhizal research*

1. Genre A, Lanfranco L, Perotto S, Bonfante P. 2020. Unique and common traits in mycorrhizal symbioses. *Nat Rev Microbiol* 18: 649-660.
https://doi.org/10.1038/s41579-020-0402-3

2. Sugiura Y, Akiyama R, Tanaka S, *et al*. 2020. Myristate can be used as a carbon and energy source for the asymbiotic growth of arbuscular mycorrhizal fungi. *Proc Natl Acad Sci USA* 117: 25779-25788. https://doi.org/10.1073/pnas.2006948117

3. Miyauchi S, Kiss E, Kuo A, *et al*. 2020. Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits. *Nat Commun* 11: 5125. https://doi.org/10.1038/s41467-020-18795-w

4. Prescott CE, Grayston SJ, Helmisaari HS, *et al*. 2020. Surplus carbon drives allocation and plant–soil interactions. *Trends Ecol Evol* 35: 1110-1118.
https://doi.org/10.1016/j.tree.2020.08.007

5. Pan S, Wang Y, Qiu Y, *et al*. 2020. Nitrogen-induced acidification, not N-nutrient, dominates suppressive N effects on arbuscular mycorrhizal fungi. *Glob Chang Biol* 26: 6568-6580. https://doi.org/10.1111/gcb.15311

6. Suetsugu K, Matsubayashi J, Tayasu I. 2020. Some mycoheterotrophic orchids depend on carbon from dead wood: novel evidence from a radiocarbon approach. *New Phytol* 227: 1519-1529. https://doi.org/10.1111/nph.16409

7. Wong-Bajracharya J, Castañeda-Gómez L, Plett KL, *et al*. 2020. Untangling the effect of roots and mutualistic ectomycorrhizal fungi on soil metabolite profiles under ambient and elevated carbon dioxide. *Soil Biol Biochem* 151: 108021.
https://doi.org/10.1016/j.soilbio.2020.108021

8. Wang T, Tian Z, Tunlid A, Persson P. 2020. Nitrogen acquisition from mineral-associated proteins by an ectomycorrhizal fungus. *New Phytol* 228: 697-711.
https://doi.org/10.1111/nph.16596

9. Plett JM, Plett KL, Wong-Bajracharya J, et al. 2020. Mycorrhizal effector PaMiSSP10b alters polyamine biosynthesis in *Eucalyptus* root cells and promotes root colonization. *New Phytol* 228: 712-727. https://doi.org/10.1111/nph.16759

10. Sánchez-García M, Ryberg M, Khan F, *et al*. K. 2020. Fruiting body form, not nutritional mode, is the major driver of diversification in mushroom-forming fungi. *Proc Natl Acad Sci USA* 117: 32528-32534. https://doi.org/10.1073/pnas.1922539117

*Selected from 154 Web of Science articles published between September – December, 2020 by: Justine Karst, Jonathan Plett, Melanie Jones, Marcel van der Heijden, Francis Martin, Jan Jansa, John Klironomos, Liang-Dong Guo, Miranda Hart, Jason Hoeksema, Judith Lundberg-Felten, Bala Chaudhary, Annegret Kohler, Joseph Cooper, Junling Zhang, and César Marín.

Research commentaries

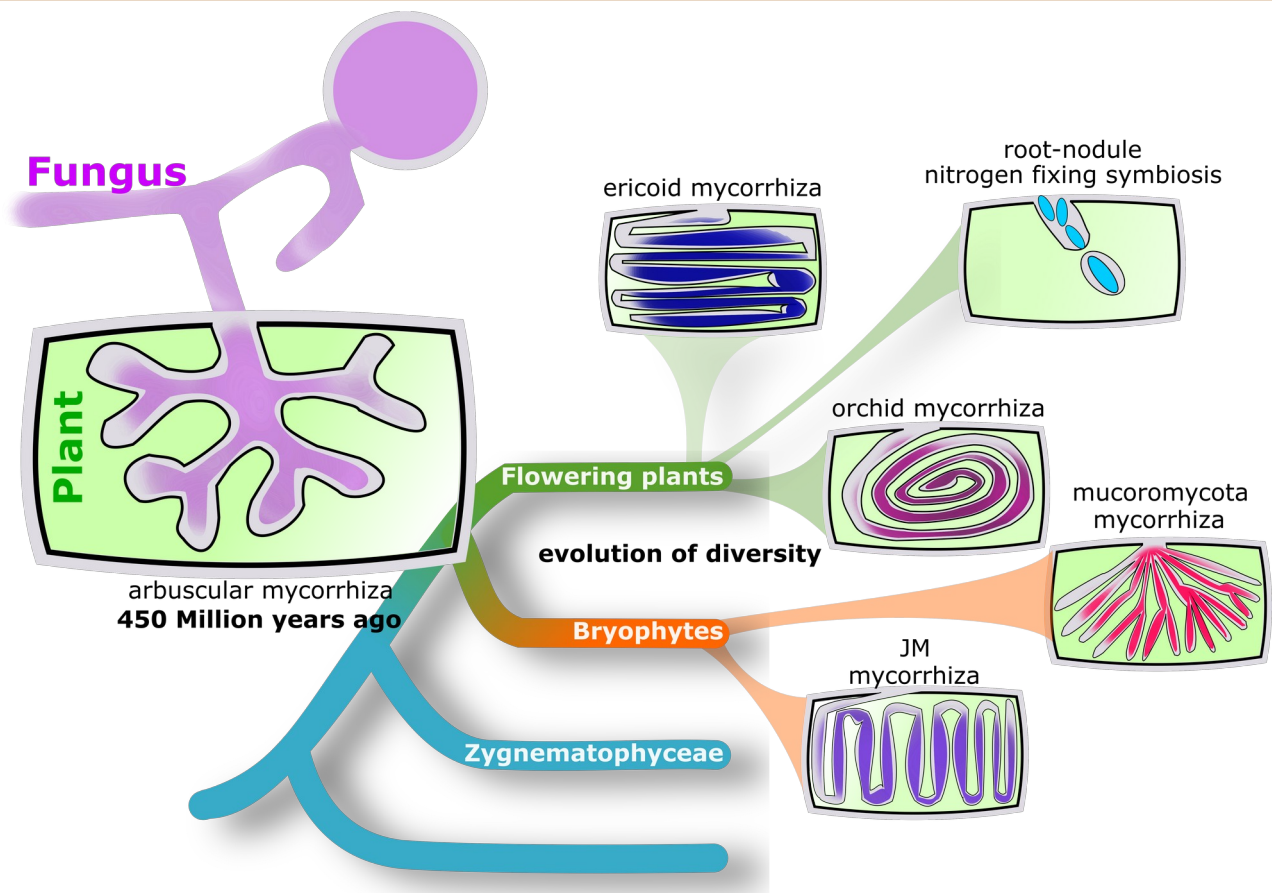## ORIGINS: origins and evolution of intracellular symbioses in plants

Pierre-Marc Delaux

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Over the last decade, the EU has been supporting the mycorrhizal scientific community through a number of funding schemes covering topics as broad as the description of a novel type of mycorrhizal symbiosis (ERC CoG *MYCOREV* to Katie Field), the dissection of arbuscular mycorrhiza (AM) formation (ERC StG *RECEIVE* to Caroline Gutjahr), the use of mycorrhizal fungi to improve plant defense against pests (ITN *MiRA*), or using mycorrhizal fungi as a phylogenetic framework to understand the evolution of organismal complexity (ERC StG *Multicellularity* to László Nagy), among others. In the latest round of ERC funding (ERC CoG 2019), my proposal *ORIGINS*, aiming at understand the evolution of mycorrhizal symbioses in plants, has been selected for funding. Building a competitive proposal is impossible without the knowledge background generated by years of intense work from a solid and diverse scientific community: you all! Therefore, it is my great pleasure to briefly present what we hope to achieve in the context of this project.

Mutualism between plants and microorganisms, such as the AM symbiosis, has been essential for the evolution of terrestrial ecosystems for millions of years. This symbiosis, by far the most widespread in land plants, results in the accommodation of the symbiotic fungus inside the plant cells. Following this initial symbiosis, multiple other intracellular symbioses have evolved in plants as diverse as orchids, Ericaceae, legumes, or the Jungermanniales, a group of Bryophytes. These symbioses provide numerous benefits, improving plant nutrient acquisition, defense against pathogens, and fitness. Despite their absolute importance in terrestrial ecosystems, the molecular mechanisms underlying the origin and subsequent evolution of intracellular symbioses in plants remain poorly understood.

The ORIGINS project will fill this knowledge gap by testing five main hypotheses. The first hypothesis, formulated originally by Pirozynski and Malloch (1975) is that the AM symbiosis evolved in the first land plants facilitating their colonization of emerged land. Testing this hypothesis will be facilitated by assessing the development of a model liverwort, *Marchantia paleacea*, which we initiated together with a talented PhD student (Guru Radhakrishnan), when we were both in Giles Oldroyd's lab, work further improved by Nicolas Vigneron (PhD student) and Mélanie Rich (Postdoc) after I started my lab in Toulouse in 2015.

Fungus

Plant

arbuscular mycorrhiza
450 Million years ago

ericoid mycorrhiza

root-nodule
nitrogen fixing symbiosis

orchid mycorrhiza

Flowering plants

evolution of diversity

mucoromycota
mycorrhiza

Bryophytes

JM
mycorrhiza

Zygnematophyceae

ORIGINS evolutionary model

The second hypothesis is a direct follow-up of the work we published with Jean-Michel Ané in 2015 (Delaux *et al.* 2015), in which using phylogenomics, we show that two genes central in symbiotic signalling (CCaMK/DMI3 and CYCLOPS/IPD3) are present in the Zygnematophyceae (a class of green algae sister to land plants). Although the genes are present in these species, they do not form any known symbiosis. We propose that these genes play a different, non-symbiotic function in algae, and that they got recruited to control symbiosis in the first land plants. Genetics and biochemistry in the Zygnematophyceae will allow us finally testing that!

The third hypothesis proposes that a single signaling pathway composed of five genes defines the blueprint for all intracellular mutualistic symbiosis in all land plants. The idea of a shared genetic

pathway across the diversity of plant symbioses is obviously reminiscent of the discovery, years ago, of the "common symbiosis pathway" regulating both the arbuscular mycorrhizal symbiosis and the nitrogen-fixing root nodule symbiosis. Martin Parniske's seminal review describing the arbuscular mycorrhizal symbiosis as "*the mother of plant endosymbioses*" (Parniske 2008) predicted the existence of such a pathway, although, this original idea was built on the diverse root-nodule nitrogen fixing symbioses that have been since reunified as a single entity (Griesmann *et al.* 2018; van Velzen *et al.* 2018). Using again phylogenomics, we have recently expanded this original hypothesis, formulated for a few dicot species and two symbioses, to all the main land plant clades and types of intracellular symbioses (Radhakrishnan *et al.* 2020), discovery that we were pleased to see in

the Top 10 papers on mycorrhizal research in the second issue (Vol. 1) of the IMS Newsletter! The symbiotic services provided by the diverse types of mycorrhizal symbioses cannot be explained by such a conserved pathway and must rely on independently evolved mechanisms to exchange nutrients. We can speculate (Hypothesis 4) that the functional specificity of each type of intracellular mutualistic symbiosis evolved *de novo*, and that this evolution is constrained by the link with the signalling module. In the ORIGINS project, we will move from comparative phylogenomics to actual genetic validations of these two hypotheses in five land plant clades. I must say that the Smith and Read book (Smith and Read 2008) and piles of papers from Paola Bonfante and her colleagues from Torino were extremely useful to define the most appropriate biological systems.

In the last section of the project, we will investigate why the evolution of intracellular symbioses is constrained to a unique genetic pathway. We hypothesize that this is due to an existing, undiscovered, additional genetic layer protecting this *entry* pathway from hijacking by pathogens. This is supported by the identification of undescribed genes following a symbiotic phylogenetic pattern. Dear reader, I hope that, here, you are really wondering what these candidate genes can be! We hope to have the definitive answer in a few years!

To sum up, through this project, combining phylogenomics, biochemistry, transcriptomics, and genetic validations in six plant lineages covering more than 500 million years of diversity, we will provide a comprehensive understanding of the molecular mechanisms underlying the evolution of intracellular mutualistic symbioses in plants. As a reciprocal reward to the community that created the context for this project, we hope to generate resources and knowledge for novel projects and discoveries to emerge.

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The role of arbuscular mycorrhizal fungi in non-native plant invasion along mountain roads

Jan Clavel

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Human disturbance is key in facilitating non-native plant invasions. We have seen across a wide diversity of ecosystems that non-native plants use more welcoming disturbed sites as their entry points towards new ecosystems. Roadsides and trails in particular, make for great pathways for these non-native plants, which use them to spread out to new ranges that would have otherwise remained unattainable (Lembrechts *et al.* 2016). However, the driving factors behind this impact of human disturbance on invasion success remain complex, and need to be further disentangled if we hope to better understand and predict the spread of non-native plants.

Amongst the already well-studied drivers of this disturbance effect, are altered plant competition, changes in nutrient availability, and increases in propagule and colonization pressures, which are all known to facilitate the invasion success of non-native plants. Another factor which has yet to be looked at closely is the potential role of mycorrhizal associations. With our increasing understanding of the importance of mycorrhizal interactions in shaping plant communities, the focus on their role in plant invasions has also steadily been on the rise (Dickie *et al.* 2017). Mycorrhizal fungi have been found to play substantial roles in shaping plant invasions across many systems, however the nature of that role is highly variable (Pringle *et al.* 2009). Both mycorrhizal limitation and facilitation of non-native success have been observed, and similarly, the impact of non-native plant species on the native mycorrhizal fungal community has also shown varied results going from stimulation, through no observable effect, to disruption of mutualism. However, less is known about the effect of disturbance on mycorrhizas in the context of invasions, as most studies have focused on the effect of physical disturbance (i.e. tillage) in agricultural ecosystems. We believe that investigating the impact of disturbance on mycorrhizal relationships in more natural systems could be an important step in disentangling the drivers of plant invasions.

As part of the Mountain Invasion Research Network – MIREN (https://www.mountaininvasions.org/) network (Dietz *et al.* 2006), we have since 2012 been studying the spread of non-native plant species along mountain roads in the mountains of the Northern Scandes (Norway). Our previous surveys have shown that non-native species in the region are currently in the process of extending their upward ranges along these mountain roads (Lembrechts *et al.* 2014). Furthermore, the non-native species we observed are typically associated with arbuscular mycorrhizal fungi (AMF) whereas the local flora is

dominated by species associated with ecto- and ericoid-mycorrhizal fungi. This allowed us to focus our sampling efforts towards AMF, as we measured both their abundance and diversity across our study system. We did so with the goal of testing three hypotheses: 1) there is a positive correlation between road disturbance and AMF abundance and diversity; we expected the roadside conditions (Müllerová *et al.* 2011) to be more favorable to AMF than the natural vegetation; 2) AMF abundance and diversity diminish towards higher elevations, which might limit the upward expansion of non-native plant species; as we have observed that non-native plant species have not yet reached higher elevations, we supposed a lack of adequate mycorrhizal fungi might play a role in limiting upward spread; 3) the presence of non-native plant species along disturbed roadsides correlates with increased presence of AMF in their surrounding roadside environment. We expected that non-native plant species could lead, independently of the direct disturbance effect, to a further increase in AMF colonisation in their surrounding vegetation.

Following a standardized plot design used by the MIREN group (Seipel *et al.* 2012), we took two categories of root samples along the mountain roads both in disturbed and undisturbed vegetation. We first sampled the background vegetation, i.e. a random selection of roots found near and away from the roads. Whenever possible we also took root samples of three focal species which are the most common non-native species observed in the region (*Achillea millefolium* L., *Trifolium repens* L., and *Trifolium pratense* L.). This was repeated for a total of 20 separate sites along three separate mountain roads. We then measured AMF root colonisation and community composition for all background and focal plant samples, respectively through microscope counting and DNA-based barcoding.

Our first and clearest result was a large disparity in AMF traits between the disturbed and undisturbed vegetation samples. AMF root length colonisation was higher across the board in samples taken along the roadsides, as was the diversity of AMF operational taxonomic units (OTUs) (Fig. 1). This result was also

Figure 1. Effects of road disturbance on AMF distribution along roadsides. **a.** Background AMF colonisation in disturbed and undisturbed vegetation. **b.** Venn-diagram of AMF OTU overlap between disturbed (brown) and undisturbed (green) vegetation background communities. **c.** Background AMF colonisation in the disturbed vegetation for plots with or without presence of non-native plant species.
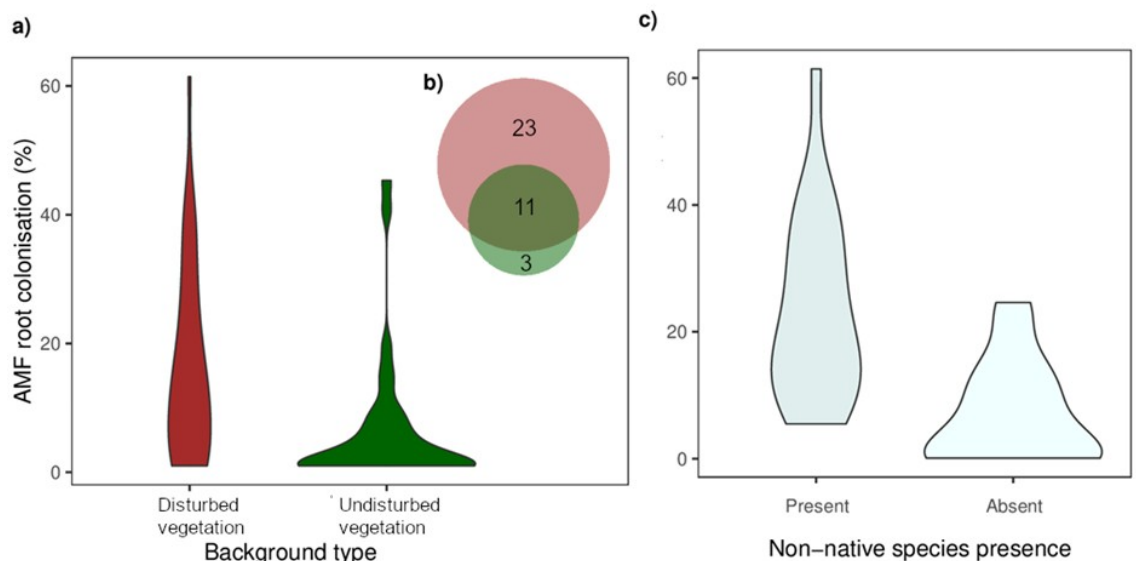
consistent across sites with and without presence of non-native species along the roadside, which suggests that the observed correlation is linked to the characteristics of the disturbed environment and not to a secondary effect of non-native species influencing their surrounding fungal community. This result was in line with our first hypothesis, and was likely a consequence of the large changes, especially in pH and nutrient availability (Müllerová *et al.* 2011), caused by disturbance making the roadside a more welcoming environment for AMF and AMF-associated species (Soudzilovskaia *et al.* 2015).

Conversely, we found no clear correlation between elevation and AMF colonisation (Fig. 2). There were also no major changes in AMF community composition, as all of the most common OTUs we observed were present across our whole elevation gradient (spawning from sea level to approximately 1000 m.a.s.l.). This disproves our second

hypothesis, meaning that the current elevation range limitation we observe in the region's non-native species is caused by other factors than a lack of adequate mycorrhizal availability, for example colder temperatures leading to reduced winter survival (Haider *et al.* 2011). Interestingly, we did observe some rarer AMF OTUs whose maximum elevation range coincided with that of the non-native species, suggesting that these species could possibly be bringing along new strains of mycorrhizas.

Finally, we did observe a pattern of overall higher AMF colonisation rates in disturbed vegetation plots when non-native plant species were present, which aligned with our third hypothesis. However due to the strictly observational nature of our study, it is difficult to disentangle to which degree non-native species presence is driving the increased colonisation rate or vice-versa (Zobel and Öpik 2014). Our current results seem to suggest that the former, plant-driven increases in background
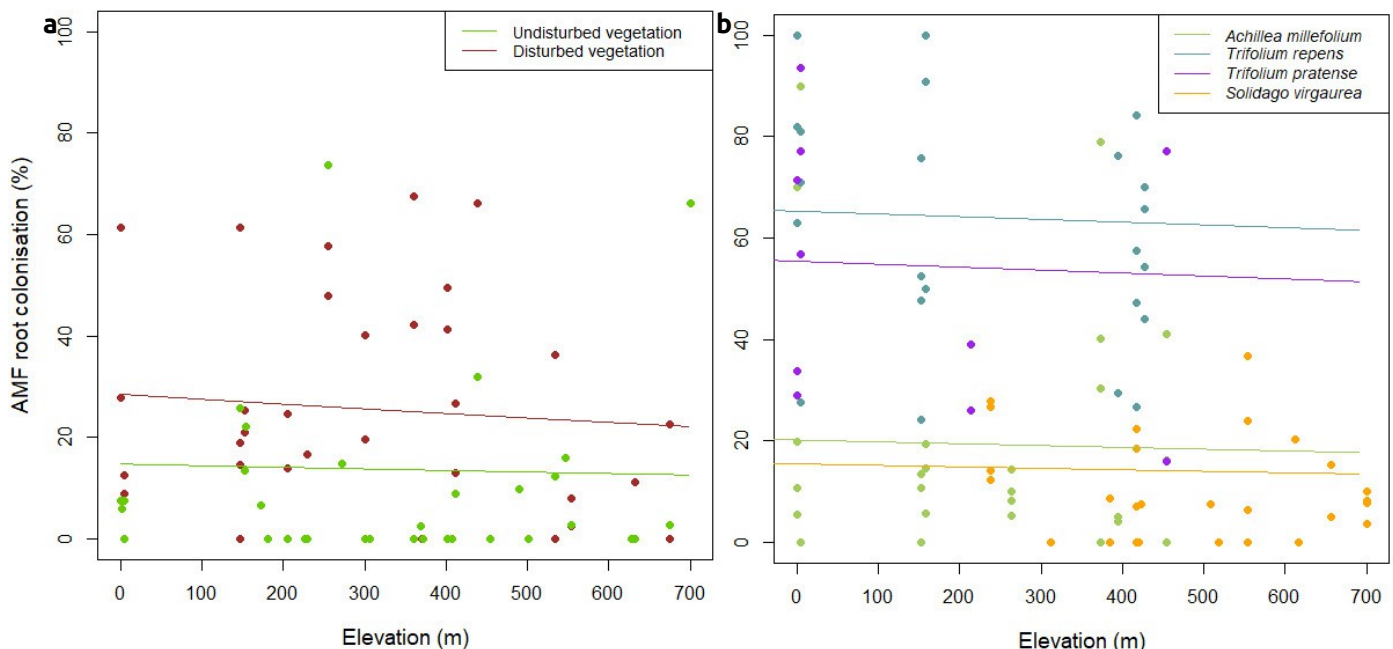
Figure 2. Elevation effect on the percentage of AMF root colonisation across the 700 m elevation gradient for background samples (**a**) including disturbed (brown) and undisturbed (green) vegetation background, as well as for four focal plant species (**b**): two obligatorily mycorrhizal non-native plant species (*Trifolium repens*, blue, and *Trifolium pratense*, purple), one facultative mycorrhizal non-native plant species (*Achillea millefolium*, green), and one facultative mycorrhizal native species (*Solidago virgaurea*, orange).

AMF colonisation, is more likely but that remains to be confirmed by further research.

Overall, our results align with a possible facilitating role of mycorrhizal fungi on the establishment success of non-native plants through disturbance, since AMF abundance was elevated along the disturbed roadsides, and increased AMF abundance correlated with high abundance of non-native plants within these roadsides. We believe these results represent an important first step in understanding the combined effects of disturbance and mycorrhizal interactions on non-native plant species invasions, and could help in the future to better predict patterns of invasion.

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Inoculation with native arbuscular mycorrhizal fungi improves vegetation restoration success in barren landscapes

Tanel Vahter

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The disparity between habitat conversion and conservation is 8:1 in temperate grasslands (Hoekstra *et al.* 2005). While conservation and restoration of indigenous grasslands should remain a priority, it is also clear that this alone will be insufficient in maintaining grassland-associated biodiversity, thus alternative habitats should be considered simultaneously (Kasari *et al.* 2016).

As post-mining landscapes are generally considered unattractive for developing alternative uses, the establishment of diverse vegetation assemblages could be seen as a positive alternative by many stakeholders. With up to 800,000 km² (that's roughly the size of the Scandinavian peninsula) impacted by mining activity globally (Cherlet *et al.* 2018), post-mining areas could represent an important habitat addition to offset grassland loss both locally and globally.

However, creation of grassland plant communities in post-mining landscapes has proven to be very difficult, with conventional restoration techniques often yielding low plant diversity (Tischew and Kirmer 2007). Focusing on plants but neglecting plant-associated microbiomes has been identified as a key factor explaining restoration failure (Koziol and Bever 2017).

Potential benefits of inoculation with diverse communities of native arbuscular mycorrhizal (AM) fungi have been proposed in a number of impressive grassland restoration studies (e.g Koziol *et al.* 2018; Middleton and Bever 2012; Wubs *et al.* 2016). However, mine restoration experiments have most often employed a narrow (one could also say commercial) selection of AM fungi, and examples of using native AM fungal communities alongside their native host plants were lacking.

Our research aimed to assess whether the introduction of native soil biota and plants, in tandem, is an efficient means of initializing vegetation recovery in barren post-mining landscapes (Vahter *et al.* 2020). To do this, we established a fully factorial experiment in three post-mining areas in Estonia, where we seeded plots with native grassland plants (14 species), and inoculated them with trap-cultured native soil biota from a similar habitat.

We measured the composition of plant and AM fungal communities in two consecutive years, hypothesizing that the addition of AM fungal propagules and plant seeds would increase the richness of both groups independently, but that a synergistic effect would be observed in co-introduction. We also expected that the inoculation would steer the plant community in a distinct direction, as previously observed by Wubs *et al.* (2016).

Our results demonstrate that the addition of either soil AM fungal inoculum or plant seeds increased plant richness independently, although the
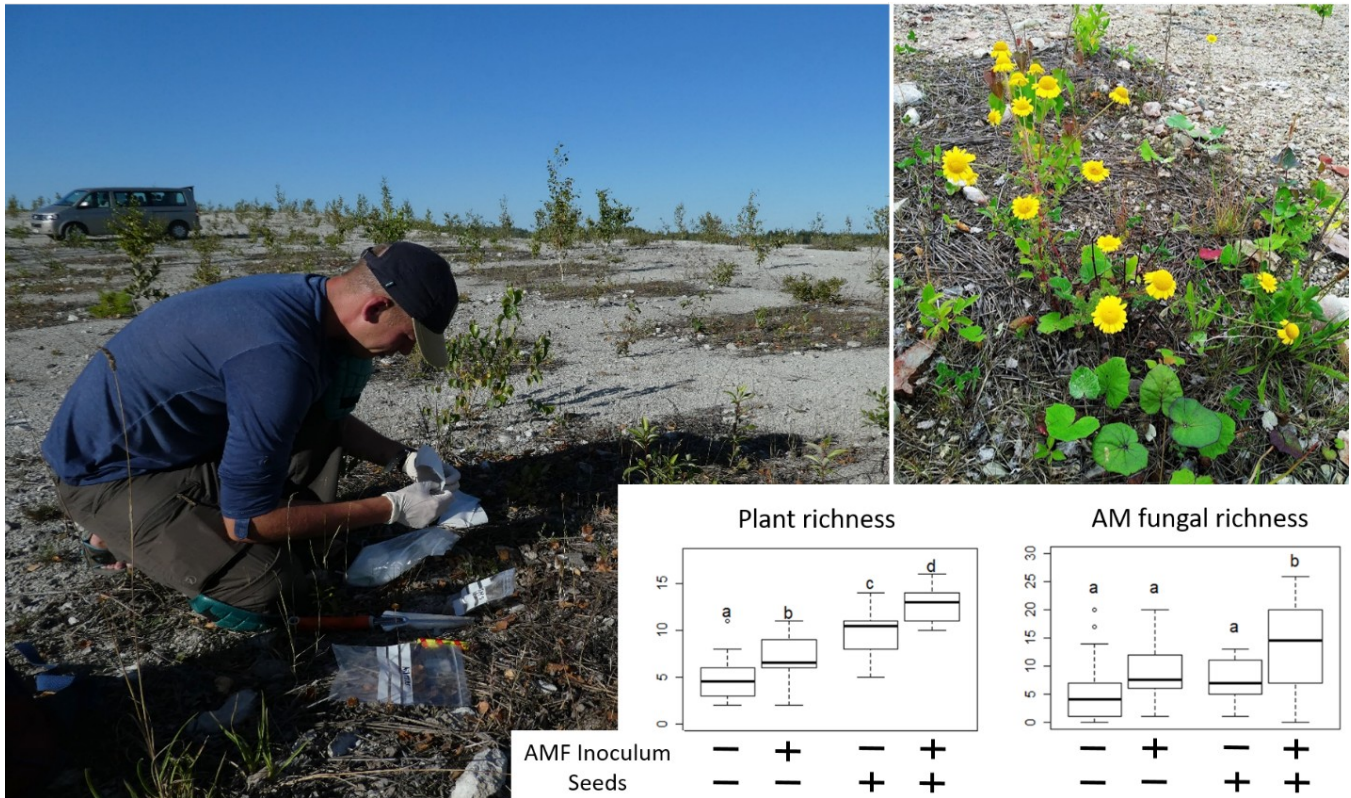
Plant and AM fungal species richness at restoration sites depends on the presence of both symbiotic partners

effect of adding seeds is well above inoculation alone. Our expectations of co-introductions being the most successful were also met, with the addition of plant seeds together with soil AM fungi resulting in the highest plant and AM fungal richness of all treatments. However, plant communities were structured mostly by the addition of seeds and not by the inoculations as we expected. We also saw that the benefit of inoculations and seed additions is rather additive than synergistic, implying that there were some propagules of both plants and fungi available in the sites but their proliferation was limited by the abundance of their partner.

While plant and AM fungal communities responded somewhat differently to the treatments, linkage in the responses of the partner groups was apparent in the plant community mycorrhization index (Moora 2014; Gerz *et al.* 2016): mycorrhizal plants were more abundant

in the inoculated and seeded plots. These results strengthen existing evidence that AM fungi are important for the development of plant communities, and that plant and fungal communities can co-vary (Neuenkamp *et al.* 2018), given that there is a source of propagules for both.

In two vegetation periods, the combination of soil microbiota and plant diaspores increased plant richness three-fold when compared to sterile substrate in the controls. Notably, even years after mining activity had ceased, the areas surrounding experimental plots had developed minimal herbaceous vegetation. Thus, single introductions of soil biota can have profound positive effects on the development of below- and aboveground diversity, with these effects increasing over time. Something to consider at the starting line of United Nations Decade on Ecosystem Restoration.

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The α,β-hydrolase KAI2/D14/DLK2 protein family in the arbuscular mycorrhizal symbiosis

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Strigolactones (SLs) and karrikins (KARs), two molecules bearing essential butenolide moieties, play important biochemical and physiological roles in plants. SLs, a class of carotenoid-derived terpenoid lactones, were first identified as germination stimulants for root parasitic plants (Cook *et al.* 1966), and later were characterized as shoot branching inhibitors (Gomez-Roldan *et al.* 2008), and as root-derived symbiotic signals for arbuscular mycorrhizal (AM) fungi (Akiyama *et al.* 2005). By contrast, KARs are abiotic and exogenous molecules of more limited occurrence derived from partial plant combustion that are reported to promote seed germination and seedling establishment (Nelson *et al.* 2010).

In the past decade, there has been a number of critical discoveries concerning the SLs/KARs perception and their functions in plants. Specialization among the members of the α,β-hydrolase protein family discriminates plant responses to KARs and SLs. DWARF14-fold hydrolase (D14) acts as a SL receptor, while a D14 paralog, known as D14-LIKE (D14L) or KARRIKIN INSENSITIVE2 (KAI2), is required for responses to KARs (Hamiaux *et al.* 2012; Guo *et al.* 2013). Recent discoveries have also begun to elucidate distinct roles for Strigolactone-related components in the AM symbiosis. It has become evident that SLs are involved in controlling pre-symbiotic events in AM formation (Akiyama *et al.* 2005), and mutants or antisense lines impaired in SL biosynthesis, or transport exhibit reduced mycorrhization (Koltai *et al.* 2010; Kretzschmar *et al.* 2012). Interestingly, a rice KAR receptor (OsD14L) is indispensable for AM formation during pre-symbiotic signalling (Gutjahr *et al.* 2015). Then, it is clear that the SL and karrikin receptors, D14 and KAI2 (D14L), respectively, are in a certain way related to the regulation of the AM symbiosis.

In a recent work, we investigated the role of the tomato *DLK2* gene (*SlDLK2*) in AM formation (Ho-Plágaro *et al.* 2021). DLK2 proteins constitute a third clade from the DWARF14 family of ß-hydrolases. They are closely related to the SLs receptor D14, and *DLK2* gene expression has consistently been used as a marker for SL and KAR signalling. Our research constitutes the first report for functional validation of DLK2 in AM symbiosis, and our results pointed out a novel role for the ß-hydrolases during arbuscule development. Expression analysis of *SlDLK2* during AM symbiosis shows that *SlDLK2* is induced after prolonged co-cultivation, suggesting a role for SlDLK2 during the later stages of the AM symbiosis. This is a novel and exciting finding as, to date, a role for D14L and SL signalling has only been shown during early AM formation stages. This suggests that ß-hydrolases also play a role during the later stages, to sustain the AM symbiosis function or maintenance. Consistently, *SlDLK2*
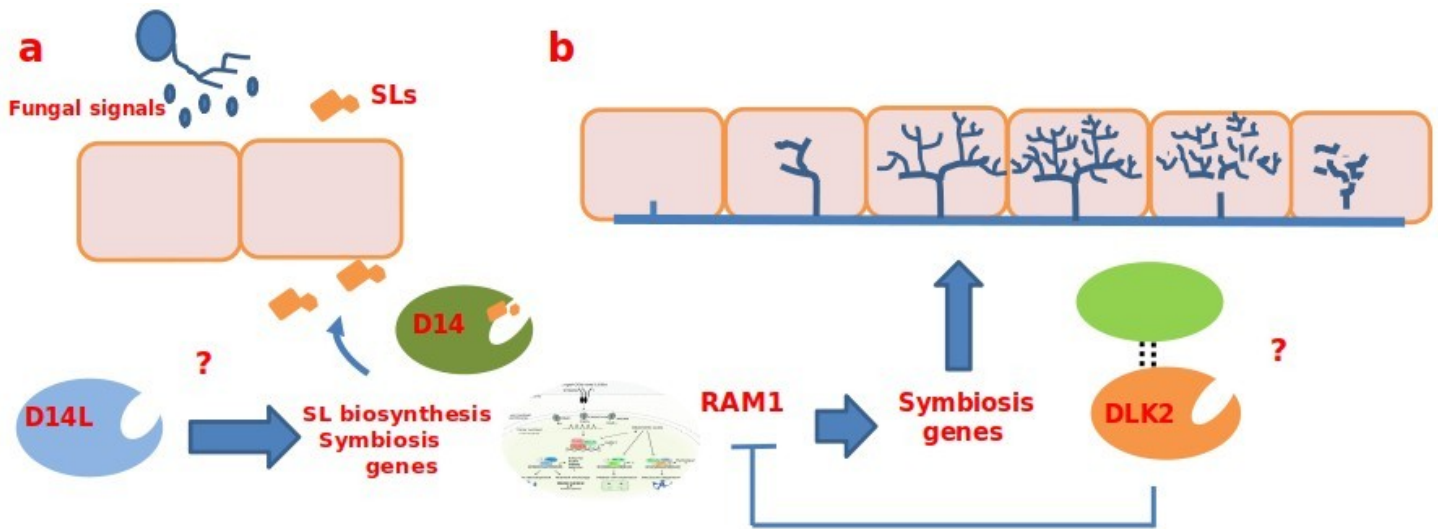
Integrative model summarizing the role of SL/KAR receptor α/β hydrolases during the AM symbiosis. Pre-symbiotic signalling **(a)** activates the D14L signalling pathway enabling AM symbiosis and SL production. Although colonization is independent of the SLs receptor D14, the production of SLs enhance successful mycorrhization on the host and is required for the punctual entry of AM fungi. A heterocomplex regulatory element with DELLA protein regulates the expression of RAM1, which enables arbuscule development and functioning **(b)**. Transcription of AM-related genes is activated in developing and mature arbuscules, including DLK2 that binds an unidentified DLK2 ligand and regulates arbuscule development, probably due to its capacity to bind DELLA and to its negative effect on RAM1.

promoter-GUS activity is associated with cells containing arbuscules.

These findings prompted us to investigate the function of *SlDLK2*. Genetic approaches were conducted to analyse *SlDLK2* expression and to understand *SlDLK2* function in the AM symbiosis. We tested the effect of *SlDLK2* silencing (RNAi) and overexpression (OE) in mycorrhizal tomato roots. *SlDLK2* RNAi roots showed a significant increase in all the mycorrhizal parameters examined, while the opposite trend was observed for the *SlDLK2* OE in roots. Interestingly, *SlDLK2* negatively regulates arbuscule morphology and *SlDLK2* ectopic expression arrests arbuscule branching. The altered AM phenotype in *SlDLK2* OE in roots resembles, although in a much less severe form, all the morphological features observed in the phenotype of the *Medicago* and *Lotus ram1* mutants, as well as that of the petunia *ata* mutants (Pimprikar *et al.* 2016; Luginbuehl *et al.* 2017).

These data suggest that SlDLK2 could have a role regulating AM development. In this scenario, SlDLK2 would act as a repressor of arbuscule branching, which signals through binding to an endogenous plant (or fungal) ligand with butenolide moieties generated during the AM symbiosis. To test this hypothesis, we used two experimental approaches. First, we determined whether SlDLK2 physically interacts with DELLA, which in turn regulates the transcription of RAM1, the GRAS protein required for arbuscule branching and for the induction of AM marker genes (Pimprikar *et al.* 2016), and then we monitored transcriptional changes directed by SlDLK2 overexpression in roots using RNA sequencing.

Co-immunoprecipitation (coIP) and luciferase assays, in combination with transient expression in *Nicotiana benthamiana* leaves, revealed a physical interaction between SlDLK2 and SlGAI1 (tomato DELLA), suggesting that SlDLK2 interacts with DELLA. We also pointed

out that about 42% of the genes that were found to be repressed by *SlDLK2* OE in non-mycorrhizal roots corresponded to genes upregulated in roots during mycorrhization, including AM-marker genes involved along several stages of arbuscule life cycle, such as the regulator RAM1. This result is in line with the remarkable alterations that we found in arbuscular morphology in *SlDLK2* OE plants.

Altogether, our data suggest that we have found a new component involved in the complex plant-mediated signalling mechanism that regulates the life cycle of arbuscules, and we proposed a model in which the function of DLK2 protein is based on its capacity to bind DELLA, and on its direct negative effect on mycorrhization genes such as *RAM1*. Although the possible signalling ligand that binds DLK2 is completely unknown, we speculate that as D14 binds the apocarotenoid molecule strigolactone, DLK2 might bind another apocarotenoid-type compound derived from the methylerythritol phosphate (MEP) pathway, which is responsible of apocarotenoid synthesis and is highly activated in mycorrhizal roots (Walter *et al.* 2000).

Future challenging tasks will be to determine the biochemical parameters and the analysis of the structure-function correlation and evolutionary relevance of SlDLK2, including the search for their natural ligand. Coming experiments will be also aimed at unveiling the meaning of the DELLA/SlDLK2 interaction and the putative SlDLK2/RAM1 relationship on the complex mechanism of arbuscule life cycle regulation.

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YouTube interviews*

- Francis Martin on a large-scale genome sequencing of mycorrhizal fungi and its evolution

César Marín interviews Francis M. Martin PhD, Distinguished Research Director at INRAE, France, and Chief Scientist at the Beijing Forestry University, China, about a large-scale genome sequencing of mycorrhizal (and saprophytic) fungi, its genomics, transcriptomics, and evolution.

Interview: https://southmycorrhizas.org/reading/november-2020/
Study: Miyauchi S, Kiss E, Kuo A, *et al*. 2020. Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits. *Nat Commun* 11: 5125. https://doi.org/10.1038/s41467-020-18795-w

- Nahuel Policelli on mycorrhizal invasions

Camille Truong interviews Nahuel Policelli, Postdoctoral associate at Boston University, who will tell us about the role of ectomycorrhizal fungi for pine invasions in the Southern hemisphere and potential applications in forest restoration.

Interview: https://southmycorrhizas.org/reading/january-2021/
Study: Policelli N, Horton TR, García RA, *et al*. 2020. Native and non-native trees can find compatible mycorrhizal partners in each other's dominated areas. *Plant Soil* 454: 285-297. https://doi.org/10.1007/s11104-020-04609-x

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MYCORRHIZAL
RESEARCH NETWORK

Tools

→ *FungalTraits*: a database for functional assignments of fungi

Põlme *et al*. (2020) present FungalTraits, a database created to "facilitate functional assignments and ecological interpretation of environmental studies", which operates at the genus and species hypothesis levels. This database captures the effort of a global mycological expert community, whom manually categorized and assigned trait information to 697,413 fungal ITS sequences,

Study: Põlme S, Abarenkov K, Henrik Nilsson R, *et al*. 2020. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Divers* 105: 1-16. https://doi.org/10.1007/s13225-020-00466-2

→ A new method to separate arbuscular mycorrhizal fungal (AMF) spores

Srisom *et al*. (2020) developed a microfluidic device which helps in the manual separation of AMF spores from a filtered and centrifuged suspension. The device uses a manual temporary flow diversion (MTFD) mechanism to select and stream single spores.

Study: Srisom K, Tittabutr P, Teaumroong N, *et al*. 2020. New method for arbuscular mycorrhizal fungus spore separation using a microfluidic device based on manual temporary flow diversion. *Mycorrhiza* 30: 789-796. https://doi.org/10.1007/s00572-020-00986-4

→ Methodological guide for the ericoid mycorrhizal symbiosis

Vohník (2020) presents a thorough compilation of methods regarding root sampling and handling, microscopic and photographic observations, root mycobiont isolation and maintenance of the obtained isolates, isolate identification, and resynthesis experiments of a less explored mycorrhizal type, the ericoids.

Study: Vohník M. 2020. Ericoid mycorrhizal symbiosis: theoretical background and methods for its comprehensive investigation. *Mycorrhiza* 30: 671-695. https://doi.org/10.1007/s00572-020-00989-1

→ *gDAT*: a bioinformatic pipeline to analyze arbuscular mycorrhizal fungal communities based on molecular data

Vasar *et al*. (2021) develop the bioinformatics tool gDAT (graphical downstream analysis tool), which uses different DNA sequencing marker regions (SSU, ITS, LSU) for the community analyses of microbial biota.

Study: Vasar M, Davison J, Neuenkamp L, *et al*. 2021. User-friendly bioinformatics pipeline gDAT (graphical downstream analysis tool) for analysing rDNA sequences. *Mol Ecol Resour* Early view. https://doi.org/10.1111/1755-0998.13340

Events

EVENTS POSTPONED DUE TO COVID-19:

- Global Symposium on Soil Biodiversity
Website
Online
19 – 22 April, 2021

Organizers: UN Food and Agriculture Organization (FAO), Global Soil Partnership (GSP), Intergovernmental Technical Panel on Soils (ITPS), UN Convention on Biological Diversity (UNCBD), Global Soil Biodiversity Initiative (GSBI).

- Soil Ecology Society Biennial Meeting 2021
Website
Online
27 May, 2021

Organizers: Soil Ecology Society (US) and local organizers.

- Eurosoil 2020 (now 2021)
Website
Online
23 – 27 August, 2021

Organizers: European Confederation of Soil Science Societies and local organizers.

- 18th International Symposium on Microbial Ecology
Website
Cape Town, South Africa
August 2021

Organizers: International Society for Microbial Ecology

- 45th *New Phytologist* Symposium: Ecological and evolutionary consequences of plant–fungal invasions
Website
Campinas, Brazil
Date not yet announced

Organizers: New Phytologist Trust and symposium organizers.

3ʳᵈ Global Soil Biodiversity Conference
Website
Clayton Hotel, Dublin, Ireland
March 2023

Organizers: Global   Soil   Biodiversity
Initiative

EVENTS AS SCHEDULED:
10ᵗʰ International Symbiosis Society Congress
Website
Lyon, France
18 – 23 July 2021

Organizers: International   Symbiosis
Society

MYCORRHIZAL EVENTS:

Session (SSS4.7) at EGU2021 (European Geosciences Union):
The role of fungi for soil functions, and soil biodiversity in land use systems
Website
Online
19 – 30 April, 2021

Convener: Edith Hammer | Co-conveners: Dimitrios Floudas, Jan Jansa, Elly Morriën, Erik Verbruggen

ICOM11
Website
China National Convention Center, Beijing, China
31 July – 5 August, 2022

Organizers: Chinese Society of Mycology and IMS

III International Symposium on Mycorrhizal Symbiosis in South America
Website
Instituto SINCHI, Leticia, Colombia
24 August – 2 September, 2023

Organizers: South American Mycorrhizal Research Network and Instituto SINCHI