

**Microbial processes in mycorrhizosphere of plants growing at
a former uranium mining site**

Dissertation

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Abbreviations

ACC – aminocyclopropane-1-carboxylate
AM(F) – arbuscular mycorrhiza(l) (fungi)
AMD – acid mine drainage
ASV – amplicon sequence variant
B – birch
BS – bulk soil
CCA – canonical correspondence analysis
ECM – ectomycorrhiza(l)
ERM – ericoid mycorrhiza
H_{BP} – Berger-Parker index
H_{GS} – Gini-Simpson index
H_{SD} – Simpson dominance index
H_{SH} – Shannon diversity index
IAA – indol-3-acetic acid
ITS – internal transcribed spacer
MHB – mycorrhiza helper bacteria
MR – mycorrhizosphere
NMDS – non-metric multidimensional scaling
O – oak
OTU – operational taxonomic unit
P – pine
PCA – principal component analysis
PGPB – plant growth-promoting bacteria
S – richness
SIMPER – similarity percentage analysis
SUB – control pot substrate
TC – the content of total carbon
TN – the content of total nitrogen
TP – the content of total phosphorus
WSA – water stable aggregates

Summary

Mining activity results in a severe disturbance of the entire ecosystem and initiates primary succession on large areas. Remediation of post-mining sites is usually aimed at reforestation as forests provide sustainability of the whole ecosystem over a long period of time. Post-mining areas represent unique environments where pioneer trees, in order to adapt to harsh abiotic conditions, must establish beneficial interactions with soil microorganisms. Although the composition of soil microbial communities and the potential of different plants to colonize mineral nutrient-poor substrates at primary succession have been in the scope of many studies, the results of these studies are mainly dissociated. Moreover, little is known regarding microbial community assembly in mycorrhizospheres of pioneer plants. Therefore, understanding the patterns of microbial community structure and trajectories of bacterial and fungal community assembly in the mycorrhizosphere during the primary succession might considerably contribute to the phytoremediation of post-mining areas.

A former uranium mining area near Ronneburg (Germany) is an example of a primary succession initiated after the drastic land surface disturbance. Topological differences within the area, enhanced by the different rate of natural chance colonization by the plants from neighbouring forest stands, resulted in the establishment of sites with different levels of successional development. The main objective of this study was to elucidate patterns of bacterial and fungal communities' assembly in mycorrhizospheres during the primary succession at a post-mining area. The role of plant presence and plant identity in the shaping of associated microbial communities was assessed by comparing the microbial community compositions in the mycorrhizosphere and the corresponding bulk soil not affected by plant roots. The present study attempted to separate the impact of soil chemical parameters from the plant influence as well. The experimental framework consisted of two parts: 1) the study of field sites of, presumably, the same age but at different levels of development, and 2) a pot experiment, which modelled an initial stage of succession with similar abiotic conditions for all trees. The structure of bacterial and fungal communities inhabiting the mycorrhizosphere of birches (*Betula* sp.), oaks (*Quercus* sp.), and pines (*Pinus* sp.) was characterized using next-generation sequencing. Overall, among the most abundant bacterial classes were Acidobacteriia, Alphaproteobacteria, Ktedonobacteria, Bacteroidia. All together, they contributed to about 50 – 60% of all bacterial sequences. The fungal community was represented mainly by Thelephoraceae, Inocybaceae, Russulaceae, which contributed to 50 – 80% of overall abundance.

The results demonstrated that the composition of bacterial and fungal communities was driven by different factors. Bacterial communities revealed close associations with soil chemical parameters, namely the content of toxic metals and content of total nitrogen as well as C/N ratio, whereas the site identity predominantly determined fungal communities, and their distribution patterns correlated with the stage of vegetation succession. Furthermore, the content of bioavailable aluminium affected the structure of both bacterial and fungal communities. Although soil pH value had a minor direct effect on the structure of microbial communities, its indirect effect through the change of mobility of several toxic metals and, consequently, the increase of their bioavailability was assumed.

At the initial stages of succession modelled in the pot experiment, plants were associated with microbial communities different from unvegetated control pot substrate's microbial community. At the same time, the rhizosphere effect was not species-specific, and different tree species established mycorrhizospheres with a similar structure. Surprisingly, the rhizosphere effect was not pronounced at the field, and no significant differences between mycorrhizosphere and bulk soil were observed.

For the first time, this study demonstrated the capability of pioneer plants to promote the complexity of microbial interactions in the mycorrhizosphere during the succession. Development of the ecosystem and establishment of sites with contrasting conditions resulted in the marked division of taxa into generalists and specialists. The particular importance of bacteria with plant growth-promoting features in the interactions within the mycorrhizosphere was shown. Moreover, ectomycorrhizal fungi (ECM) *Inocybe* and *Lactarius* played an important role at the local scale of network communication and could be considered as potential cores in mycorrhizosphere interaction.

ECM fungi can considerably facilitate colonization of mineral substrate by the pioneer host plants through the development and/or differentiation of extramatrical mycelium. Morphotyping revealed that the ascomycete *Meliniomyces bicolor* was the only species common for all field trees as well as among the most representative ones. The present study demonstrated that exploration strategies of field ECM fungi reflected the differences in environmental conditions determined by the level of ecosystem development. Furthermore, changes in abiotic conditions in the pot experiment resulted in the formation of rhizomorphs by ECM fungi associated with pot oaks and pines, indicating the high capacity of the ECM community to respond to disturbances.

The high survival rate of birches and pines in the pot experiment coupled with network analysis demonstrated their high potential to colonize new substrate and adapt to new conditions as well as their capability to establish and support communication with beneficial bacteria and fungi. Additional experiment related to inoculation of plants with a commercial ECM fungal blend promoted the survival and growth of oaks and pines.

This work evaluates the potential of three trees for phytoremediation of post-mining areas and their capability to facilitate the development of disturbed ecosystems during the primary succession. The role of the trees in the shaping of associated microbial communities and the capacity to establish, promote or support associations of beneficial microorganisms contributes to the predicting and understanding of ecosystem succession.

Zusammenfassung

Die Bergbautätigkeit führt zu einer drastischen Störung des gesamten Ökosystems und initiiert die Primärsukzession auf großen Flächen. Die Sanierung von Bergbaufolgestandorten zielt normalerweise auf die Wiederaufforstung des Gebiets ab, da Wälder die nachhaltige Nutzung des gesamten Ökosystems über einen langen Zeitraum gewährleisten. Bergbaufolgelandschaften stellen einzigartige Umgebungen dar, in denen Pionierpflanzen, um sich an harte abiotische Bedingungen anzupassen, vorteilhafte Interaktionen mit Bodenmikroorganismen aufbauen müssen. Obwohl das Potenzial verschiedener Pflanzen zur Kolonisierung mineralischer, nährstoffarmer Substrate sowie die Zusammensetzung der mikrobiellen Bodengemeinschaften bei der Primärsukzession im Rahmen vieler Studien beschrieben wurden, sind die Ergebnisse dieser Studien eher sehr unterschiedlich. Außerdem ist wenig über die mikrobielle Gemeinschaft in Mykorrhizosphären von Pionierpflanzen bekannt. Deshalb könnte das Verständnis der Muster der mikrobiellen Gemeinschaftsstruktur und der Entwicklung des Zusammenbaus von Bakterien- und Pilzgemeinschaften in der Mykorrhizosphäre während der Primärsukzession erheblich zur Verbesserung der Phytoremediation von Bergbaufolgestandorten beitragen.

Ein ehemaliges Uranbergbaugebiet in der Nähe von Ronneburg (Deutschland) ist ein Beispiel für eine Primärsukzession, die nach den drastischen Störungen der Landoberfläche initiiert wurde. Topologische Unterschiede innerhalb dieses Gebietes, verstärkt durch die unterschiedliche Rate der natürlichen Zufallsbesiedlung mit den Pflanzen aus benachbarten Waldbeständen, führten zur Gründung von Standorten mit unterschiedlichem Sukzessionsentwicklungsstand. Das Hauptziel dieser Studie war es, Muster des Zusammenbaus von Bakterien- und Pilzgemeinschaften in Mykorrhizosphären während der Primärsukzession im Bergbaufolgestandort aufzuklären. Die Rolle der Pflanzengegenwart und der Pflanzenidentität bei der Bildung assoziierter mikrobieller Gemeinschaften wurde durch einen Vergleich der Zusammensetzung der mikrobiellen Gemeinschaft in der Mykorrhizosphäre und im freien Boden, der nicht durch Pflanzenwurzeln beeinflusst wurde, bewertet. Es wurde auch versucht, den Einfluss bodenchemischer Parameter vom Pflanzeneinfluss zu trennen. Der experimentelle Rahmen bestand aus zwei Teilen: 1) der Untersuchung von Feldstandorten mutmaßlich gleichen Alters, aber unterschiedlichen Entwicklungsständen, und 2) einem Topfexperiment, in dem eine Anfangsphase der Sukzession mit gleichen abiotischen Bedingungen modelliert wurde. Die Struktur von Bakterien- und Pilzgemeinschaften in der Mykorrhizosphäre von

Birken (*Betula* sp.), Eichen (*Quercus* sp.) und Kiefern (*Pinus* sp.) wurde mittels Next-Generation Sequenz Analyse charakterisiert. Insgesamt gehörten zu den am häufigsten Bakterienklassen Acidobacteriia, Alphaproteobacteria, Ktedonobacteria, Bacteroidia. Alle zusammen trugen sie zu etwa 50 – 60 % aller bakteriellen Sequenzen bei. Die Pilzgemeinschaft wurde hauptsächlich von Thelephoraceae, Inocybaceae und Russulaceae repräsentiert, die 50 bis 80% der Gesamtfülle beitrugen.

Die Ergebnisse zeigten, dass die Zusammensetzung von Bakterien- und Pilzgemeinschaften von verschiedenen Faktoren bestimmt wurde. Bakteriengemeinschaften zeigten enge Verbindungen mit bodenchemischen Parametern, nämlich dem Gehalt an toxischen Metallen und Gesamtstickstoff sowie dem C/N-Verhältnis, während Pilzgemeinschaften überwiegend von der Standortidentität bestimmt wurden und ihre Verbreitungsmuster dem Stand der Vegetationssukzession zugeschrieben wurden. Der Gehalt an bioverfügbarem Aluminium beeinflusste die Struktur von Bakterien- und Pilzgemeinschaften. Obwohl der pH-Wert des Bodens einen geringen direkten Einfluss auf die Struktur mikrobieller Gemeinschaften hatte, wurde sein indirekter Einfluss durch die Veränderung der Mobilität einiger toxischer Metalle und damit die Erhöhung ihrer Bioverfügbarkeit angenommen.

In den Anfangsphasen der Sukzession im Topfexperiment waren die Pflanzen mit mikrobiellen Gemeinschaften assoziiert, die sich von der mikrobiellen Gemeinschaft des unbepflanzten Kontrolltopfsubstrats unterschieden; der Rhizosphäreneffekt war jedoch nicht spezies-spezifisch, und verschiedene Baumspesies gründeten Mykorrhizosphären, die ähnlich in der Struktur waren. Überraschenderweise war der Rhizosphäreneffekt der Feldpflanzen nicht ausgeprägt und es wurden keine signifikanten Unterschiede zwischen Mykorrhizosphäre und freiem Boden beobachtet. Auf dem Feld waren die Unterschiede zwischen den Baumarten ausgeprägter; diese Unterschiede wurden jedoch von der Standortidentität/Sukzessionsphase bestimmt.

Diese Studie zeigte zum ersten Mal die Fähigkeit der Pionierpflanzen, die Komplexität der mikrobiellen Interaktionen in der Mykorrhizosphäre während der Sukzession zu fördern. Die Entwicklung des Ökosystems und die Etablierung von Standorten mit sehr unterschiedlichen Bedingungen führten zu einer deutlichen Aufteilung der Taxa in Generalisten und Spezialisten. Eine besondere Bedeutung von pflanzenwachstumsfördernden Bakterien bei den Interaktionen innerhalb der Mykorrhizosphäre wurde gezeigt. Außerdem spielten die Ektomykorrhiza-Pilzen *Inocybe* und *Lactarius* eine wichtige Rolle auf lokaler Ebene der Netzwerkinteraktion und könnten als potenzielle Kerne der Mykorrhizosphärenkommunikation angesehen werden.

Ektomykorrhiza-Pilze können die Kolonisierung des mineralischen Substrats von den Pionierwirtsplanzen durch die Entwicklung und/oder Differenzierung von extramatralem Mycel erheblich erleichtern. Die Morphotypisierung zeigte, dass der Ascomycet *Meliniomyces bicolor* nicht nur die einzige Spezies war, die allen Feldbäumen gemeinsam war, sondern auch zu den repräsentativsten gehörte. Die vorliegende Studie zeigte, dass die Explorationsstrategien von Feldektomykorrhiza-Pilzen die Unterschiede in den Umweltbedingungen widerspiegeln, die durch den Stand der Ökosystementwicklung bestimmt werden. Veränderungen der abiotischen Bedingungen im Topfexperiment führten zur Bildung von Rhizomorphen bei den Ektomykorrhiza-Pilzen, die mit Eichen und Kiefern assoziiert sind, und könnten auf die hohe Fähigkeit der Ektomykorrhiza-Gemeinschaft hinweisen, auf Störungen zu reagieren.

Eine hohe Überlebensrate von Birken und Kiefern in Topfexperiment in Verbindung mit Netzwerkanalyse zeigte ihr hohes Potenzial, neues Substrat zu kolonisieren und sich an neue Bedingungen anzupassen, sowie ihre Fähigkeit, die Kommunikation mit nützlichen Bakterien und Pilzen aufzubauen und zu unterstützen. Ein zusätzliches Experiment im Zusammenhang mit der Inokulation von Pflanzen mit einer kommerziellen Ektomykorrhiza-Pilzmischung förderte die Überwachung und das Wachstum von Eichen und Kiefern. Diese Arbeit bewertet das Potenzial von drei Bäumen für die Phytoremediation von Bergbaufolgestandorten und ihre Fähigkeit, die Entwicklung gestörter Ökosysteme während der Primärsukzession zu erleichtern. Die Rolle der Bäume bei der Gestaltung assoziierter mikrobieller Gemeinschaften sowie die Fähigkeit, Konsortien nützlicher Mikroorganismen

zu etablieren, zu fördern oder zu unterstützen, tragen zur Vorhersage und zum Verständnis der Ökosystemsukzession bei.

1. Introduction

1.1. Post-mining areas. A study area

Post-mining areas are an example of extensive disturbances of the ecosystem. One of the most drastic environmental outcomes of mining activity is extensive soil damage (Spasić et al., 2021). During the open-cast mining, a large amount of rock material is excavated and deposited in the form of spoil heaps. The excavated substrate is constantly exposed to erosive processes caused by surface runoff and wind. This substrate is generally nutrient deficient, depleted of organic matter, and might contain a high amount of toxic metals (Gebhardt et al., 2007). Moreover, the oxidation of sulfide minerals facilitated by water, oxygen and microorganisms' activity results in acid mine drainage (AMD) and leads to soil acidification (Gagnon et al., 2020b). Due to considerable compaction, soil structure is enormously affected and characterized by low porosity, decreased water-holding capacity and the lack of water-stable aggregates (Kałucka and Jagodziński, 2017). As a result of the low content of clay-humic complexes and acidic pH, metals are almost not subjected to absorption or sedimentation, which might lead to their high migration and transfer to the food chains and, therefore, represent a public health threat (Gagnon et al., 2020b).

The most active development of uranium mines was connected mainly with the exploitation of uranium ores in the 40-50s of XX. The environmental standards of the first uranium mines were very low. After completing of mine works, the mining sites were often left without any remedial actions. The shutdown of exploitation did not mean the interruption of its adverse effects; ore deposits were exposed to the influence of the atmosphere and changed during the weathering and erosion. As a result, different toxic compounds, including heavy metals, were generated and accumulated (Sasmaz et al., 2016), so that abandoned mines might still release harmful compounds into the environment through mine wastes and drainage (Merten et al., 2005; Sitte et al., 2010; Huang et al., 2012; Othmani et al., 2015; Demková et al., 2017). Nowadays, exploited mining areas in the European Union are required to be rehabilitated to reduce severe effects on the environment and human health (Directive 2006/21/EC, 2009; Scannell, 2012). An example of an ongoing program of a post-mining area restoration is a former uranium mining site near Ronneburg (Germany, Thuringia) performed by WISMUT GmbH. Within a period of exploitation of uranium ore mines from 1946 to 1990, a total of 231 000 tonnes of uranium were produced here (Becker et al., 2011). Mining activity had a considerable devastating effect on the environment and was accompanied by disturbances of all its components: relief, soil and plant cover, hydrosphere and atmosphere. According to Jakubick et al. (1998), the hazardous effect was caused by the high amount of sulphide minerals, the absence of an impermeable base liner under the piles and the lack of cover on the piles. Leaching of low-grade uranium ores followed by infiltration of surface water, rich in toxic metals, led to extensive contamination of neighbouring soils with heavy metals. Remediation of this area included next steps: 1) decontamination of mines from oil, greases and chemicals followed by their flooding to natural groundwater level, 2) backfilling of open pits with the waste rock material, 3) overlaying of waste rock material with a designed topsoil cover layer to minimize the infiltration of precipitation into waste rock material, to diminish the production of seepage water, and to prevent direct contact of waste rock material with the components of the ecosystem, and, finally, 4) establishing the vegetation cover (Becker et al., 2011). Overlayed waste rock material was further used for forestry to re-create a species-rich stable ecosystem (Federal Ministry of Economics and Technology, 2007). Nonetheless, because of extreme fluctuations of abiotic conditions (toxic metals, acidity, low nutrients, soil moisture etc.), reforestation was successful only locally, leaving large spots of topsoil unvegetated.

1.2. Phytoremediation of post-mining areas

The uppermost goal of remediation of post-mining lands is to re-establish an appropriate plant cover to quicken soil formation, facilitate the accumulation of organic matter, hinder erosive processes, commence nutrient cycling and, in this way, establish a stable ecosystem (Li et al., 2014).

Phytoremediation is a complex of techniques that involves the living plants to extract and remove or immobilize toxicants from contaminated soils and water (Salt et al., 1998). Phytoremediation techniques are divided into five subgroups based on processes mediated by plants: phytoextraction, rhizofiltration, phytostabilization, phytodegradation, phytovolatilization (Salt et al., 1998; Pulford and Watson, 2003). In terms of metal-contaminated lands, phytoextraction, phytostabilization and phytovolatilization (mainly for Hg, As and Se) are considered (Suman et al., 2018).

There is a growing interest in the use of woody plants for phytoremediation. Trees, in general, exhibit a high ability to grow in metal contaminated soils, which, however, can negatively affect their growth rate (Pulford and Watson, 2003). The establishment of woody vegetation influences the physical stabilization of contaminated soil, especially on sloping surfaces: trees significantly hinder wind and water erosion of soils; extensive root systems bind the soil particles, contributing to soil structure; considerable rate of evapotranspiration by the trees diminishes the downward flow of water through the soil, decreasing in this way amount of pollutants transported to groundwater. Moreover, litter and dead tree roots increase the organic matter pool in the soil, promoting nutrient cycling. Woody species have deep root systems, which makes metal uptake more effective; moreover, they produce abundant harvestable biomass that might be more efficient in terms of in-situ remediation (Johnson et al., 1994; Pulford and Watson, 2003; Capuana, 2011; Šnajdr et al., 2013).

As former mining areas often comprise sites with highly contrasting conditions, the success of reforestation strategies will depend on the ability of individual tree species to adapt to particular habitat conditions at contaminated areas (Pietrzykowski, 2019). Based on these differences, tree species are classified as pioneer, or early successional, and target, or climax or late successional (Pietrzykowski, 2019). Pioneer tree species succeed at the early stages of succession due to their fast growth rates, high tolerance to unfavourable conditions (toxic metals, fluctuations in water content and temperature, high insolation etc.), low nutrient demands (Pietrzykowski, 2019). Moreover, some species establish symbiotic relationships with N₂-fixing bacteria, which help these trees to overcome the lack of nitrogen in soils (Borišev et al., 2018). Among woody tree species well adapted to growth at post-mining lands are poplars (*Populus sp.*), willows (*Salix sp.*), locusts (*Robinia sp.*), birches (*Betula sp.*), alders (*Alnus sp.*), pines (*Pinus sp.*). Although the role of pioneer trees as the main carbon providers at the early stages of succession is incontestable, target tree species produce high biomass, significantly contribute to nutrient cycling and, therefore, promote ecosystem stability. Oak (*Quercus sp.*) is an example of a climax tree species.

1.3. Succession definition. Primary succession at post-mining areas

Mining activity results in a severe disturbance of the entire ecosystem and brings the vast areas to primary succession. The term "succession" is defined by Johnson and Miyanishi (2021) as "an orderly unidirectional process of community change in which communities replace each other sequentially until a stable (self-reproducing) community is reached" (Figure 1). The term is used nowadays in many different aspects: population, community, ecosystem, process. Related to the reclamation of post-mining areas, a concept of "primary succession" is often used. Primary succession is defined by Kałucka and Jagodziński (2017) as "the process of colonization and establishment of vegetation on newly exposed substrates, usually scarce in nutrients, barren and devoid of autochthonous organic matter, although a considerable amount of allochthonous, windborne biological materials, in form of living organisms or organic detritus coming from abrasion of surrounding established ecosystems, may be deposited". On the example of a former uranium mining area near Ronneburg, described above, it was shown that reforestation with selected species might be not always successful. In this case, natural

regeneration with air born seeds might occur (Macdonald et al., 2015; Märten, 2017). Therefore, post-mining areas can consist of sites at different stages of succession, including bare unvegetated spots and the sites with the climax vegetation.

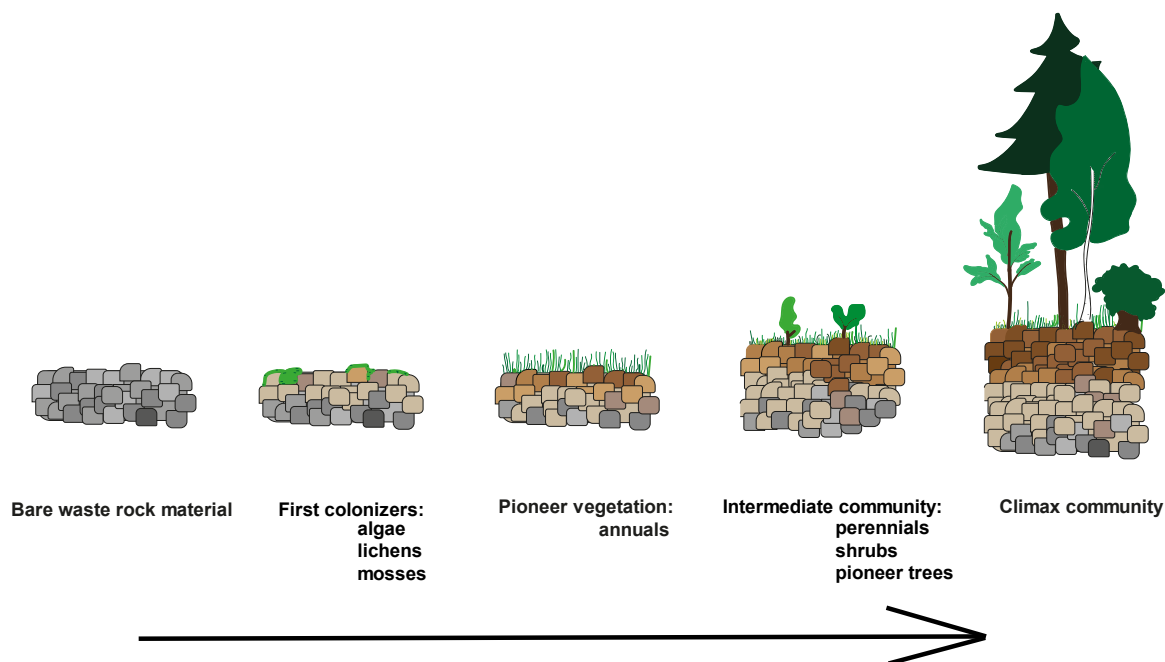


Figure 1. A succession at a post-mining area, starting on bare waste rock material.

Soil represents a unique habitat for various organisms. The specificity of the soil is determined by the interplay of solid, liquid and gaseous constituents what makes the soil a very complex discontinuous system (Totsche et al., 2010). Natural soil heterogeneity creates a variety of environments that differ from each other in physicochemical and biological properties both in space and time and, thus, maintains high diversity of microbial communities (Schulz et al., 2013; Ildó et al., 2018).

During primary succession, microorganisms play a central role in soil formation and soil development, initiating elemental transformation, developing initial carbon and nitrogen cycles, and, in this way, promoting colonization of substrate by pioneer plants. (Schulz et al., 2013). The solid mineral substrate is exposed to the constant influence of solar radiation, desiccation and re-hydration, considerable fluctuations in temperature (McFadden et al., 2005; Gorbushina, 2007; Ciccazzo et al., 2016). Only organisms with a high level of tolerance to multiple and fluctuating stress (also known as poikilo-tolerant) can survive under these conditions (Gorbushina, 2007). The formation of biofilms is a mechanism that ensures the survival of microflora. Biofilm can be defined as an aggregate of microorganisms bound with each other and adhered to the underlying surface through produced extracellular polymeric substances (EPS) (Gorbushina, 2007; Flemming et al., 2016). Secreted extracellular material ensures spatial physiological differentiation of biofilms, determining in this way viability of cells; enables the capture and allocation of nutrients; protects from drought-induced stress, acting as hydrogel (Flemming et al., 2016). Biofilm communities consist of a wide range of pro- and eukaryotes and often comprise phototrophic cyanobacteria and algae as well as a group of melanized microcolonial ascomycetes (Gorbushina, 2007; Gorbushina and Broughton, 2009). Along with that, biofilms not only promote the survival of microorganisms in the initial substrate but also represent an area where bioweathering takes place. Microorganisms produce a wide range of organic acids, siderophores, and chelating agents, which react with rocks and lead to their dissolution (Ciccazzo et al., 2016). Moreover, a mechanical separation of rock grains due to cell growth as well as desiccation/hydration cycles of biofilm matrix occurs (Gorbushina, 2007). At the initial stages of succession, free-living diazotrophs are of substantial

importance as they significantly contribute to nitrogen accumulation (Edwards et al., 2006; Knelman et al., 2012; Ciccazzo et al., 2016).

1.4. Role of plants in primary succession

Chemical and mechanical transformation of waste rock material followed by a release of macronutrients and enrichment of substrate with carbon and nitrogen due to the activity of microbiota create a medium for colonization by pioneer plants. Plants play a crucial role in soil development during primary succession. Vegetation modifies the soil architecture as growing roots bind or disintegrate soil particles and influence, in this way, soil porosity, aeration and hydraulic conductivity. Plant roots, therefore, contribute to soil structural and functional complexity and create specific habitats for microorganisms (de B. Richter et al., 2007; Bardgett et al., 2014; de la Fuente Cantó et al., 2020). In addition, plants modify their soil environment as they release organic compounds via root exudates as well as provide dead plant biomass, which contributes significantly to organic matter accumulation at early stages.

The narrow region of the soil around roots and, thus, directly affected by plant roots is known as the "rhizosphere". Although the rhizosphere is usually defined as a fraction of soil under the direct influence of root exudates, to determine precisely the boundaries of this influence is highly unlikely. Depending on the course of study, the rhizosphere expands to the millimetre for microbial communities and immobile nutrients, tens of millimetres for mobile nutrients and water, and several tens of millimetres for volatile compounds produced by the plant roots (Gregory, 2006).

Plant roots release a wide range of various compounds that can affect physicochemical properties in the rhizosphere (Nguyen, 2003; de la Fuente Cantó et al., 2020). The exudates include carbohydrates, organic acids, phenolic compounds, fatty acids, enzymes, vitamins, hormones, and nucleotides (Jones et al., 2009; Dennis et al., 2010). Root exudates represent up to 30% of the total photosynthate produced by the plant (Walker et al., 2003; de la Fuente Cantó et al., 2020). The quantity and chemical composition of the released compounds are determined by the plant species, a stage of plant development as well as soil conditions (Rambelli, 1973; Fan et al., 2001; Nguyen, 2003; Chaparro et al., 2013; Oburger et al., 2014; Canarini et al., 2016; Oburger and Jones, 2018). Polymerized sugars excreted from the root cap cells, known as mucilage, determine the root tip penetration ability (de la Fuente Cantó et al., 2020). By coating the soil particles and binding them together, root exudates and mucilage contribute to the formation of water stable aggregates, improve soil structure and, therefore, influence water content in general (Bardgett et al., 2014; Carminati et al., 2017). The exudation of organic acids, mainly malic, citric and oxalic acids, can change soil pH values and redox potential, which, therefore, might affect mobilization and availability of nutrients (Hinsinger and Gilkes, 1997; Watanabe and Osaki, 2002; Khorassani et al., 2011; Lei et al., 2016; de la Fuente Cantó et al., 2020). Moreover, organic acids chelate effectively metal cations such as Al and Fe and might contribute to mineral weathering and decrease metal toxicity (Pellet et al., 1995; de B. Richter et al., 2007; Furukawa et al., 2007; Chen and Liao, 2016; Osmolovskaya et al., 2018).

The rhizosphere is involved in weathering of minerals and soil formation via the release of organic acids by roots (de B. Richter et al., 2007). Protons and anions originated from these organic acids replace cations in crystal lattices, leading to mineral destabilization (Gregory, 2006). A vessel experiment, where one-dimensional macroscopic rhizosphere of Italian ryegrass (*Lolium multiflorum*) was simulated, demonstrated the weathering of phlogopite mica through the release of potassium (Hinsinger et al., 1992). Moreover, after 4 days of the experiment, a vermiculitization of phlogopite was observed up to 1.5 mm from the root surface. Similarly, during a 100-days experiment, it was demonstrated that the exudation of organic acids in the rhizosphere of corn resulted in the release of structural K⁺ from mineral lattice (Khormali et al., 2015). April and Keller (1990) showed that mineral grains attached to the roots

were fractured and aligned with their long axis tangential to root surface. Breakage of minerals led to the increase of the mineral surface area exposed to weathering processes. At the same time, tangential relocation of minerals toward root-induced chemical gradient intensified their degradation. These results suggest the involvement of growing roots in the pedogenic process.

Pioneer plants represent hotspots that reduce deficiency of resources and neutralize the harsh abiotic conditions (Cicciazzo et al., 2016). Plant roots have a considerable effect on soil microorganisms, or the "rhizosphere effect", establishing a specific microbial community around the root system. Rhizomicrobial community is usually characterized by increased activity and reduced diversity compared to the bulk soil (Marilley et al., 1998; Blagodatskaya et al., 2014; de la Fuente Cantó et al., 2020). At the early successional stages, large areas are generally unvegetated or covered with sparse vegetation. At these sites, the rhizosphere effect is determined mainly by the plant presence but might not be related to plant species (Tscherko et al., 2005; Brown and Jumpponen, 2014; Cicciazzo et al., 2016). Moreover, harsh environmental conditions might conceal the rhizosphere effect, as young vegetation is predominantly defined by soil chemical parameters, age and site identity (Tscherko et al., 2005; Noll and Wellinger, 2008; Harantová et al., 2017; Kolaříková et al., 2017; Cicciazzo et al., 2014b). Root exudates represent growth substrates, structural material or signalling compounds for associated microorganisms (Barea et al., 2005; Hartmann et al., 2008; Uroz et al., 2010). The rhizosphere is inhabited by a large number of organisms, including bacteria, fungi, oomycetes, archaea, algae, viruses, and microfauna (Mendes et al., 2013). It is believed that plants either stimulate the selection of particular rhizobiome beneficial for plant growth and health (Cook et al., 1995; Mendes et al., 2013) or passively attract microorganisms from the bulk soil via chemotaxis (Hartmann et al., 2008).

1.5. Mycorrhizosphere concept

Since nearly 95% of terrestrial plants in natural ecosystems form mycorrhiza – a symbiotic association with mycorrhizal fungi – the "mycorrhizosphere" concept was proposed (Johansson et al., 2004) (Figure 2).

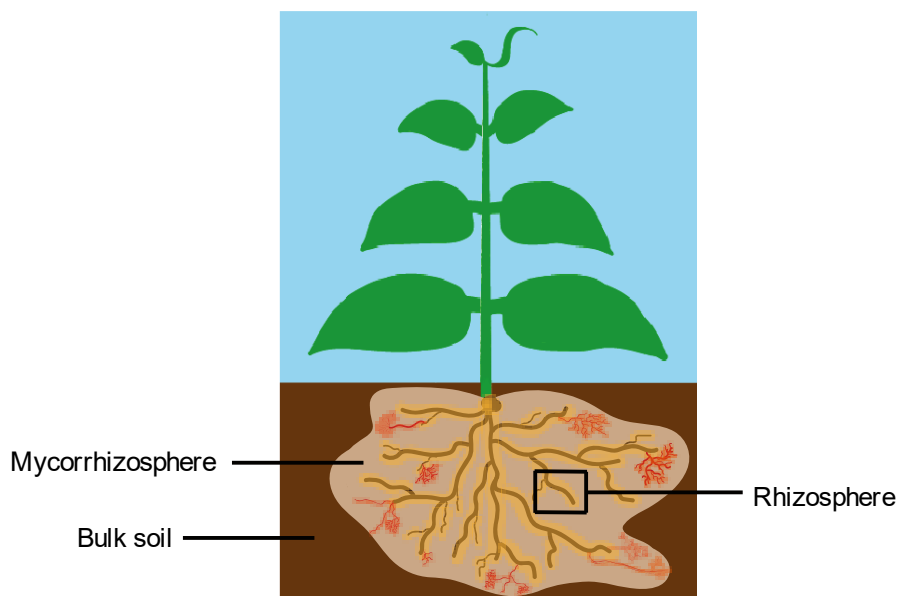


Figure 2. Rhizosphere vs mycorrhizosphere and zone of their influence.

Rambelli (1973) described in detail the mechanism of mycorrhizosphere formation. First, root exudates contact with mycelial fragments or fungal propagules, stimulating their growth and development. The development of hyphae is directed towards zones with the highest rate of root exudation – root tips. After the root tip is reached, the mycorrhizal fungus colonizes the root surface and consumes root

exudates as a carbon source, competing with rhizosphere microorganisms. At this point, the mycorrhizal fungus envelops the root tip forming a dense sheath. After that, mycelium penetrates into root tissues and initiates mycorrhizal symbiosis. From this moment rhizosphere is transformed. Root exudates now are filtered by the fungal sheath and, to a certain extent, used by the fungus for nutritional purposes. Therefore, the root exudates are modified considerably. The change of the root exudates' chemical composition, presence of mycelium as a carbon source for rhizosphere microorganisms as well as alterations of physical properties of soil surrounding the roots lead to the shift in the diversity and activity of microbial communities (Garbaye, 1991; Barea et al., 2002; Linderman, 2008).

New microhabitats created by mycorrhizal fungi in the mycorrhizosphere require different interactive strategies that might allow root-associated microbial communities to benefit from new conditions (Nazir et al., 2009). Similar to plant roots, growing mycelium alters soil structure through physical pressure on soil particles which causes their relocation and disintegration. Andrade et al. (1998) demonstrated a significant positive correlation between the presence of hyphae of arbuscular mycorrhizal (AM) fungi and water stable soil aggregates (WSA). WSA stability affected microbial communities: the total bacterial count and the number of phosphorus-solubilizing bacteria were significantly higher than those evaluated in unstable fraction. Moreover, compounds released by hyphae contribute to soil aggregation. Bacteria inhabit surfaces of extraradical mycelium and mycorrhizal roots (Nurmiaho-Lassila et al., 1997; Mogge et al., 2000; Agerer et al., 2012; Vik et al., 2013; Marupakula et al., 2017; Emmett et al., 2021), spores (Walley and Germida, 1995; Roesti et al., 2005; Gopal et al., 2012) as well as fruiting bodies (Sbrana et al., 2000; Liu et al., 2018).

1.6. Microbial interactions in the mycorrhizosphere

Nazir et al. (2009) suggested that the main mechanism underlying the interactions between mycorrhizal fungi and associated soil bacteria has a nutritional basis. According to Leveau and Preston (2008), bacteria can benefit through 1) the lysis of the fungal cells and taking up the content of the cells (mycophagy or extracellular necrophagy), 2) consuming of soluble organic compounds released by fungal hyphae (extracellular biotrophy), or 3) living inside fungal hyphae (endocellular biotrophy).

As de Boer et al. (2005) reviewed, numerous taxonomically distinct bacteria can lyse fungal cells. Among these bacteria are actinomycetes, Betaproteobacteria (for example, *Collimonas*), myxobacteria, paenibacilli. The distinctive feature of almost all mycolytic bacteria is their ability to produce polymer hydrolyzing enzymes (e.g., chitinases, lipases, glucanases, proteases) and toxins that cause fungal inhibition and cell death (de Boer et al., 2005; Leveau and Preston, 2008).

Chemical compounds released by fungi supply carbon sources and serve as nutrients for bacteria (de Boer et al., 2005). Bacteria-saprotrophs do not depend on the root, do not compete for nutrients with mycorrhizospheric inhabitants, and use a wide range of organic substrates excreted by plant roots and hyphae (Garbaye, 1991).

On the other hand, it is believed that fungi-derived substrates have a qualitative and/or quantitative effect on the bacterial community. Fungal exudates comprise a wide range of sugars (trehalose, inositol, mannitol, xylitol) and amino acids (glycine, glutamic acid and aspartic acid) as well as polyols, oxalate, acetate, formate, glycogen (Griffiths et al., 1994; de Boer et al., 2005; Medeiros et al., 2006; Leveau and Preston, 2008; Nazir et al., 2009). Several studies demonstrated that the chemical composition of fungal exudates explained fungal selectivity for particular bacteria in the mycorrhizosphere. For example, Rangel-Castro et al. (2002) proposed that trehalose and mannitol, released by ECM fungus *Cantharellus cibarius*, could be used by bacteria for growth and reproduction along the vegetative hyphae or inside fruit bodies. Sun et al. (1999) suggested that organic compounds released by *S. bovinus*, including mannitol, might serve as a prerequisite for the structure of the bacterial community associated with the hyphal tips. *Laccaria bicolor* selectively stimulated the growth of *Pseudomonas fluorescens* isolated from Douglas fir-*Laccaria bicolor* mycorrhizas and mycorrhizosphere by the

release of trehalose (Frey et al., 1997). In the *in vitro* system, it was demonstrated that the exudates of AM fungus *Glomus* sp. significantly stimulated the growth and vitality of bacteria and changed the structure of bacterial communities with the enrichment of several Gammaproteobacteria (Toljander et al., 2007). An exceptionally high abundance of Gammaproteobacteria was attributed to their ability to increase mycorrhizal colonization and nutrient uptake in plants.

It was shown that mycorrhizal fungi select for particular bacterial taxa in the mycorrhizosphere (Linderman, 1987; de Boer et al., 2005; Leveau and Preston, 2008). Garbaye (1991) referred these bacteria to *specialized mycorrhizosphere organisms*, which are completely dependent on the organic compounds produced by both plant and fungal symbionts and, thus, compete with each other for the nutrient resource. Prevalence of several bacterial species belonging to such genera as *Pseudomonas*, *Burkholderia*, *Bacillus* in mycorrhizosphere was reported (Timonen et al., 1998; de Boer et al., 2005; Timonen and Marschner, 2006; Uroz et al., 2007; Uroz et al., 2012; Navarro-Ródenas et al., 2016). Most specialized mycorrhizosphere bacteria considerably promote plant growth, protect plants against pathogens, and are attributed to so-called plant growth-promoting bacteria (PGPB) (Kloepper and Schroth, 1978). PGPB possess a wide range of mechanisms that affect plant growth (de Souza et al., 2015). Among plant growth-promoting mechanisms are:

- *biological nitrogen fixation*, a process of reduction of N_2 to ammonia through the enzymatic activity of nitrogenase. The process is carried out in symbiosis between bacteria from Rhizobiaceae family ("rhizobia") and legumes or between actinomycetes of genus *Frankia* and non-legume actinorhizal plants. Symbiotic diazotrophs form nodules on the plant roots where nitrogen is directly provided to plants in exchange for carbon (Roley, 2021). The vast majority of diazotrophs are free-living bacteria (e.g., *Azospirillum*, *Burkholderia*, *Azotobacter*, *Klebsiella* etc.), including cyanobacteria (e.g., *Nostoc*, *Anabaena*) as well as some methanogenic archaea (Prasanna et al., 2009; de Souza et al., 2015; Bae et al., 2018; Smercina et al., 2019; Mishra et al., 2021; Roley, 2021);
- *production of indolic compounds*, mainly auxins, which represent a group of phytohormones. Indol-3-acetic acid (IAA) is the most studied auxin so far. IAA promotes seed germination, mediates responses to light, thus affecting photosynthesis and pigment production (Glick, 2012). It has been shown that plant roots are susceptible to fluctuations in the amount of IAA and respond to its presence by elongation and branching of roots via cell elongation and division as well as initiation of lateral roots (Leveau and Lindow, 2005). Intensification of root growth enlarges the root surface and, therefore, might contribute to water acquisition and nutrient uptake (Dimkpa et al., 2009b). Apart from bacteria, the ability to produce IAA was shown for several ectomycorrhizal fungi. For example, Krause et al. (2015) demonstrated that IAA produced by *Tricholoma vaccinum* not only induced hyphal branching but also enhanced colonization of host plant roots and promoted Hartig net formation in ectomycorrhiza;
- *production of siderophores*, low-molecular-mass molecules with a high affinity to Fe^{3+} . Siderophores chelate iron and transport it into cells, allowing bacteria to overcome the nutritional Fe limitation. Analysis of 2 211 bacterial isolates from seven independent PGPB datasets revealed that more than half of all isolates were able to produce siderophores (da Costa et al., 2014). High production of siderophores was observed for *Burkholderia*, *Enterobacter*, and *Grimontella*, whereas *Klebsiella*, *Stenotrophomonas*, *Rhizobium*, *Herbaspirillum*, and *Citrobacter* were associated with low production of siderophores. Most importantly, Dimkpa et al. (2009a) demonstrated that microbial siderophores could reduce metal-induced oxidative stress in plants. The authors suggested that siderophores had a bioprotective effect as they chelated toxic metal what led to the decrease of metal concentration;
- *production of 1-Aminocyclopropane 1-Carboxylic Acid (ACC) deaminase*, which is related to the lowering of ethylene production in plants. Ethylene considerably inhibits plant growth, acting as a stress phytohormone at high concentrations. Microbial ACC deaminase controls plant ethylene production by metabolizing ACC (the immediate precursor of ethylene biosynthesis in higher

plants) into α -ketobutyric acid and ammonia (de Souza et al., 2015). ACC deaminase was found among many bacteria and is considered as a rather common feature for rhizosphere microorganisms (Glick et al., 2007). ACC deaminase is particularly involved in the alleviation of stress induced by high salinity (Jalili et al., 2009; Bal et al., 2013; Ali et al., 2014) and drought (Zahir et al., 2008). Moreover, Arshad et al. (2007) suggested that the ability of ACC deaminase to reduce the ethylene level also contributed to an extensive root system development. Therefore, plant root growth can result in the increased uptake of heavy metals;

- *solubilization of phosphorus*, an essential nutrient for plants, mainly presented in insoluble and, therefore, unavailable forms (de Souza et al., 2015). Production of organic acids, particularly gluconic and citric, is the main mechanism by which phosphate-solubilizing bacteria dissolve inorganic phosphates and, hence, contribute to plant nutrition (Goldstein, 1995; Kim et al., 1997; Qin et al., 2011; Glick, 2012; de Souza et al., 2015). A wide range of taxonomically distinct rhizosphere bacteria demonstrated the ability to solubilize inorganic phosphates (Rodríguez and Fraga, 1999; Chen et al., 2006; Qin et al., 2011; Estrada et al., 2013).

An example of beneficial bacteria in the mycorrhizosphere is a group of so-called mycorrhiza helper bacteria (MHB) (Duponnois and Garbaye, 1991). MHB embrace taxonomically distinct bacteria, such as Proteobacteria (*Agrobacterium*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Bradyrhizobium*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, *Rhizobium* etc.), Firmicutes (*Bacillus*, *Brevibacillus*, *Paenibacillus* etc.) and Actinobacteria (*Rhodococcus*, *Streptomyces*, *Arthrobacter* etc.) (Frey-Klett et al., 2007). According to Garbaye (1994), there are several ways by which MHB participate in the process of mycorrhiza establishment and development: 1) direct trophic stimulation through the production of a large number of organic substances which might be either a carbon source or growth factor, 2) detoxification of toxic secondary metabolites, accumulated in rhizosphere during the growth of ectomycorrhizal fungi, 3) transformation of root exudate composition or alteration of the amount of produced root exudates, 4) promotion of the susceptibility of the plant root to infection by the mycorrhizal fungus, and 5) participation in recognition mechanisms between a plant and symbiotic fungus. In addition, numerous studies showed that such parameters as density and localization of bacterial cells relative to fungal mycelium (Aspray et al., 2006a; Aspray et al., 2006b), fungus specificity (Duponnois et al., 1993; Aspray et al., 2006a), ability to promote the mycorrhiza formation by young secondary roots (Poole et al., 2001) are also important for the process of mycorrhiza establishment.

Endocellular bacteria depend on their fungal host for nutrients (Leveau and Preston, 2008). Some endocellular bacteria are vertically transmitted and, therefore, obligate; others invade fungal cells and represent an example of sporadic co-existence (de Boer et al., 2005; Leveau and Preston, 2008). The most studied endobacterium *Candidatus Glomeribacter gigasporarum* is a constant inhabitant of the fungus *Gigaspora margarita* and taxonomically is closely related to *Burkholderia* (Bonfante and Anca, 2009). Among the mechanisms underlying this type of interaction is a supply with nutritional benefits for the fungal host through the fixation of nitrogen (Leveau and Preston, 2008). Another possible function might be related to the inability of numerous ectomycorrhizal fungi to produce invertase, the enzyme responsible for the breakdown of sucrose (Nurmiaho-Lassila et al., 1997). Therefore, endobacteria, producing invertase, might contribute to fungal nutrition via hydrolysis of sucrose to glucose and fructose, which are used after by a "host" fungus.

Several studies demonstrated the presence of endocellular bacteria in live cells of ectomycorrhizal fungi as well. For example, Bertaux et al. (2005) detected the community of endocellular bacteria, mainly belonging to Alphaproteobacteria in non-axenic cultures of the ectomycorrhizal fungus *Laccaria bicolor*. Staining with acridine orange confirmed the presence of intracellular bacteria in both live and dead fungal cells in ectomycorrhiza, fungal mats and fruit bodies. The authors proposed the environmental acquisition of endobacteria as a mechanism of the internal colonization of cells. Nurmiaho-Lassila et al. (1997) studied bacterial diversity and colonization patterns in *Suillus bovinus* and *Paxillus involutus*.

The presence of endocellular bacteria was observed for *S. bovinus* but not for *P. involutus*. This result was explained by possible differences in physiologies between studied fungi.

1.7. Ectomycorrhiza characteristics

Mycorrhiza is a mutualistic association between a fungus and the roots of a higher plant. Mycorrhizal symbiosis is the most widespread type of symbiosis on the planet. Approximately 8000 plant species form ectomycorrhiza (van der Heijden et al., 2015). Although it constitutes a relatively small part of the total number of plants, these species (mostly from *Betulaceae*, *Pinaceae*, *Fagaceae*, and *Dipterocaraceae*) are the dominants of temperate and boreal forests, occupying a disproportionately large area of terrestrial ecosystems (Dahlberg, 2001). There are approximately from 7 000 to 10 000 (according to different authors) fungal species that form ectomycorrhizal symbiosis and belong mainly to Basidiomycota and Ascomycota as well as to a genus *Endogone* from Zygomycota (Molina et al., 1992; Dahlberg, 2001; Bücking et al., 2012; van der Heijden et al., 2015). The majority of plants can establish ectomycorrhiza with a great number of ectomycorrhizal fungi from distinct taxonomic groups (Molina et al., 1992; Bruns et al., 2002).

The ectomycorrhizal association is mutually beneficial for both partners of symbiosis: the fungus provides the host plant with absorbed soil-derived mineral nutrients with low mobility (especially phosphorus and nitrogen), and water, alleviates stress induced by unfavourable abiotic conditions (drought, salinity, heavy metals), increases resistance against pathogens; in return, the host plant transports photosynthetically fixed carbon to root tissues and then to mycorrhizal fungus (Bruns et al., 2002; Bücking et al., 2012).

The establishment of ectomycorrhiza causes different morphological changes of roots (Bücking et al., 2012). Ectomycorrhizal roots are usually characterized by a decrease in root hair formation and an enlargement of cortical cells. The ectomycorrhiza includes three main structural components: the hyphal mantle, the Hartig net, and the extraradical mycelium. The hyphal sheath encloses the lateral roots and might range from thin to very thick, forming pseudoparenchyma, from smooth to hairy with emerging hyphae. The ectomycorrhizal mantle plays an important role in nutrient transport between the fungus and the host plant via close contact with soil (Morel et al., 2005). Fungal hyphae penetrate between cortical and epidermal root cells and surround them, forming a branched structure, the Hartig net. Ectomycorrhizal fungi form mycelia that extend from the mantle into the soil, establishing extramatrical (extraradical) mycelium. Hyphae can grow in parallel and assemble in aggregates called rhizomorphs.

Agerer proposed a classification of functional traits of ectomycorrhiza, which reflect how fungi explore and interact with the environment (Agerer, 2001). Based on the differentiation and distribution of extramatrical mycelium, several so-called *exploration types* of ectomycorrhiza were distinguished (Agerer, 2001). The main characteristics of exploration types are summarized in Table 1. Two exploration strategies have been proposed to characterize general carbon and nitrogen distribution patterns within the plant-fungal system (Hobbie and Agerer, 2010). The first strategy is common for the nutrient-rich environment, where nitrogen acquisition depends on labile nitrogen forms such as amino acids, ammonium, and nitrate. Under these conditions, contact and short-distance exploration are of less energy demand to their host, and, therefore, they might be favoured (Lilleskov et al., 2011; Moeller et al., 2014). When nutrient sources are spatially dispersed and/or represented as insoluble complex organic matter, the second strategy, which involves long-distance rhizomorphs, is preferred. Long-distance transport requires hydrophobic rhizomorphs to prevent leakage of solutes during the transport (Hobbie and Agerer, 2010).

Table 1. Characteristics of exploration types of mycorrhiza according to (Agerer 2001).

Exploration type	Hyphal mantle	Emanating hyphae	Rhizomorphs	Hydrophobicity	Contact with a substrate
<i>Contact</i>	Smooth	Rare	No	Hydrophilic	Close contact with a substrate via mantle
<i>Short-distance</i>	Different types	Voluminous	No	Hydrophilic	Close contact with a substrate via mantle and emanating hyphae
<i>Medium-distance fringe subtype</i>	Different types	Voluminous	Fans of ramified and repeatedly interconnected rhizomorphs	Hydrophilic	Extended contact with the soil through rhizomorphs
<i>Medium-distance mat subtype</i>	Different types	Presented	Mats of densely packed undifferentiated rhizomorphs	Hydrophobic	Extended contact with the soil through rhizomorphs
<i>Medium-distance smooth subtype</i>	Smooth	Rare	Rhizomorphs un- or slightly differentiated with a thick central hypha	Hydrophobic	Extended contact with the soil through rhizomorphs
<i>Long-distance</i>	Smooth	Presented	Rare but highly differentiated	Hydrophobic	Long distribution in the soil

Many authors tend to refer particular ECM taxa to so-called "early-stage" or "late-stage" fungi according to the classification introduced by Mason et al. (1983). These stages are attributed to the changes in the fungal community during ecosystem development: from the dominance of *Inocybe*, *Laccaria*, *Thelephora* at "early stages" to the dominance of *Lactarius*, *Cortinarius*, *Russula*, *Tricholoma* at "late stages". Nevertheless, this classification was extensively criticized. For example, Newton (1992) mentioned that many ECM species could not be easily designated to either stage. Besides, particular environmental conditions can affect the behaviour and morphology of some species. Despite these limitations, Iordache et al. (2009) suggested that categorizing ECM species into "early-stage" or "late-stage" might be reasonable for early stages of primary succession but not for more advanced successional stages.

Artificial mycorrhization of plants both in microcosms and under natural conditions has been shown to exert mainly a positive effect on plant performance, as it stimulated the plant growth, intensified photosynthetic rate and root respiration, increased accumulation of nitrogen and phosphorus in plant biomass, and raised the survival rate of plants during afforestation compared to non-inoculated plants (Cairney and Chambers, 1997; Baxter and Dighton, 2001; Menkis et al., 2007; Makita et al., 2012; Oliveira et al., 2012; Sanchez-Zabala et al., 2013).

It has been shown that some ectomycorrhizal species demonstrate a high tolerance to environmental stress, including metal toxicity and low pH values, and, therefore, can be applied in soil remediation programs (Dodd and Thomson, 1994; Cullings and Makhija, 2001). Inoculation of plants with ectomycorrhizal fungi alone or together with associated bacteria might either intensify phytoextraction by plants and lead to the accumulation of heavy metals in aboveground biomass (Baum et al., 2006; Kozdrój et al., 2007; Zimmer et al., 2009), or, opposite, immobilize and sequester metal ions in fungal biomass and, therefore, hamper accumulation of metals in plant biomass compared to non-inoculated plants (Colpaert and Van Assche, 1992; Krupa and Piotrowska-Seget, 2003; Hryniewicz et al., 2012; Fernández-Fuego et al., 2017). Mycorrhization of plants growing in metal contaminated soil might stimulate plant growth, improve nutrient uptake, and increase total chlorophyll content (Baum et al., 2006; Fernández-Fuego et al., 2017).

1.8. Common patterns of microbial succession at post-mining areas

Microbial community assembly is determined by processes, grouped in two classes: *deterministic* processes, which include niche-based "environmental filtering" as well as biotic interactions, and *stochastic* processes, which are determined predominantly by probabilistic dispersal (Stegen et al., 2012). It is believed that soil bacterial and fungal communities assemble differently during ecosystem

development (Trowbridge and Jumpponen, 2004; Brown and Jumpponen, 2014; Schmidt et al., 2014; Harantová et al., 2017). One of the reasons is that bacteria can use different nutritional strategies, including autotrophy and heterotrophy, as well as can fix nitrogen and, therefore, might successfully colonize oligotrophic substrates, whereas fungi are all heterotrophs (Schmidt et al., 2014; Harantová et al., 2017). That makes fungi more dependent on carbon and nitrogen sources and limits the number of habitats they can occupy under oligotrophic conditions. For example, Brown and Jumpponen (2014) demonstrated that along the chronosequence in retreating glacier soils, fungi with a higher proportion of nonrandomly distributed OTUs than bacteria exhibited specific habitat requirements which, highly likely, might be explained by the presence of organic matter sources.

It has been discussed that at initial sites at primary succession, stochastic processes are of great importance. Due to their small size and unicellular organization, bacteria are prone to a great extent to spatial dispersal (Schmidt et al., 2014). Brown and Jumpponen (2014) used the term "propagule rain" to emphasize the significance of stochastic processes in non-vegetated soils. At the same time, plant colonization leads to homogenization of substrate and determines the prevalence of deterministic processes at the site (Schmidt et al., 2014). This suggests that as vegetation succession proceeds and the ecosystem develops, selection driven by deterministic processes will become dominant (Brown and Jumpponen, 2014; Dini-Andreote et al., 2015). These results imply that vegetation plays an important role as a driver of microbial community structure.

Ecosystem development is determined by the reciprocal interactions between abiotic conditions, communities' structure, and their activities (Kyaschenko et al., 2017). These activities, in turn, have a great effect on the environment what might either stabilize the ecosystem or result in directional development. There are many studies devoted to the drivers of microbial community throughout ecosystem development; however, these data are rather inconsistent. The principal differences in bacterial and fungal physiological and ecological strategies might imply that structure and distribution patterns of each group would be determined by different edaphic factors (Lauber et al., 2008). For example, one could expect that fungi are more dependent on vegetation type than bacteria. Being a symbiotic partner of the plant and/or a decomposer of plant litter might define the importance of vegetation in the assembly of fungal community at early stages of succession (Harantová et al., 2017; Kolaříková et al., 2017). Moreover, bacteria and fungi might be involved in the mineralization of different types of carbon substrates, and, therefore, changes in the carbon pool might exert contrasting effects on these organisms (Lauber et al., 2008).

Considerable fluctuations in abiotic factors typical for primary succession (for example, soil acidity at post-mining areas) as well as high structural heterogeneity might represent ecological filtering and, therefore, determine the structure and activity of microbial communities (Schmidt et al., 2014). Most of the works emphasize the particular importance of the content of organic carbon and total nitrogen as well as soil pH values on the bacterial and fungal communities. Interestingly, changes in these parameters are often connected to the vegetation succession and/or attributed to the indirect effect of the plants. Knelman et al. (2012) determined that pH was the only soil chemical parameter significantly associated with bacterial community structure. Interestingly, variance partitioning revealed that pH was a vegetation-derived effect. Authors suggested that vegetation had the greatest impact on bacterial community structure by decreasing pH through litter deposit, organic acid exudation and proton release. Chodak and Niklińska (2010) demonstrated that there were significant differences in the content of organic carbon and total nitrogen between organic and mineral soil horizons in reclaimed mine soils, which might be attributed to the level of soil development. These differences determined the most pronounced effect of soil pH value and total nitrogen content on microbial properties (microbial biomass, basal respiration, nitrogen mineralization and enzymatic activity) in the organic horizon. In contrast, only the amount of accumulated carbon in mineral soil was important for microbial enzymatic activities. The increased soil acidification was attributed to the release of H^+ ions in the rhizosphere due to ammonification and oxidative carboxylation of organic acids. Tscherko et al. (2003) demonstrated that

the content of organic carbon and total nitrogen explained most of the variation in functional diversity of soil microflora across two glacier forelands at primary succession, whereas pH value and the content of available phosphorus just weakly contributed to the remaining variation of the microbial activity.

In contrast, some studies demonstrated that despite the changes in soil chemistry during the succession, no chemical parameters significantly influenced the microbial community. For example, Krüger et al. (2017) discussed that soil physical parameters, e.g., soil texture and structure, might undergo considerable changes during the ecosystem development. Therefore, their effect on microbial communities should be taken into account consideration as well.

As the ecosystem develops and soil formation proceeds, vegetation covers more soil surface and produces more aboveground biomass (Tscherko et al., 2005). As a result, the diversity of vegetation increases, and the shift from annual vegetation to perennial vegetation occurs (Cicczazzo et al., 2016). Along with that, nitrogen and phosphorus cycles undergo alterations. Thus, nitrogen at early successional stages is practically absent, but its content in soil increases due to the activity of diazotrophs and plant residues decomposition. At later stages, the percentage of N₂-fixing bacteria decreases, and recalcitrant organic matter accumulates (Dickie et al., 2013). Phosphorus, on the contrary, is relatively not limited during the primary succession due to the weathering of rock material (Dickie et al., 2013; Schulz et al., 2013). During succession, the content of bioavailable phosphorus decreases due to leaching, mineral transformations and accumulation in biomass (Dickie et al., 2013). Therefore, succession is accompanied by the nitrogen limitation at early stages, co-limitation of nitrogen and phosphorus at more mature stages, and, eventually, phosphorus limitations at the late stages (Dickie et al., 2013). The decrease of bioavailable phosphorus along the succession favours the establishment of mycorrhizal symbiosis (Schulz et al., 2013). As reviewed by Dickie et al. (2013), a commonly discussed trend of mycorrhizal succession implies a shift from the prevalence of non-mycorrhizal plants at initial stages to arbuscular mycorrhizal plant species at more advanced stages and, finally, to ectomycorrhizal trees with ericoid mycorrhizal understory in the climax community. Nevertheless, the authors summarized the results of numerous studies demonstrating that all mycorrhizal types can be observed at all stages of succession.

Late successional stages are characterized by increased organic carbon and nitrogen content, the development of distinct soil horizons, and intensified microbial activity. The steady input of fresh litter affects soil quality and considerably contributes to the structure of soil microbial community due to differences in chemical composition determined by plant species (Thoms et al., 2010; Knelman et al., 2012; Urbanová et al., 2015).

Soil development, which results in the formation of vertically stratified horizons, seems to be an important driver of fungal niche differentiation (McGuire et al., 2010; Dickie et al., 2013). Different functional fungal guilds colonize distinct niches within an organic layer (de Boer et al., 2005; Kyaschenko et al., 2017). Litter is predominantly occupied by saprotrophic fungi involved in the decay of plant-derived litter through the release of extracellular enzymes (Kyaschenko et al., 2017). Recalcitrant organic matter in the deeper humus horizon is colonized by mycorrhizal fungi (Kyaschenko et al., 2017). Although it is generally accepted that mycorrhizal fungi are less efficient decomposers than saprotrophic fungi due to a lesser number of genes encoding plant cell wall-degrading enzymes, they possess various abilities to degrade lignocellulose (Kohler et al., 2015; Wagner et al., 2015; Kyaschenko et al., 2017). Certain genera of Basidiomycota, namely white-rot fungi, are particularly important in the breakdown of lignin via the production of laccases and peroxidases (de Boer et al., 2005).

A higher fungi/bacteria ratio is typical for late-successional stages due to the high vegetation diversity and accumulation of recalcitrant organic matter (Bardgett and Walker, 2004; de Boer et al., 2005; Harantová et al., 2017).

After a stable plant community has formed, a positive feedback loop develops (Schulz et al., 2013). Plants provide fixed carbon through root exudation and dead plant material supporting the decomposers community. During the mineralization of organic matter, plenty of nitrogen is released, which is again available for the plants (Schulz et al., 2013). Eventually, the development of the ecosystem reduces the harmful effects of unfavourable environmental conditions.

Post-mining substrates are commonly characterized by high concentrations of toxic metals. It is generally accepted that microorganisms and fungi found in metal-contaminated soils are exposed to strong environmental selective pressure (Bruins et al., 2000; Epelde et al., 2015). This environmental filtering leads to the adaptation to metal contamination by developing particular metal resistance mechanisms (Epelde et al., 2015). Most survival mechanisms are based on the modification of metal speciation that leads to decreased or increased metal mobility (Gadd, 2010). Further characteristics of these mechanisms is a summary of the review of Gadd (2004). Mobilization of metals leads to the transformation of insoluble metal compounds and minerals to soluble forms and can be a result of *bioleaching*, which involves proton efflux via plasma membrane H⁺-ATPases as well as the release of organic acids, which provide both protons and chelates (e.g., citric, oxalic acids), *excretion of siderophores*, *biomethylation* (mainly related to Hg, As, Se, Sn, Te and Pb). Immobilization results in the binding of metals with biomass and can involve 1) *biosorption*, mediated, mainly, by peptidoglycan carboxyl groups for Gram-positive bacteria, phosphate groups for Gram-negative bacteria, and chitin and its derivatives, melanin bound to chitin, and chitosan for fungi; 2) *bioaccumulation* that involves active transport of metals inside the cells followed with the binding to biomolecules with metal-binding properties and/or intracellular compartmentalization. These metal-binding compounds can be represented by non-specific substances (simple organic acids and alcohols, humic and fulvic acids, polysaccharides and proteins) and specific metal-binding proteins, namely metallothioneins and phytochelatins; 3) *organic and inorganic precipitation*. Redox reactions can reduce or increase the mobility of metals depending on the metal species involved.

Most of the studies on microbial community structure in soils at post-mining areas focus on successional processes and shifts in the microbial community composition and the effect of vegetation on associated microorganisms. Surprisingly, not so many studies aim at metal contamination of post-mining areas and its effect on soil microflora. Several works revealed strong associations between soil microbial communities and the content of heavy metals. For example, Bierza et al. (2020) stated that only well-adapted ectomycorrhizal species could tolerate toxic conditions and establish mycorrhiza with trees growing in metal-polluted soils. Thus, the authors demonstrated that the toxicity index was the only significant factor that explained the variability of ECM fungal communities between contaminated and non-contaminated soils. Moreover, the presence of *Scleroderma* sp., *Russula* sp., *Cortinarius* correlated with the high heavy metal content in the soil. Epelde et al. (2015) revealed that the total concentration of Cd, Pb and Zn, typical for an abandoned Pb-Zn mine site, strongly influenced the composition of the microbial community and resulted in the decrease of several taxa belonging to Actinobacteria and Acidobacteria. In contrast, the relative abundance of Chloroflexi and Ktedonobacteria increased in the most contaminated samples, which was explained by their local adaptation due to high metabolic plasticity. Sun et al. (2018) revealed that the interactive effect of pioneer plants and such soil parameters as the content of Cu and As as well as water content altered the bacterial communities in rhizosphere and bulk soil in copper mine tailings. Moreover, Alphaproteobacteria and Chlorobi had a strong positive correlation with pH value.

1.9. Aims of the study

Primary succession studies are historically focused mainly on the development of vegetation on the newly exposed, void of autochthonous organic matter substrates. At the same time, mycorrhizal fungi

and microorganisms inhabiting the mycorrhizosphere undergo successional changes as well. Microbial community structure is determined by vegetation (species diversity, vegetation type (deciduous vs coniferous, herbaceous vs woody), the density of cover, age, etc.) and soil physicochemical parameters. Nevertheless, as Brown and Jumpponen (2014) rightly stated, most studies on the role of vegetation and substrate quality in microbial succession during early stages of ecosystem development are rather decoupled, focusing on either bacterial or fungal succession along with the ecosystem development. Furthermore, the number of works on microbial succession at the post-mining areas, where disturbance resulted in drastic changes of the entire ecosystem and initiated a primary succession, is even less.

The first objective of this work is to characterize the structure of microbial communities (both bacterial and fungal) inhabiting mycorrhizospheres of trees growing at a former uranium mining site that undergoes primary succession and determine factors driving microbial community composition.

ECM fungi depend on their host plant, which provides fungal symbiont with carbon. As ECM fungal hyphae grow into the soil, they expand the zone of plant influence on the soil. This close link between the plant and a symbiotic fungus implies a great role of the vegetation in building the fungal community (Harantová et al., 2017). On the other hand, direct contact with the surrounding substrate suggests that ECM fungal community will respond to abiotic variables as well as to any disturbances, which lead to the alterations of these variables. Thus, such traits of ECM fungi as their exploration strategies might reflect abiotic conditions in their environment.

The second objective of this work is to characterize morphological features of ECM fungi associated with plants at the early successional stage and to describe any shifts in functional diversity of ECM community during succession, launched by re-planting of field trees (modelled in pot experiment).

A positive effect of inoculation of plants, growing in heavy metal polluted soils, with ECM fungi has been documented.

The third objective of this work is to estimate the influence of additional inoculation of plants with the ECM blend on the plant performance under unfavourable conditions, which plants and associated fungal community face during primary succession.

Here following hypotheses were tested:

- (1) Plant presence exerts a rhizosphere effect and shapes microbial community that is different in structure from the microbial community in bulk soil
- (2) Rhizosphere effect is tree species-specific and determines establishment of distinctive mycorrhizospheres
- (3) Abiotic conditions play a predominant role in the structuring of the bacterial community, while vegetation defines fungal community structure at the field
- (4) Inoculation of the plants with the blend of ECM fungi promotes the plant growth and improves their performance at primary succession

To check the proposed hypothesis, the next-generation sequence was used to determine the structure of bacterial and fungal communities. The content of bioavailable fractions of toxic metals (Al and heavy metals) and the content of total carbon, total nitrogen and total phosphorus were chosen as putative drivers of microbial community structure. Pooling the datasets of soil chemical parameters and datasets of taxonomical data in different combinations enables to perform multivariate analyses and ascertain the patterns of microbial community composition. Moreover, network analysis based on sequence data elucidates the interactions between bacteria and fungi in the mycorrhizosphere and demonstrates the changes of these interactions during succession, launched by re-planting of field trees (modelled in pot experiment).

Microbial and plant communities represent complex systems that are involved in a wide range of processes. The long-term field studies on vegetation and microbial succession might be considerably affected by fluctuations in environmental conditions. Therefore, a pot experiment is a good approach to decrease the complexity of the natural system and create conditions required for the research.

In this study, a pot experiment was established for several purposes: 1) to eliminate the differences in local conditions, typical for a study area, and to bring plants under similar conditions; 2) to study the potential of the ECM community to respond to disturbance, which might take under natural conditions; 3) to estimate the value of additional inoculation with the ECM blend for the plant performance during primary succession at a post-mining area.

This work significantly contributes to understanding the processes that occur at the initial stage of primary succession at a post-mining area. The results of this study can be practically implemented into the phytoremediation of disturbed areas.

2. Materials and Methods

2.1. Experiment set-up

Test field site Kanigsberg is a part of a former uranium mining area located near Ronneburg (Thuringia, Germany). The soil is characterized by the high content of heavy metals, low pH values, high structural heterogeneity due to the inclusion of coarse material.

To investigate the influence of plants and soil characteristics on microbial community structure, birch (*Betula sp.*), oak (*Quercus sp.*), and pine (*Pinus sp.*) highly represented at the test field were chosen for the experiment. To characterize the whole root system of each plant and avoid a bias in the root sampling process, young trees (ca. 2-3 years old) were selected for the experiment. For that, visually undamaged trees of approximately the same height were chosen: birches of 25-30 cm, oaks of 7-10 cm, pines of 7-11 cm. Sampling sites location coordinates are summarized in Table 2.

Table 2. Field sampling sites' location coordinates.

Sampling site	Location
Birches sampling site	50°49'35.35"N, 12°9'11.68"E
Soil substrate sampling site	
Oaks sampling site	50°49'36.13"N, 12°9'12.56"E
Pines sampling site	50°49'39.65"N, 12°9'17.87"E

To characterize chemical parameters and microbial community structure of soil affected by a plant (field plant mycorrhizosphere (MR)), the topsoil layer was carefully removed to expose the plant root system, several soil aggregates directly attached to the roots (along the whole root system when possible) were carefully sampled. Sampling was performed for three trees of each species, which were treated further as three individual samples (replicates). To eliminate the influence of the plant as a factor, soil next to the plant but potentially not influenced by plant root system (bulk soil or BS) was sampled: the soil was collected from three spatially distributed points around the plant root system and mixed to prepare a representative composite sample (Figure 3A).

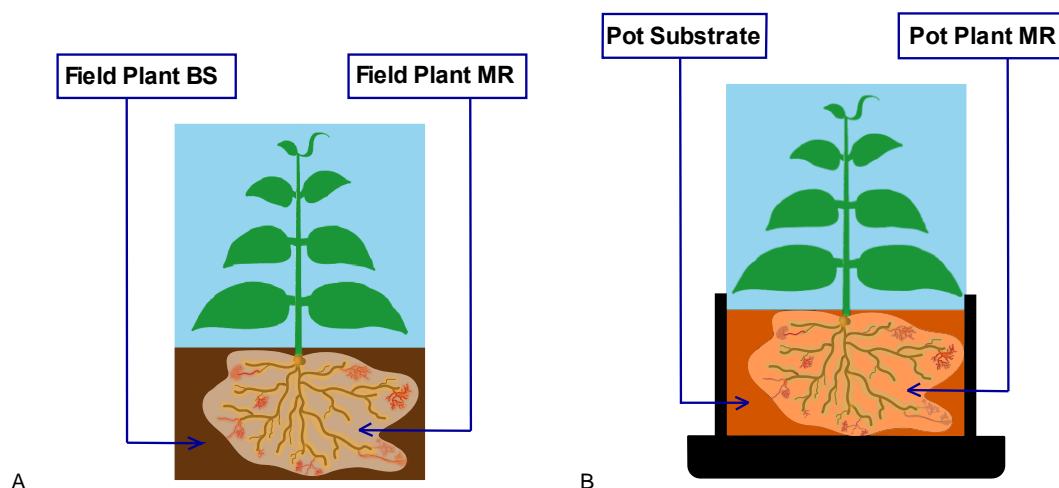


Figure 3. Schemes of soil sampling. **A** – at the test field, **B** – from pots. MR – mycorrhizosphere, BS – bulk soil.

2.2. Pot experiment set-up

A pot experiment was established in greenhouse of Thuringian State Office for Agriculture and Rural Areas (Thüringer Landesamt für Landwirtschaft und Ländlichen Raum), Jena, Germany.

In September 2017, soil from the test field (from now on referred to as “control pot substrate”) was collected (50°49'35.27"N, 12° 9'12.13"E) and pre-sieved to remove coarse material and plant residues. After that the soil substrate was dried for three days and then sieved (< 2.8 mm). Mitscherlich pots (12

l) were used for the experiment (Figures 4A-C). To prevent direct contact of soil substrate and pot metal walls, pots were inlaid with polyethylene film in a way to keep further water excess infiltration possible. Trees for the pot experiment were sampled in soil cores 15x15 cm (to keep roots from drying) from the test field in September 2017. Soil cores were lately carefully removed, keeping adjacent to the roots' surface soil. Pots were filled with the processed soil substrate to ~ 2/3 of the volume, then two trees of one species were placed inside. The root system was horizontally spread. Roots and surrounding pot substrate were watered with 100 ml of deionized water to prevent drying of roots. Remained 1/3 part of the soil was carefully added to the pot. The total amount of soil in each pot constituted 6 kg.

To estimate if inoculation of plants with ectomycorrhizal fungi can improve plants performance during the initial stages of succession, additional pots with plants were inoculated with a commercial mixture of ectomycorrhizal fungi INOQ Forst (INOQ GmbH, Germany) (Figure 4D). This mixture represents a blend of mycorrhizal fungi in highmoor peat substrate. According to the manufacturer, this soil amendment improves soil structure, increases humus content and intensifies biological activity. The mixture on the date of the order in August 2017 contained the ectomycorrhizal fungi *Amanita muscaria*, *Boletus edulis*, *Hebeloma crustuliniforme*, *Paxillus involutus*, *Pisolithus tinctorius*, *Cenococcum geophilum*, *Pisolithus arrhenius* and the endomycorrhizal fungus *Rhizopagus irregularis*. Inoculation was performed according to the manufacturer's recommendations.

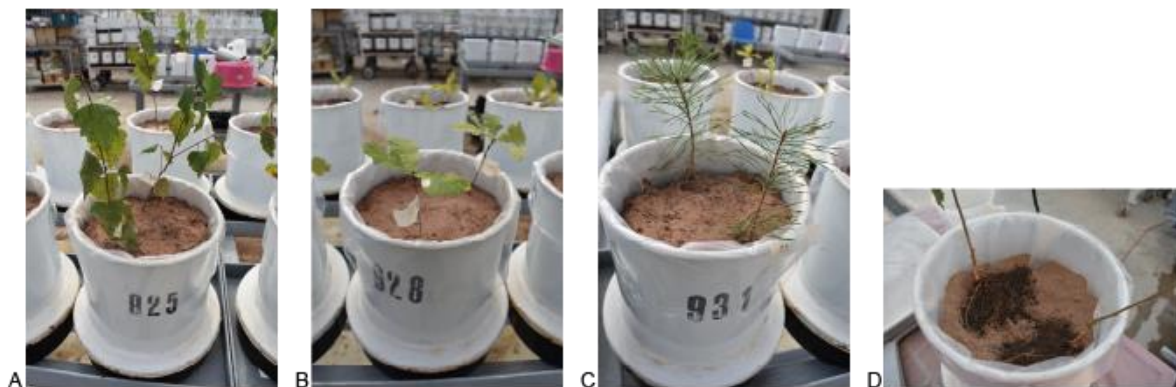


Figure 4. Images of pot variants. **A** – pot birches, **B** – pot oaks, **C** – pot pines. **D** – the process of inoculation of plants with the ECM blend.

All pots, including control variants, were watered with deionized water to approximately 15% vol. water content. Watering was performed weekly in autumn-winter seasons and twice per week in spring-summer seasons. After watering, the very upper layer of soil (0.7 – 1 cm) formed a dense crust in the pot. To improve air exchange, loosening of the topsoil layer was carefully performed with non-metal equipment the next day after watering.

In sum, four different variants of the pot experiment were established (Table 3):

- 1) Three pots with pot substrate without any treatment (control)
- 2) Five to six pots with pot substrate and two seedlings of one species (in sum, 10 to 12 plant replicates for each tree species)
- 3) Three pots with the pot substrate mixed with the ECM blend without any treatment (control)
- 4) Five to seven pots with the pot substrate and two seedlings of one species inoculated with the ECM blend (in sum, 10 to 14 plant replicates for each tree species).

Table 3. Overview of pot experiment variants.

Non-inoculated variants				Inoculated variants			
control	birch	oak	pine	control	birch	oak	pine
3 pots	6 pots	5 pots	5 pots	3 pots	7 pots	7 pots	5 pots
-	12 plants	10 plants	10 plants	-	14 plants	14 plants	10 plants

After two years of pot experiment, soil adjacent to pot plant root system was sampled as described above to characterize chemical properties and microbial community structure of soil affected by a pot plant (Figure 3B).

2.3. Soil chemical analysis

To perform chemical analysis, the soil was pre-treated. First, it was air-dried at 40 °C. After soil samples reached constant weight, they were crushed if coarse soil aggregates formed during the drying and sieved (< 2.0 mm) to remove coarse soil material and plant residues.

To measure pH value, 5 g of pre-treated soil were mixed with 25 ml of deionized water. The soil suspension was shaken for 1 h. pH was measured with pH electrode SenTix 81 (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim) three times for each sample (technical replicates). The mean value for each sample was then calculated. For every variant of the experiment, the pH value of three samples was measured.

For elemental analysis, air-dried soil was finely ground in an agate mortar to produce a powder-like material. The ground material was stored in 50 ml air-tight falcon tubes in darkness. Content of total carbon (TC) and total nitrogen (TN) was measured with VarioMAX CN Element Analyzer at the Laboratory "Routine measurements and Analysis" (Elementar Analysensysteme GmbH, Hanau, Germany), Max Planck Institute for Biogeochemistry, Jena, Germany. The resulted values were re-calculated for the absolute dry weight of the samples measured at 105 °C to eliminate differences in water content between the samples. Content of total phosphorus (TP) was determined with ICP-Atomic Emission Spectrometer "Optima 3300 DV" (PerkinElmer, USA) at the Laboratory for Spectrometry, Max Planck Institute for Biogeochemistry, Jena, Germany. Bioavailable fractions of toxic metals were determined with ICP-OES spectrometer 725ES (Agilent, Waldbronn, Germany) and Quadrupole-ICP-MS-spectrometer XSeries II (Thermo Scientific, Bremen, Germany) after sequential extraction (fractions I and II according to Zeien and Brümmer (1989)) at the Institute of Geosciences, Friedrich Schiller University, Jena, Germany. For every variant of the experiment, elemental analysis was performed in triplicates.

2.4. Plant biomass chemical analysis

To evaluate the content of toxic metals in plant material, aboveground green biomass was used. After harvesting, plant biomass was immediately properly rinsed, first with distilled water and, after, three times with deionized water. Plant biomass was dried at 40 °C for one week and then ground in an agate mortar to produce a powder-like material.

The sum content of toxic metals in aboveground biomass was determined with Mars Xpress-System (CEM, Kamp-Lintfort, Germany) at the Institute of Geosciences, Friedrich Schiller University, Jena, Germany.

2.5. Morphotyping

To analyze ECM fungal communities inhabiting the mycorrhizosphere of field plants, in September 2017, trees of chosen species were sampled in soil cores (20x20 cm, 10-15 cm deep, ca. 50-100 cm apart from each other) to prevent drying of roots and kept in a laboratory in plastic pots before processing. Root systems of five plants of each tree species were observed. To perform morphotyping,

the roots of plants were soaked overnight in tap water at 4 °C. After that, roots were carefully washed on a sieve to remove soil material and cut for 1-3 cm long pieces. Finally, fine roots of plants were separated and observed with a dissecting microscope (Stemi, 2000-C, Zeiss, Germany).

To analyze ECM fungal communities inhabiting the mycorrhizosphere of pot plants, in September 2019, the soil was carefully removed from pots by portions to prevent disturbance of the root system, and a plant was taken out. The plant was transferred to the laboratory and immediately processed as described above. Root systems of three plants of each tree species (both non-inoculated and inoculated variants) were observed.

Description of mycorrhizal roots morphology was performed according to Agerer (1987-2006). Characteristics chosen for description were colour of ectomycorrhiza, type of ramification, the shape of unramified ends, mantle surface, characteristics of rhizomorphs (presence/absence, colour, shape, point of connection with a hyphal mantle), characteristics of emanating hyphae (presence/absence, colour). The abundance of each morphotype per total length of ectomycorrhiza was assessed.

Mycorrhizal roots of different morphology were collected for further molecular identification.

2.6. Molecular methods of ectomycorrhiza identification

Molecular identification of ectomycorrhizal fungi was based on the amplification and following sequence analysis of internal transcribed spacer (ITS) of rDNA.

Before molecular analysis of ectomycorrhiza, pre-treatment of samples was performed. Selected short roots were placed in 1.5 ml microcentrifuge tubes containing 0.5 ml sterile distilled water and vortexed for 30 sec to remove remained soil particles. After that, roots were transferred to 1.5 ml microcentrifuge tubes with 200 µl 30% H₂O₂ and vortexed for 10 sec for surface sterilizing. Roots were then rinsed in sterile distilled water three times to remove the rest of H₂O₂, transferred in the end into a sterile 1.5 ml microcentrifuge tube and kept at -20 °C for further analyses.

To identify the ectomycorrhizal species, direct PCR was performed according to Iotti and Zambonelli (2006). Shortly, after pre-treatment of the sample, a small piece of the hyphal mantle was taken under binocular and transferred to a 0.5 ml PCR tube containing 20 µl of sterile distilled water and kept at -20 °C for further analysis. To perform PCR, samples were thawed at room temperature. Bovine serum albumin (Carl Roth, Karlsruhe, Germany) was added to the samples (final concentration 0.4 µg/µl) before other PCR reagents to prevent possible binding of inhibiting compounds to *Taq* polymerase and mixed by pipetting. Final 50 µl volume PCR reaction contained 6.25 µl 10x Dream Taq Buffer, 10 mM µl dNTPs, 10 µM forward primer, 10 µM reverse primer, 1.25 U *Taq* polymerase. Universal primers ITS1 (5' -TTCGTAGGTGAACCTGCGG - 3') and ITS4 (5' - TCCTCCGCTTATTGATATGC - 3') were used (Gardes and Bruns, 1993). Amplification conditions were pre-heating at 95 °C for 5 min followed by 35 cycles of 95 °C 30 s, 56 °C 30 s, 72 °C 50 s, final elongation at 72 °C for 10 min. Amplified DNA was visualized with agarose gel electrophoresis. 5 µl of PCR products were loaded on 1.0% agarose gel prepared in 1xTAE buffer and run under electrical voltage 100 V for 20 minutes. After that, the gel was stained in 1 µg/ml ethidium bromide for 10 min and visualized under UV light by Infinity Video Documentationsystem. Images were elaborated with Infinity-Capt Software (version 14.1a).

Samples containing PCR product of the right size were purified using QIAquick PCR Purification Kit (Qiagen, Germany), checked in 1.0% gel, and sent for sequence analysis.

In the case of unsuccessful ITS amplification after direct PCR, DNA from the same morphotype was extracted with PowerSoil DNA Isolation Kit (MoBio, Carlsbad, USA). Root samples were thawed, sterile glass beads (Sigma Aldrich, USA) were added to the sample, and the sample was milled using a plastic pestle. After that 100 µl of sterile distilled water were added to the sample and vortexed for 30 sec. The supernatant containing water and disrupted roots was transferred to a PowerSoil bead tube. Following DNA extraction steps were performed according to the manufacturer's instructions. Extracted DNA was used for PCR under the conditions described above.

Sequencing of amplified DNA was performed by GATC Biotech (Konstanz, Germany) with forward primer ITS1. Final sequences were compared to those in NCBI (<http://www.ncbi.nlm.nih.gov/>) and UNITE (Nilsson et al., 2019) databases.

2.7. Soil DNA extraction

Soil from the test field or pots was kept in sterile Falcon tubes and processed immediately after the sampling. Total soil genomic DNA was extracted from 0.25 g of soil sample using PowerSoil DNA Isolation kit (MoBio, Carlsbad, USA) according to manufacturer's instructions in triplicates. The concentration of extracted DNA was measured with DeNovix DS-11 Spectrophotometer (Biozym, Hessisch Oldendorf). Since the resulted yield of DNA was very low, no additional DNA purification step was performed. Triplicates of each sample were then pooled into one and sequenced using the Illumina MiSeq platform at StarSEQ (Mainz, Germany) after amplification of 16S rDNA region (bacteria) using primers 27F (5' - AGA GTT TGA TCC TGG CTC AG - 3') and 534R (5' - ATT ACC GCG GCT GCT GG - 3'), and ITS1 region (fungi) using primers ITS1F (5' - CTT GGT CAT TTA GAG GAA GTA A - 3') and ITS2 (5' - GCT GCG TTC TTC ATC GAT GC - 3') (Wagner et al., 2019).

2.8. Sequence analysis

Sequence analysis was performed using the open-source bioinformatic platform Quantitative Insight Into Microbial Ecology 2 (QIIME 2) (Bolyen et al., 2019). Quality filtering was applied to raw sequence data and included the trimming of low-quality regions, removing short-length sequences, demultiplexing and discarding sequences containing ambiguous bases and putative chimeras. Bacterial sequences were rarefied to 34321 reads per sample, and fungal sequences were rarefied to 50286 reads per sample. Remained high-quality sequences with more than 97% of similarity were joined into amplicon sequence variants (ASVs). Taxonomic assignment was performed with SILVA database (Quast et al., 2012) for bacterial sequences and UNITE database (Nilsson et al., 2019) for fungal sequences. Bacterial community structure was characterized at class level (with representability of classes more than 1%). Fungal community structure was characterized at the family level (with representability of families more than 1%).

2.9. Data process and statistical analysis

To characterize the similarity/dissimilarity of the experiment variants based on soil chemical characteristics, principal component analysis (PCA) was performed. Soil parameters data were standardized to have a mean of zero and a standard deviation of 1 according to formula (a z-score)

$$z_i = \frac{x_i - \bar{x}}{s},$$

Where

z_i – standardized variable,

x_i – a measured value,

\bar{x} – mean value of a sample,

s – standard deviation of a sample

ECM communities of field trees were characterized with community diversity indices (Shannon diversity index (H_{SH})), Gini-Simpson index (H_{GS}), Simpson dominance index (H_{SD}), Berger-Parker index (H_{BP})) based on the relative abundance of morphotypes. Trees of one species were compared to each other within each variant of the experiment (field trees, non-inoculated pot trees, inoculated pot trees) to

determine the similarity/dissimilarity of ECM communities with Sørensen, Jaccard, Bray-Curtis indices and to estimate representability of replicates.

Bacterial and fungal communities of each soil sample were characterized with community diversity indices ((richness (S), Shannon diversity index (H_{SH}), Gini-Simpson index (H_{GS}), Simpson dominance index (H_{SD}), Berger-Parker index (H_{BP})) based on the frequency of ASVs.

Soil replicates within a variant of the experiment (field MR, field BS, pot plant MR, control pot substrate) were compared to each other to determine the similarity/dissimilarity of microbial communities with Sørensen, Jaccard, Bray-Curtis indices and to estimate the representability of replicates.

Non-metric dimensional scaling (NMDS) based on the Bray-Curtis similarity index was used to visualize the similarity/dissimilarity of the experiment variants. The similarity percentages breakdown (SIMPER) procedure was applied to estimate the contribution of individual taxa in dissimilarity between compared groups of experiment variants based on the Bray-Curtis similarity index.

Taxonomical dataset and dataset of soil chemical parameters were split into subsets called herein as variants of the experiment: field plant MR, field plant BS, pot plant MR, control pot substrate, field plant sampling site (pooled data for field plant MR and corresponding BS). To determine which parameters contribute the most to the structuring associated with plants bacterial and fungal communities, comparisons of variants of the experiment in different combinations were performed (Table 4).

Table 4. Comparison of the variants if the experiment

Driving parameter	Combination of comparison	Explanation
Plant role	FIELD PLANTS MRs vs BSs (within a tree species)	<ul style="list-style-type: none"> - Differences between a field mycorrhizosphere and its corresponding bulk soil are determined by the plant. - Natural fluctuations in abiotic conditions might cause differences as well.
	FIELD PLANTS MRs (between tree species)	<ul style="list-style-type: none"> - Differences between the mycorrhizospheres of field plants are determined by the plant's species. - Natural fluctuations in abiotic conditions, as well as site identity, might affect the microbial community as well.
	POT PLANTS MRs vs CONTROL POT SUBSTRATE	<ul style="list-style-type: none"> - Differences determined by natural fluctuations in abiotic conditions as well as site identity are eliminated. - Differences between a pot plant mycorrhizosphere and a control pot substrate are determined by plant presence. - Differences between pot plants mycorrhizospheres are determined by plant species.
Soil role	FIELD SAMPLING SITES (combined MR with BS)	<ul style="list-style-type: none"> - Differences between field sampling sites are determined by site identity.
Stage of succession (determined by vegetation succession and level of soil development)	FIELD MR vs POT PLANT MR vs CONTROL POT SUBSTRATE	<ul style="list-style-type: none"> - A sequence <i>control pot substrate</i> → <i>pot plant MR</i> → <i>field plant MR</i> simulates a chronosequence of soil/site development. - The differences between the variants are determined by age and the state of soil development.

MR – mycorrhizosphere, BS – bulk soil

To elucidate which processes determine the assembly of bacterial and fungal community in mycorrhizosphere, the changes in microbial community structure along the simulated succession *control pot substrate* → *pot plant MR* → *field plant MR* were estimated. Impact of age (“0” – unvegetated control pot substrate, “initial” – colonization of the substrate by a plant (modelled in pots), “primary” – development of pioneer vegetation cover (observed at the test field)) as well as such soil parameters as content of total carbon, total nitrogen, total phosphorus, C/N ratio and pH, reflecting the state of soil development, was assessed.

Canonical correspondence analysis (CCA) and Spearman's rank correlation analysis were performed to estimate the correlation of the most representative bacterial and fungal taxa, diversity indices,

exploration types of ectomycorrhiza and field morphotypes with environmental variables. A p-value lower than 0.05 was considered as significant.

To estimate microbial interactions within mycorrhizospheres, a co-occurrence network was constructed based on Spearman's rank correlations between bacterial and fungal genera. To reduce network complexity only statistically significant ($p < 0.05$) positive correlations with the Spearman's correlation coefficient > 0.6 (strong correlation) were considered.

Next subsets of taxonomic data were considered:

1. Overall co-occurrence patterns in
 - 1.1. Field. Overall Field MR network subset consisted of taxonomic data for each field plant MRs. Only taxa with the relative abundance $\geq 0.1\%$ represented at least at one field sampling site in all three MRs replicates were included in the final dataset.
 - 1.2. Pot. Overall Pot MR network subset consisted of taxonomic data for each pot plant MRs. For pot birches and pines, only taxa with the relative abundance $\geq 0.1\%$ represented at least in one pot plant variant in all three MRs replicates were included in the final dataset. As only two pot oaks were included in the calculations, taxa which were represented only in one replicate with the relative abundance $> 1\%$ were additionally included in further calculations.
2. Co-occurrence patterns for each tree species in
 - 1.1. Field. A sampling site network subset consisted of taxonomic data for MR and BS of a particular tree species pooled together. Only taxa with the relative abundance $\geq 0.1\%$ represented at least in five habitats (3 MRs and 2 BSs or 2 MRs and 3 BSs) were included in the final dataset.
 - 1.2. Pot. A pot plant network subset consisted of taxonomic data for MR of a particular pot plant and control pot substrate pooled together. Filtering steps for pot plants MRs were the same as described for the overall pot MR subset. For control pot substrate, only taxa with the relative abundance $\geq 0.1\%$ represented in all three replicates were included in the final dataset.

All taxa included in datasets were categorized as “generalists” if represented in all variants of respective network or “specialists” if preferentially represented in one particular variant of the respective network.

Networks were visualized using an open-source software platform Cytoscape 3.8.2 (Shannon et al., 2003) and analyzed with an integrated into the Cytoscape platform plugin NetworkAnalyzer. Table 5 summarizes the characteristics of considered network parameters.

Table 5. Overview of network parameters.

Network parameter	Network parameter interpretation
Degree	The node degree of a node n is the number of edges linked to n (NetworkAnalyzer Online Help 2018). A node with a high degree value plays a significant role in the network.
Betweenness centrality	Betweenness centrality represents the ability of the node to connect two or more non-adjacent nodes and measures the extent of how often the node appears on the shortest paths between other nodes (Vernocchi et al. 2020). The betweenness centrality of a node reflects the amount of influence and control over the network. Node with a high betweenness centrality value can be considered as a bridge between different parts of the network. Therefore, the removal of this node from the network might disrupt interaction among other nodes as this node occurs on the largest number of paths in the network (Vernocchi et al. 2020). Betweenness centrality is a value between 0 and 1. The betweenness centrality of isolated nodes is equal to 0.
Closeness centrality	Closeness centrality is a measure of how fast information spreads from a given node to other reachable nodes in the network (NetworkAnalyzer Online Help 2018). Closeness centrality is a value between 0 and 1. The closeness centrality of isolated nodes is equal to 0.

Clustering coefficient	<p>The clustering coefficient is a ratio N / M, where N is the number of edges between the neighbours of a node n, and M is the maximum number of edges that could exist between the neighbours of n. (NetworkAnalyzer Online Help 2018).</p> <p>The clustering coefficient represents the ability to form cores.</p> <p>The clustering coefficient is a value between 0 and 1. If the degree of the node is zero or one, then the clustering coefficient equals zero (Vernocchi et al. 2020).</p>
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All multivariate analyses as well as Spearman's rank correlation analysis were performed with an open-source software PAST 4.03. Significance of multivariate analyses results was checked with one-way analysis of similarities (ANOSIM) and one-way permutational analysis of variance (PERMANOVA). The significance was calculated by permutation of group membership ($N = 9999$). Bonferroni correction was applied.

JASP 0.14.0.0 open-source software was used to perform Shapiro-Wilks's test of normality. If the data were normally distributed, one-way ANOVA analysis with post-hoc Tukey test was applied to identify significant differences between groups of values (relative abundance of taxa, diversity indices or environmental variables value). If Shapiro-Wilks's test failed, non-parametric Kruskal-Wallis test with Bonferroni correction was performed.

Diversity indices were calculated with PAST 4.03. Platform SPADE R online (Species Prediction And Diversity Estimation) (Chao et al., 2014) was used to calculate community similarity indices (Sørensen, Jaccard, Bray-Curtis) for each variant. Number of bootstrap replications was 100.

Microsoft Excel was used to calculate mean values and standard deviation of the variables, calculate and depict relative abundance of the most representative taxa as well as differences between groups of variants. Output graphs produced in PAST 4.03 were adjusted for better representability with Adobe Illustrator.

3. Results

3.1. Soil chemistry

Table 6 summarizes the data on the content of total carbon (TC), total nitrogen (TN) and total phosphorus (TP) in the soil as well as soil pH values.

Table 6. Selected soil chemical parameters.

Variant of experiment	pH	TC (%)	TN (%)	P (mg/kg)
B_MR	4.44 ± 0.32	0.87 ± 0.24	0.11 ± 0.01	669 ± 173
B_BS	3.75 ± 0.30	1.07 ± 0.05	0.13 ± 0.01	603 ± 198
B_POT	6.03 ± 0.36	0.39 ± 0.05	0.07 ± 0.00	769 ± 285
O_MR	3.54 ± 0.10	3.45 ± 0.35	0.31 ± 0.05	786 ± 127
O_BS	3.56 ± 0.09	2.60 ± 0.85	0.27 ± 0.07	802 ± 193
O_POT	5.32 ± 0.42	0.36 ± 0.01	0.07 ± 0.00	843 ± 100
P_MR	5.20 ± 1.47	0.87 ± 0.13	0.09 ± 0.01	347 ± 61
P_BS	6.21 ± 0.53	1.11 ± 0.44	0.10 ± 0.03	369 ± 37
P_POT	5.52 ± 0.22	0.37 ± 0.01	0.07 ± 0.00	718 ± 178
SUB	3.47 ± 0.01	0.33 ± 0.01	0.07 ± 0.00	801 ± 121

B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, SUB – control pot substrate.

3.1.1. Content of total carbon in soil

Overall, the soil at the field oaks sampling site contained significantly higher TC than field birches and pines sampling sites (Figure 5A). There were no significant differences in TC between mycorrhizosphere and bulk soil among the trees of one species (Figure 5A). TC in the mycorrhizospheres of field plants was significantly higher than in mycorrhizospheres of corresponding pot plants (Figure 5B). Mycorrhizospheres of pot plants did not differ from each other (Figure 5C). Pot oaks and pines mycorrhizospheres contained a significantly higher amount of TC compared to control pot substrate. Comparison of trees in different experiment variants revealed significant differences in TC only between field variants (Figure 5D). Table S1 contains tests output for the significance of differences in TC between variants of the experiment.

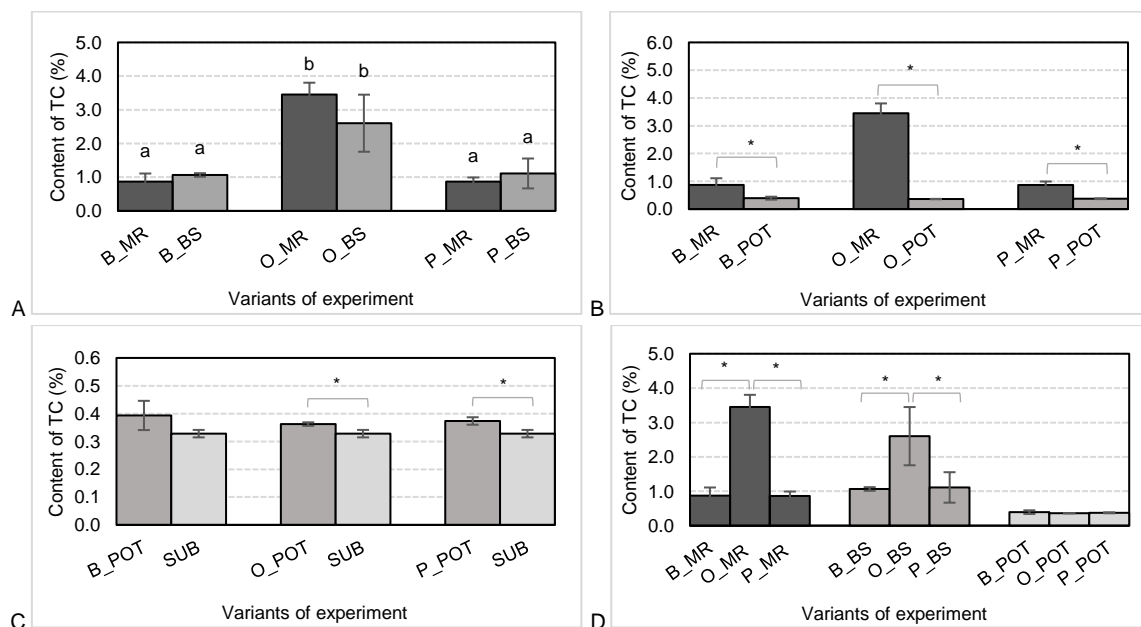


Figure 5. Content of total carbon in soil – comparison of the experiment variants: **A** – field plants mycorrhizospheres and bulk soil, **B** – comparison of field plants mycorrhizospheres and pot plants mycorrhizospheres, **C** – comparison of pot plants mycorrhizospheres and control pot substrate, **D** – comparison of trees in different variants. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks and different letters represent significant differences ($p < 0.05$).

3.1.2. Content of total nitrogen in soil

Overall, the soil at the test field was characterized with a very low amount of TN: the highest values were determined at the oaks sampling site with 0.31% in mycorrhizosphere and 0.27% in bulk soil (Figure 6A). No significant differences in TN between mycorrhizosphere and bulk soil of field plants were determined (Figure 6A). TN was higher in mycorrhizospheres of field plants compared to plants in pots (Figure 6B); however, the significance of differences was confirmed only for birches and pines. Comparison of trees in different variants showed significant differences in TN at the field between birches and oaks as well as oaks and pines (Figure 6C). Mycorrhizospheres of pot plants did not differ from each other (Figure 6C). Table S2 contains tests output for the significance of differences in TN between variants of the experiment.

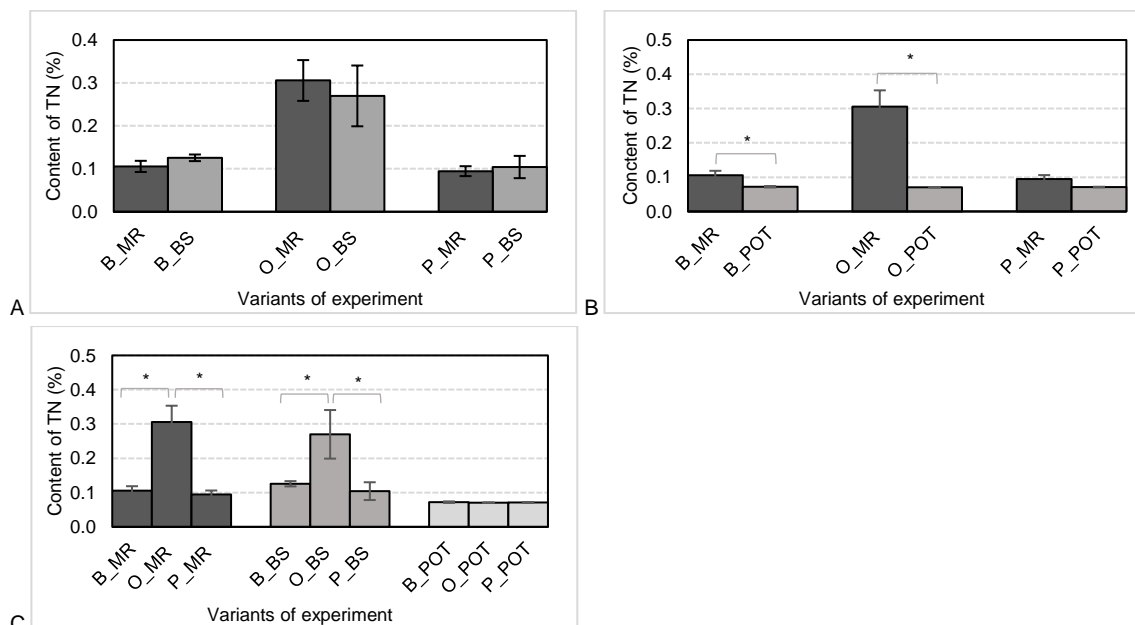


Figure 6. Content of total nitrogen in soil – comparison of the experiment variants: **A** – field plants mycorrhizospheres and bulk soil, **B** – field plants mycorrhizospheres and pot plants mycorrhizospheres, **C** – comparison of trees in different variants. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant differences ($p < 0.05$).

3.1.3. Content of total phosphorus in soil

The highest content of TP was determined at the field oaks sampling site as well as in pot variants. There were no significant differences in TP between mycorrhizosphere and bulk soil within one tree species (Figure 7A). Although field pine variants contained lower TP compared to other field sites, the significance of these differences was not confirmed (Figure 7A). TP in the mycorrhizospheres of pot plants was higher compared to corresponding field plants mycorrhizospheres; however, the significance of differences was shown only for pines (Figure 7B). There were no significant differences in TP between pot plant mycorrhizospheres and control pot substrate (Figure 7C). Comparison of trees in different variants showed significant differences in TP between field oaks and pines (Figure 7D). Mycorrhizospheres of pot plants did not differ from each other (Figure 7D). Table S3 contains tests output for the significance of differences in TP between variants of the experiment.

3.1.4. Soil pH value

According to the Soil Science Division Staff (2017) classification, measured pH values characterized field birches and oaks sampling sites as extremely acidic, field pines sampling site as moderately acidic, pot variants as strongly to moderately acidic and control pot substrate as extremely acidic. No significant

differences in pH values between mycorrhizosphere and bulk soil for field trees of one species were determined (Figure 8A). pH values in pot plant mycorrhizospheres were higher compared to field plant mycorrhizospheres (Figure 8B); the significance of differences was confirmed for birches and oaks. Control pot substrate was significantly more acidic than pot tree mycorrhizospheres (Figure 8C). Field pine variants were characterized with higher pH values compared to field birches and oaks variants; however, the significance of differences was shown only for bulk soil between these trees (Figure 8D). Mycorrhizospheres of pot plants did not differ from each other (Figure 8D). Table S4 contains tests output for the significance of differences between experiment variants in soil pH values.

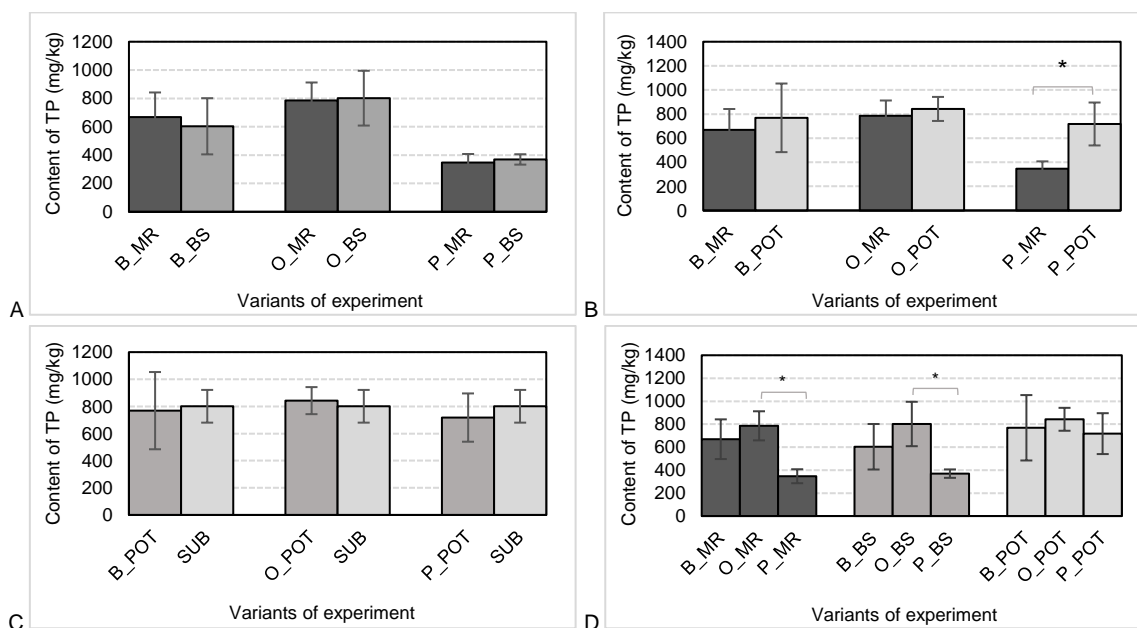


Figure 7. Content of total phosphorus in soil – comparison of the experiment variants: **A** – field plants mycorrhizospheres and bulk soil, **B** – field plants mycorrhizospheres and pot plants mycorrhizospheres, **C** – pot plants mycorrhizospheres and control pot substrate, **D** – comparison of trees in different variants. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant differences ($p < 0.05$).

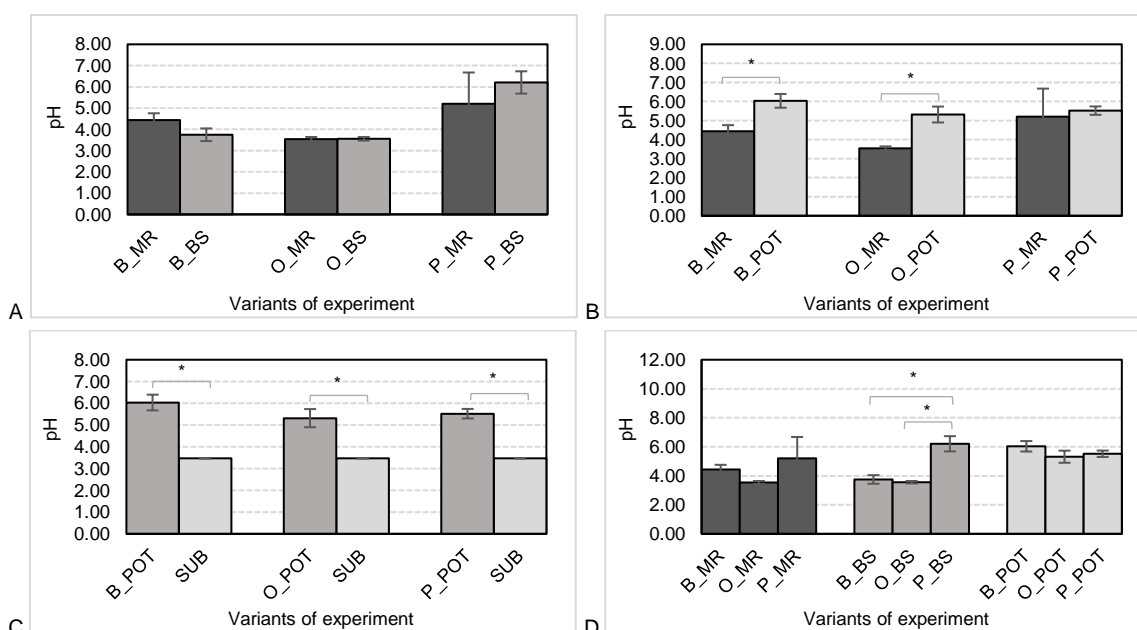


Figure 8. Soil pH values – comparison of the experiment variants: **A** – field plants mycorrhizospheres and bulk soil, **B** – field plants mycorrhizospheres and pot plants mycorrhizospheres, **C** – pot plants mycorrhizospheres and control pot substrate, **D** – comparison of trees in different variants. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant differences ($p < 0.05$).

3.1.5. Content of toxic metals in soil

Field sampling sites differed from each other in the content of toxic metals. The most prominent differences between trees were observed in the content of Al, Fe and Mn (Figure 9).

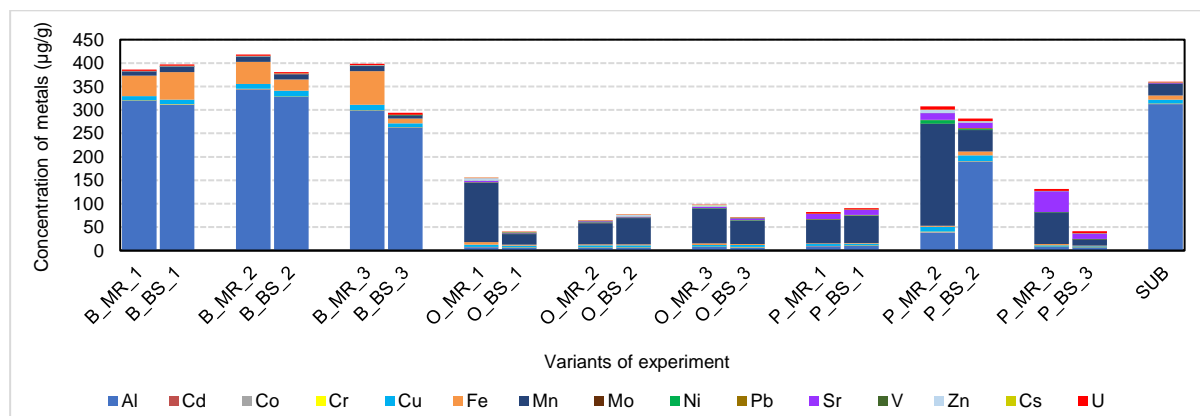


Figure 9. Sum content of toxic metals' bioavailable fractions determined in test field soil. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, SUB – control pot substrate.

Mycorrhizospheres of field birches contained significantly higher amounts of Al, Cu, Fe, U compared to oaks mycorrhizospheres, and Al, Cr, Fe compared to pines mycorrhizospheres (Table S5). Mycorrhizospheres of birches contained significantly lower amounts of Mn, Mo, Ni and Sr than oaks mycorrhizospheres and Pb compared to pines mycorrhizospheres. Mycorrhizospheres of field oaks and pines differed only in the content of Mo (significantly higher for oaks) and Pb and U (significantly higher for pines). The field pine sampling site was characterized by high variability in the content of metals. Mycorrhizosphere and bulk soil did not differ in metals' content among one tree species (Figure S1, Table S6).

A comparison of variants of the experiment within one tree species is displayed in Figure S1.

Results on chemical analyses regarding the experiment with additional inoculation of pot plants with ECM blend are displayed in Figure S2. The significance of differences between variants is included in Tables S1-S4.

Results on the content of toxic metals' bioavailable fractions regarding the experiment with additional inoculation of pot plants with the ECM blend are displayed in Figure S1. The significance of differences between variants is included in Table S6.

3.1.6. Patterns of soil characteristics in variants of the experiment

PCA did not demonstrate separation of field plants mycorrhizospheres and corresponding bulk soil (Figure 10A, Tables S7-S10). Ordination of the sites was based rather on the site identity (birches, oaks or pines sampling sites) than on the sampling variant (mycorrhizosphere or bulk soil).

PCA of field and pot variants showed distinctive separation of a cluster formed by all pot plants and field birches mycorrhizospheres from a cluster formed by field oaks mycorrhizospheres and two field pines mycorrhizospheres (Figure 10B). Separation was explained mainly by the content of Al, Cu, Mn, Pb as well as TC, TN and C/N ratio (Table S11). Nevertheless, ANOSIM and PERMANOVA tests did not confirm the significance of this separation (Tables S12-S15).

Although PCA demonstrated the distinction between a cluster formed by control pot substrate replicates, a cluster formed by pot oaks, pot pines₁ and ₂, pot birch₁, and a cluster formed by pot birches₂, ₃ and pot pine₃ (Figure 10C), ANOSIM (Tables S17-S18) and PERMANOVA (Tables S19-S20) tests did not confirm the significance of this separation. Separation of control pot substrate from pot plants mycorrhizospheres was determined by soil pH values and TP (Table S21).

Significant ordination was revealed only for the variant “FIELD SITES,” when field plants mycorrhizospheres and their corresponding bulk soil data were pooled. PCA plot (Figure 10D) depicts distinctive ordination of field birches and oaks sampling sites. Field pines sampling sites did not form a separate cluster.

The first three PCs explained approximately 77% of total variation and had eigenvalues higher than 1, providing a good ordination of the original dataset. The scree plot (Figure S3) displayed the importance of the first three components as well. A table of loadings (Table S22) contains data on the correlations between important principal components and soil characteristics (bootstrapping applied, N = 999).

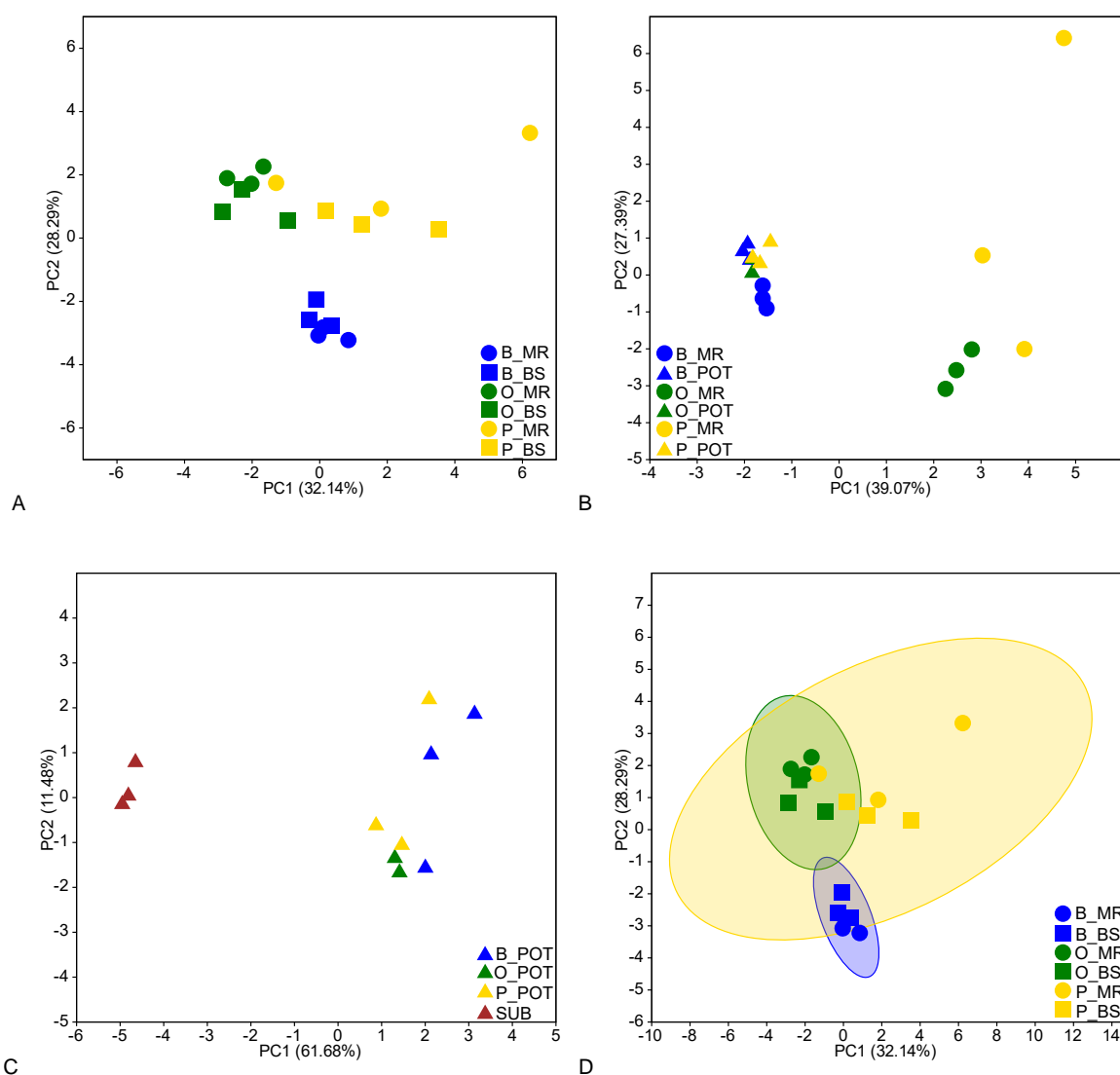


Figure 10. Principal component analysis to represent the difference between the experiment variants based on soil chemical parameters: **A** –field plants mycorrhizospheres and bulk soil, **B** – field plants mycorrhizospheres and pot plants mycorrhizospheres, **C** – pot plants mycorrhizospheres and control pot substrate, **D** – comparison of sampling sites. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

PCA biplot (Figure 11) displays the ordination of field sampling sites in correlation with soil characteristics. The cluster formed by field birches was separated from the field oaks cluster and the field pines cluster along the PC2. This pattern indicates the importance of such soil parameters as the concentration of Mn, Ni, Al, Fe, Cu, and TC, TN and C/N ratio in the ordination of sampling sites. The cluster formed by field oaks and the cluster formed by field pines were separated along the PC1 showing considerable input of such soil parameters as the concentration of U, Co, Ni, TC, TN, TP, and soil pH value.

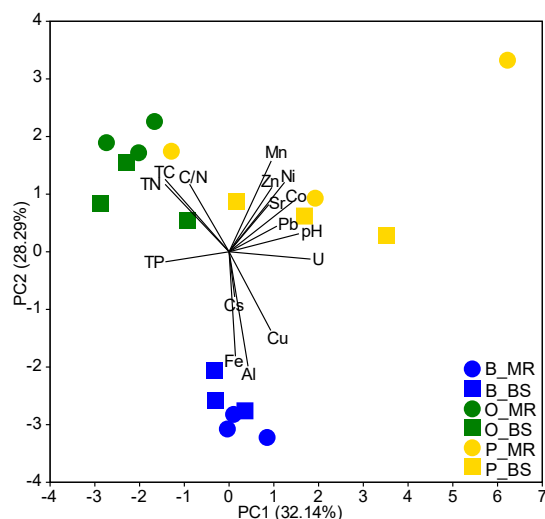


Figure 11. Principal component analysis biplot representing the input of soil parameters in the ordination of field sampling sites. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

ANOSIM test ($R = 0.64$, $p = 0.0001$) corroborated significance of ordination. ANOSIM R-statistic (Table 7) of pairwise comparison between field birches and field oaks sampling sites was 0.96, confirming high dissimilarity in soil characteristics between these sites. In contrast, R-values of pairwise comparisons between field birches and field pines sampling sites and field oaks and field pines sampling sites were 0.55 and 0.46, respectively, suggesting overlaps in soil parameters.

Table 7. Matrix table representing R-statistic of pairwise ANOSIM of field test sampling sites based on soil chemical parameters. Asterisks represent significant differences ($p < 0.05$).

	Field Birches sampling site	Field Oaks sampling site	Field Pines sampling site
Field Birches sampling site			
Field Oaks sampling site	0.96*		
Field Pines sampling site	0.55*	0.46*	

3.2. ECM community analysis

3.2.1. Morphotyping

In sum, 11 morphotypes for field plants were described: three for birches, four for oaks, four for pines. Direct ITS PCR was successful for 10 of 11 morphotypes (Tables S23-S24). The most abundant morphotypes for all field trees were dark-coloured mycorrhizas described as *Cenococcum geophilum* (with voluminous black emanating hyphae) and *Pinirhiza bicolorata* (with light brown tips) according to Agerer (1987-2006) and determined by sequence analysis as *Meliniomyces bicolor*. Overall, *M. bicolor* formed three dark-coloured morphotypes different in the shape of unramified ends and presence/absence of emanating hyphae.

Field birches ectomycorrhiza was formed predominantly by two almost equally presented taxa – ascomycete *M. bicolor* (*C. geophilum*-type) and basidiomycete *Lactarius* sp. The least presented morphotype was formed by basidiomycete *Mallocybe* sp. (Figure 12 A-C).

For field oaks, the most representative ECM taxon *M. bicolor* was described as *Pinirhiza bicolorata* according to Agerer (1987-2006) and formed two morphotypes, different in presence/absence of emanating hyphae (Figure 12 D-G). Subdominant morphotype O_F_MT2 characterized with the smooth hyphal mantle of light brown to beige colour without emanating hyphae was not determined by

sequence analysis and remained unidentified. The least represented morphotype was formed by basidiomycete *Cortinarius* sp.

For field pines, the most abundant morphotypes were formed by *M. bicolor* (*C. geophillum*-type) and a basidiomycete defined at the level of family Thelephoraceae (Figure 12 H-K). Among minor representatives were basidiomycetes *Rhizopogon mohelensis* and *Tricholoma argyraceum*.

For non-inoculated pot plants, 12 morphotypes were described: five for birches, three for oaks, four for pines (Figure 13). For inoculated pot plants, 10 morphotypes were described: three for birches, three for oaks, four for pines (Figure 14). Sequence analysis was unsuccessful for most of the pot mycorrhiza (Table S24). Among identified for non-inoculated pot plants were oak morphotype O_non_inoc_MT3 defined at the level of family Hyaloscyphaceae, and subdominant for non-inoculated pines morphotype P_non_inoc_MT1 defined as *Mallocybe* sp.

All morphotypes observed on inoculated pot pines were successfully sequenced: the most representative dark-coloured with voluminous emanating hyphae morphotype was formed by *M. bicolor*, followed by *Mallocybe* sp. The least presented morphotypes were formed by *Rhizopogon mohelnensis* and a member of the family Hyaloscyphaceae.

Pisolithus sp. was the only defined morphotype on inoculated pot oaks.

Morphotypes found on pot birches remained unidentified. ECM community of non-inoculated birches was characterized with the highest number of morphotypes – 5. Three morphotypes had a dark-coloured hyphal mantle, one of them formed abundant emanating hyphae. Morphotypes B_non_inoc_MT1 and B_non_inoc_MT3 had light brown to beige colour and rare white emanating hyphae. In inoculated pots, three morphotypes were described. One morphotype had light brown colour and a smooth mantle. Two other morphotypes were characterized by dark colour and moderate emanating hyphae; morphotype B_inoc_MT2 formed unramified mycorrhiza while B_inoc_MT3 had monopodial-pinnate branching.

Mycorrhiza observed on pot plants was characterized by a wrinkled mantle surface and light brown to reddish colour. For all trees in the glasshouse experiment amount of dark-coloured mycorrhiza decreased.

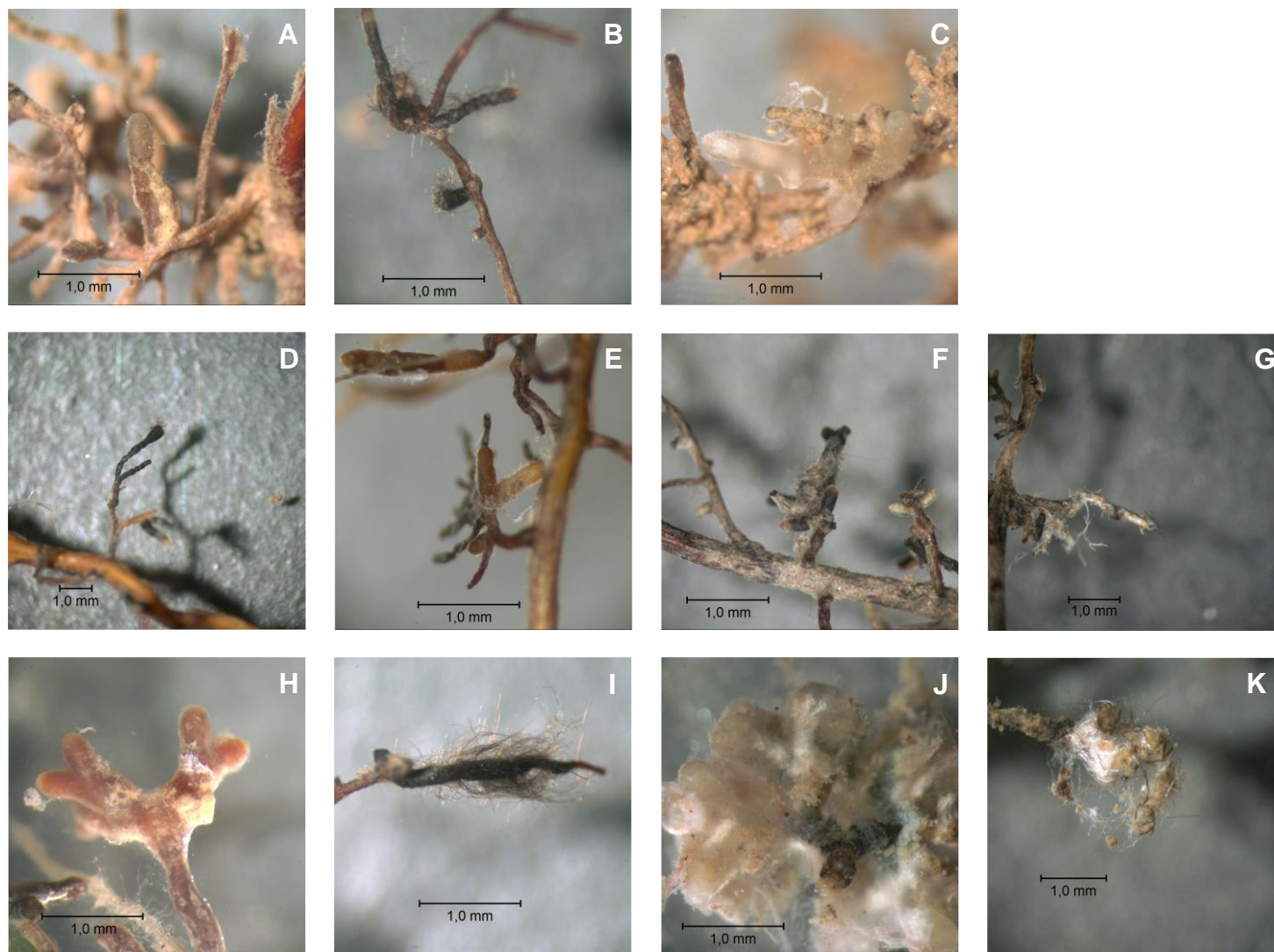


Figure 12. ECM morphotypes observed on field plants. Birch roots with (A) *Lactarius* sp., (B) *Meliniomyces bicolor* (*Pinirhiza bicolorata*-type with emanating hyphae) and (C) *Mallocybe* sp.; oak roots with (D) *Meliniomyces bicolor* (*Cenococcum geophilum*-type), (E) non-identified O_F_MT2, (F) *Meliniomyces bicolor* (*Pinirhiza bicolorata*-type w/o emanating hyphae) and (G) *Cortinarius* sp.; pine roots with (H) Thelephoraceae, (I) *Meliniomyces bicolor* (*C. geophilum*-type), (J) *Rhizopogon mohelnensis* and (K) *Tricholoma argyraceum*

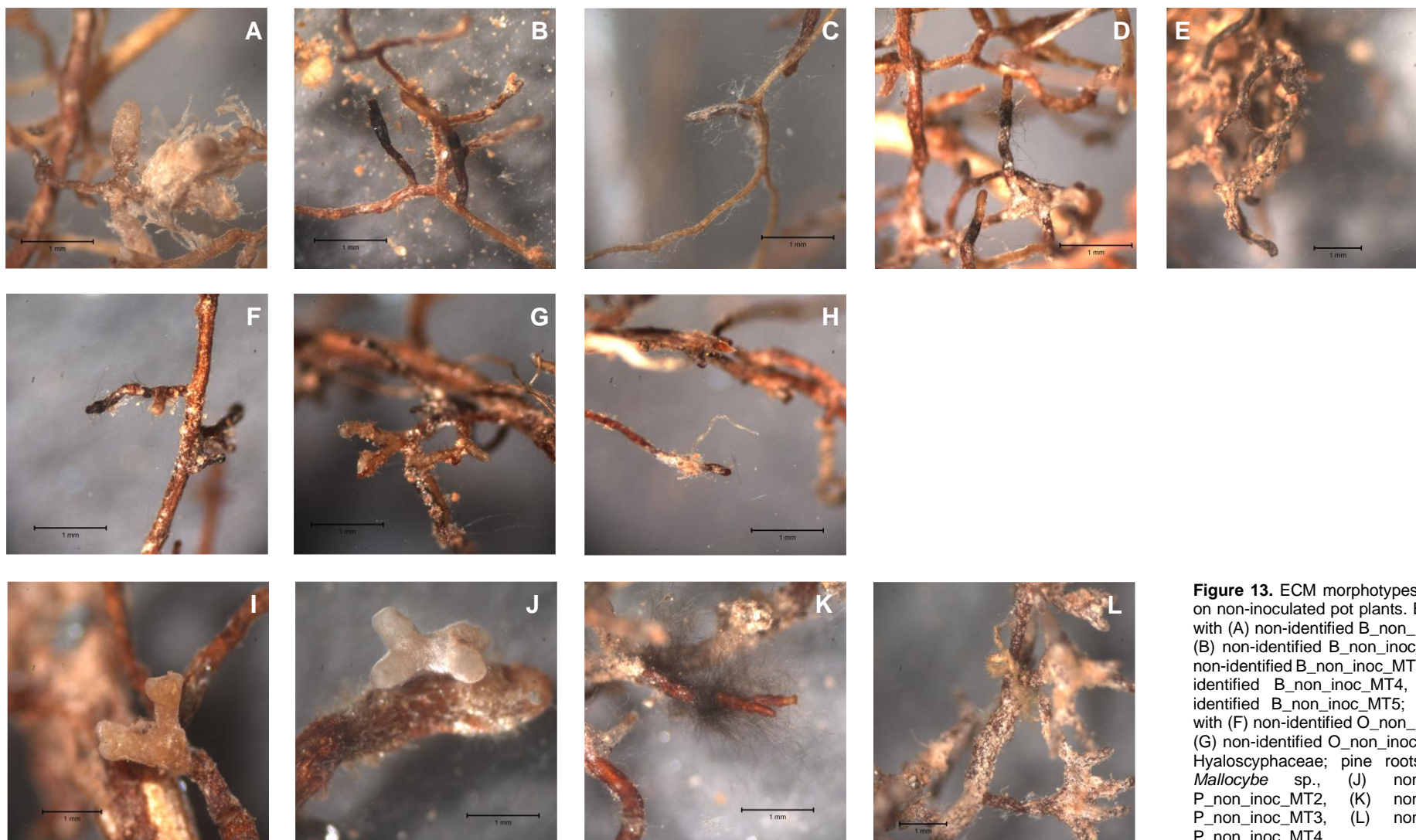


Figure 13. ECM morphotypes observed on non-inoculated pot plants. Birch roots with (A) non-identified B_non_inoc_MT1, (B) non-identified B_non_inoc_MT2, (C) non-identified B_non_inoc_MT3, (D) non-identified B_non_inoc_MT4, (E) non-identified B_non_inoc_MT5; oak roots with (F) non-identified O_non_inoc_MT1, (G) non-identified O_non_inoc_MT2, (H) Hyaloscyphaceae; pine roots with (I) *Mallocybe* sp., (J) non-identified P_non_inoc_MT2, (K) non-identified P_non_inoc_MT3, (L) non-identified P_non_inoc_MT4

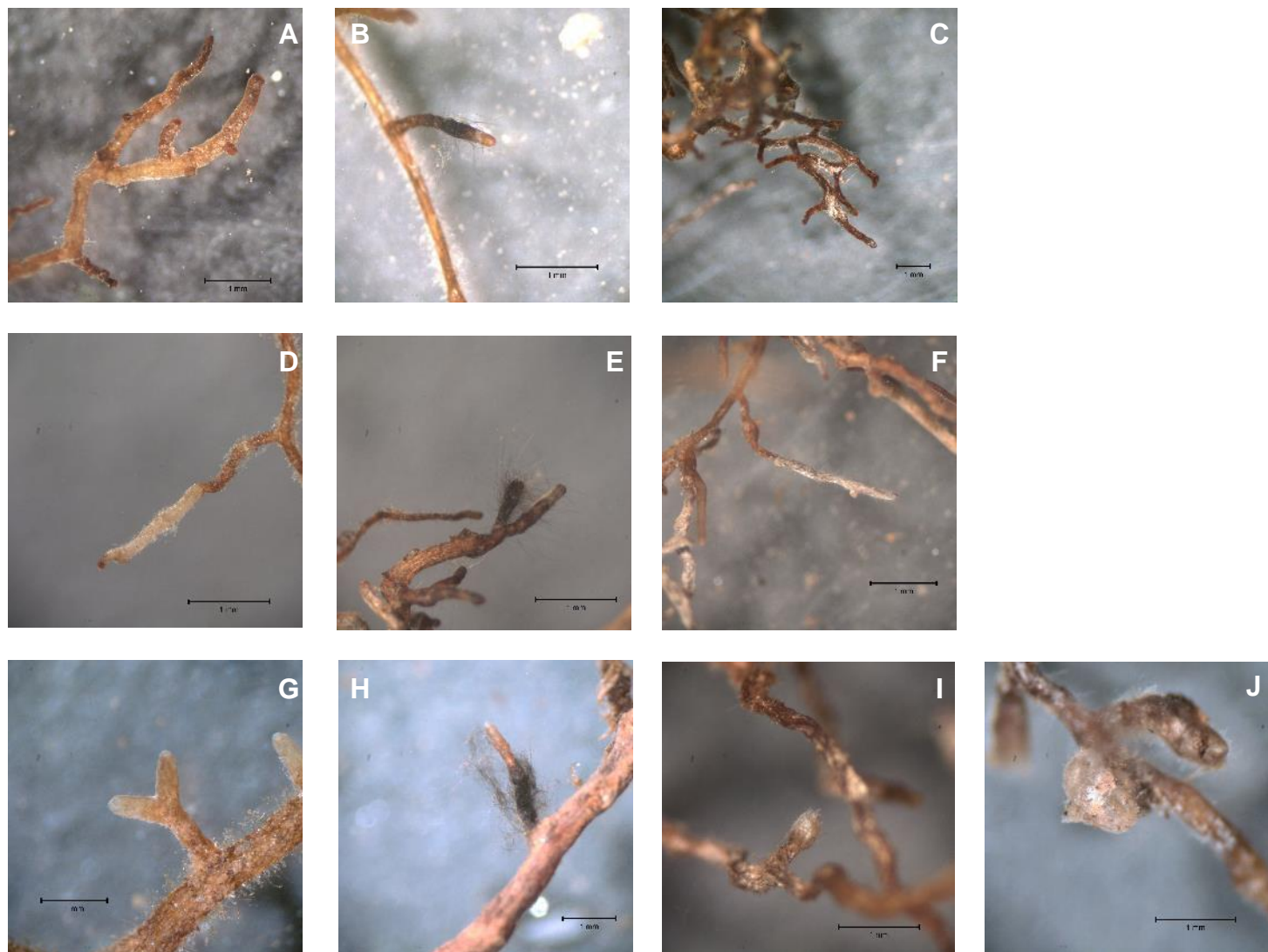


Figure 14. ECM morphotypes observed on inoculated pot plants. Birch roots with (A) B_inoc_MT1, (B) B_inoc_MT2, (C) B_inoc_MT3; oaks roots with (D) O_inoc_MT1, (E) O_inoc_MT2, (F) *Pisolithus* sp.; pine roots with (G) *Mallocybe* sp., (H) Hyaloscyphaceae, (I) *Meliniomyces bicolor*, (J) *Rhizopogon mohelnensis*

3.2.2. ECM community diversity

Table 8 summarizes data on ECM community diversity indices. ECM fungal community described for non-inoculated pot plants pots was characterized, in general, with higher diversity compared to other variants of the experiment. The highest values of Shannon diversity (1.03) and Gini-Simpson indices (0.61) were calculated for non-inoculated pot oaks, and the lowest values of these indices were determined for field oaks and inoculated pot birches. Berger-Parker index had the highest values for inoculated pot birches (0.75) and field oaks, suggesting that common morphotypes mainly dominated ECM communities in these variants. ECM community diversity of trees of the same species did not differ among each other (Table S25).

Table 8. Diversity indices calculated for ECM communities in different variants of the experiment.

Variant	H_{SD}	H_{GS}	H_{SH}	H_{BP}
Field Birches	0.60 ± 0.11	0.39 ± 0.11	0.64 ± 0.16	0.72 ± 0.13
Field Oaks	0.66 ± 0.20	0.34 ± 0.20	0.55 ± 0.30	0.73 ± 0.21
Field Pines	0.55 ± 0.14	0.45 ± 0.14	0.71 ± 0.24	0.66 ± 0.15
Non-inoculated pot Birches	0.48 ± 0.05	0.52 ± 0.05	0.90 ± 0.13	0.60 ± 0.07
Non-inoculated pot Oaks	0.39 ± 0.06	0.61 ± 0.06	1.03 ± 0.08	0.50 ± 0.13
Non-inoculated pot Pines	0.57 ± 0.13	0.43 ± 0.13	0.77 ± 0.16	0.69 ± 0.16
Inoculated pot Birches	0.68 ± 0.36	0.32 ± 0.36	0.56 ± 0.55	0.75 ± 0.31
Inoculated pot Oaks	0.51 ± 0.11	0.49 ± 0.11	0.77 ± 0.18	0.59 ± 0.14
Inoculated pot Pines	0.49 ± 0.13	0.51 ± 0.17	0.82 ± 0.21	0.57 ± 0.17

S – richness, H_{SH} – Shannon diversity index, H_{GS} – Gini-Simpson index, H_{SD} – Simpson dominance index, H_{BP} – Berger-Parker index.

Comparison of similarity indices revealed that ECM communities among the trees in one variant were similar (Table S26). Sørensen similarity index had high values and ranged from 0.833 (determined for field oaks, and field and inoculated pot pines) to 1.000 (determined for non-inoculated pot oaks), suggesting high similarity of ECM communities described for trees within one variant. Similarly, the Bray-Curtis similarity index, which considers not only the presence/absence of species but also their abundance, had high values from 0.852 for inoculated pot pines to 1.000 calculated for field and inoculated pot birches, pot oaks and non-inoculated pot pines. Thus, ECM communities within trees of one species were similar in observed morphotypes. Low values of the Jaccard coefficient were calculated for field oaks (0.500) and field pines (0.500), suggesting that these trees had only 50% of common species. For trees in other variants, the Jaccard coefficient ranged from 0.700 to 1.000 representing high similarity within one tree species.

3.2.3. Functional diversity of ECM community

Among all field trees, the most abundant exploration types were contact and short-distance exploration types (Figure 15). *Lactarius* sp. and *Mallochybe* sp. observed on field birches, unidentified field oak morphotype O_F_MT2 with smooth hyphal mantle and no rhizomorphs, dark-coloured without emanating hyphae field oak morphotype belonging to Helotiales, and field pine morphotype formed by a member of family Thelephoraceae, contributed to contact exploration type.

Dark-coloured with voluminous black emanating hyphae *M. bicolor* constituted all mycorrhiza with short-distance exploration type at the field. *Cortinarius* sp. observed on field oaks formed fans of ramified rhizomorphs and was attributed to medium-distance fringe subtype exploration type. On one field pine, *T. argyraceum* formed white-coloured mycorrhiza with hairy hyphal mantle, extensive emanating hyphae and interconnected rhizomorphs, and was related to medium-distance fringe subtype exploration type. Pine was the only tree species with observed long-distance exploration type, which was formed by *R. mohelnensis*. It had coralloid ramification and moderately presented hairy rhizomorphs.

Pot birches had mycorrhiza grouped into contact and short-distance exploration types. The ratio of relative abundance of exploration types in pots changed compared to field plants. While for non-inoculated birches amount of short-distance exploration type significantly increased to 45.5%, for inoculated variant contact exploration type constituted 95%.



Figure 15. Relative abundance of exploration types of mycorrhiza in different variants of the experiment. Contact – contact exploration type, short – short-distance exploration types, medium – medium-distance exploration type, long – long-distance exploration type.

Pot oaks were dominated by contact exploration type. The relative abundance of short-distance exploration type for non-inoculated pot variant was less, compared to field variant; however, according to the Kruskal-Wallis test, the difference was not significant (Table S27). The medium-distance exploration type observed in non-inoculated pots was formed by the fungus related to the family Hyaloscyphaceae. Relative abundance of this exploration type significantly increased compared to field plants. Inoculation of oaks with the ECM blend led to the loss of medium-distance exploration type mycorrhiza. Subdominant short-distance exploration type in inoculated pots was formed by *Pisolithus* sp. and characterized by the woolly hyphal mantle with moderately presented emanating hyphae. Non-inoculated pines in pots formed mycorrhiza with grainy hyphal mantle and elongated well-differentiated rhizomorphs and was classified as long-distance exploration type. Unidentified short-distance exploration type mycorrhiza with dark-coloured voluminous emanating hyphae was rare and observed only on one tree. Two morphotypes formed contact exploration type with smooth hyphal mantle, mainly dichotomous branching and no emanating hyphae, one of which was identified as *Mallocybe* sp. Inoculation of pot pines resulted in the total removal of long-distance exploration type mycorrhiza and led to the dominance of contact and short-distance exploration types. Contact exploration type was represented by *Mallocybe* sp. Short-distance exploration type comprised three morphotypes of different morphology. The most representative morphotype with short-distance exploration type was formed by *M. bicolor* and had different morphology compared to morphotypes formed by this species on other trees in all variants: mycorrhiza had a brown colour, woolly hyphal mantle with rare white emanating hyphae. Morphotype with abundant dark-coloured emanating hyphae was observed only on one inoculated tree and identified at the level of the Hyaloscyphaceae family. The least presented morphotype was formed by *R. mohelnensis* had a woolly hyphal mantle and short emanating hyphae. Additional inoculation of pines did not significantly change the relative abundance of contact and short-distance exploration types compared with field and non-inoculated pot plants. Overall, additional inoculation of all plants in pots caused the loss of rhizomorphs by plant roots.

3.3. Bacterial community analysis

Rarefaction curves showed that all samples reached the plateau with increased sequencing depth, suggesting that saturation in sequencing was achieved (Figure 16). At a similar sequencing depth, the highest numbers of ASVs were revealed in field oaks and pines samples. The lowest numbers of ASVs were observed in control pot substrate, pot birches and pot pines samples.

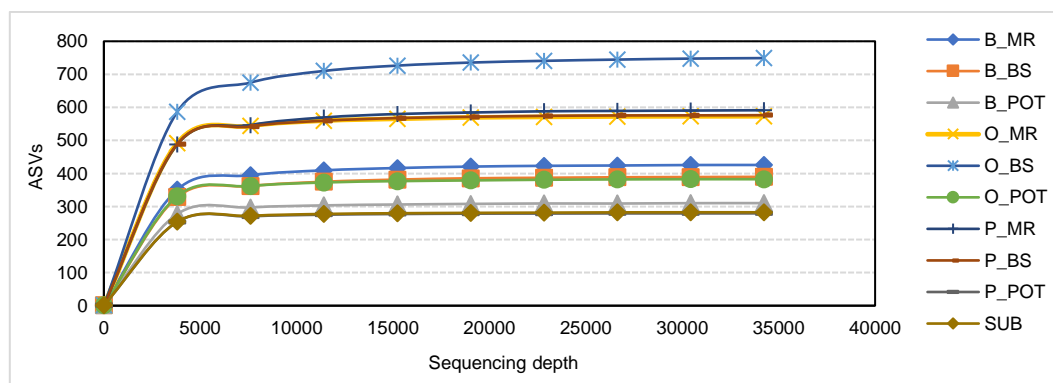


Figure 16. Rarefaction curves for bacterial sequences. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

3.3.1. Bacterial community structure

The most representative classes among all experiment variants were Alphaproteobacteria, Ktedonobacteria, Acidobacteriia, Bacteroidia, Phycisphaerae, Gammaproteobacteria (Figure 17). All together, they contributed from 53 to 79% of the bacterial community. On the other hand, Actinobacteria, Verrucomicrobiae, Planctomycetacia, Oxyphotobacteria, Thermoleophilia, Blastocatellia, Deltaproteobacteria, Gemmatimonadetes, Acidimicrobiia, Holophagae, Anaerolineae, Chloroflexia were represented to the less extent.

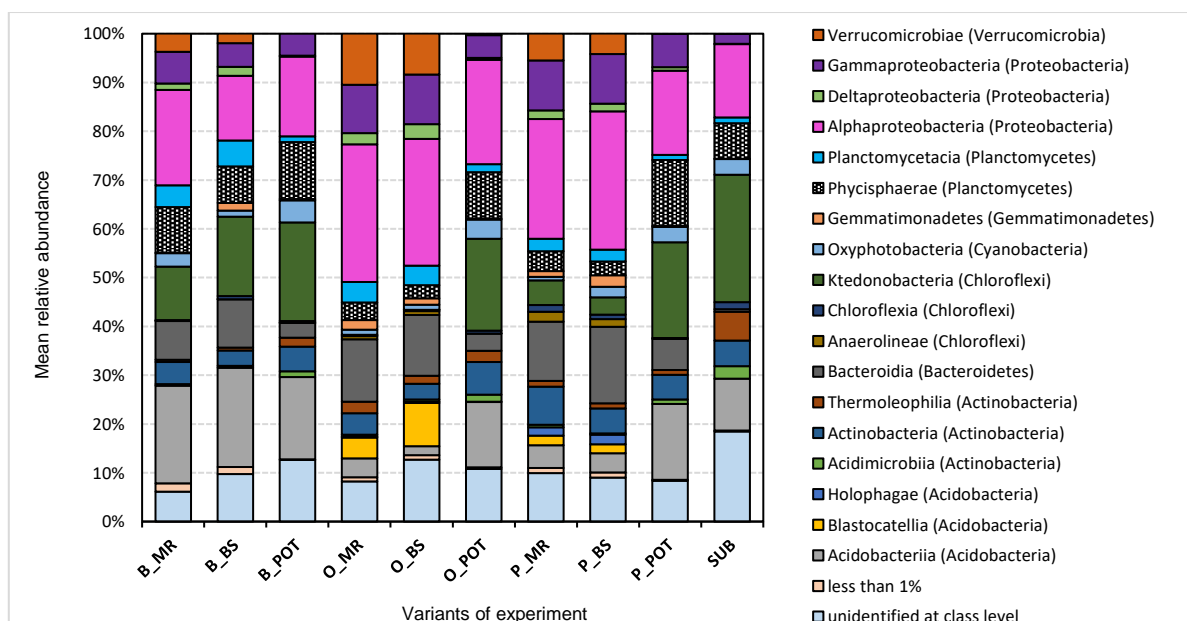


Figure 17. Structure of bacterial communities in different variants of the experiment. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Bacterial classes with a relative abundance of more than 1% are shown.

Common for all variants of the experiment bacterial classes were Alphaproteobacteria, Acidobacteriia, Phycisphaerae, Gammaproteobacteria, Actinobacteria, Planctomycetacia, Thermoleophilia. Classes

Verrucomicrobiae and Gemmatimonadetes were not observed in any pot plant variants as well as control pot substrate. Deltaproteobacteria was missing in pot birches and pot oaks mycorrhizospheres and control pot substrate; however, it was observed in pine pot variant with low relative abundance. Class Bacteroidia was defined in all variants except control pot substrate. Acidimicrobiia was observed in pot birch and oak variant as well as in control pot substrate, but not in pot pine variant. Blastocatellia was indicative for field oaks and pines (both mycorrhizosphere and bulk soil). Classes Holophagae and Anaerolineae were exclusively defined for field pines. Chloroflexia was typical for field pines variants and control pot substrate. Ktedonobacteria was the only class missing for field oak variants. The most representative bacterial classes were characterized at the genus level. The data are presented in Figure S4.

3.3.2. Bacterial community diversity

The highest richness (number of ASVs) was described for field variants of the experiment (Table 9). Similarly, the highest diversity was defined for field variants: Shannon index had the highest values for field birches mycorrhizospheres, field oaks and pines (both mycorrhizosphere and bulk soil). At the same time, all experiment variants were characterized by similar values of the Gini-Simpson index. Pot variants had high values of the Berger-Parker index, suggesting that bacterial communities in pot soil were less even, compared to the field bacterial communities.

Table 9. Diversity indices calculated for bacterial communities in different variants of the experiment.

Variant	S	H_{SD}	H_{GS}	H_{SH}	H_{BP}
B_MR	434.67±64.61	0.01±0.00	0.99±0.00	5.01±0.17	0.06±0.01
B_BS	393.67±50.85	0.02±0.00	0.98±0.00	4.91±0.12	0.06±0.00
B_POT	312.67±29.50	0.02±0.00	0.98±0.00	4.68±0.09	0.08±0.01
O_MR	576.67±81.05	0.01±0.00	0.99±0.00	5.58±0.13	0.03±0.01
O_BS	755.67±106.88	0.01±0.01	0.99±0.00	5.64±0.19	0.04±0.07
O_POT	385.00±89.10	0.02±0.00	0.98±0.00	4.96±0.31	0.06±0.01
P_MR	597.33±264.47	0.02±0.01	0.98±0.01	5.32±0.64	0.08±0.05
P_BS	582.33±27.43	0.01±0.00	0.99±0.00	5.49±0.10	0.04±0.01
P_POT	280.67±22.37	0.02±0.00	0.98±0.00	4.71±0.11	0.05±0.01
SUB	284.00±21.00	0.03±0.00	0.97±0.00	4.52±0.07	0.12±0.01

S – richness, H_{SH} – Shannon diversity index, H_{GS} – Gini-Simpson index, H_{SD} – Simpson dominance index, H_{BP} – Berger-Parker index. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

Pairwise comparisons revealed differences between particular variants of the experiment (Table S28). Similar to taxonomical data, no significant differences in diversity indices neither between field plants mycorrhizospheres and their bulk soil nor between mycorrhizospheres of field plants compared to each other were determined (Figure S5-S6). Bacterial communities, colonizing pot plants mycorrhizospheres, were more diverse (Gini-Simpson index) than in control pot substrate (Figure S7). ANOVA test revealed a significant impact of the most abundant taxa in the bacterial community of control pot substrate compared to pot plants mycorrhizospheres (Simpson dominance and Berger-Parker index).

Bacterial communities within one tree species in field variants were less similar, whereas pot plants of the same species were rather similar in diversity (Table S29). Bacterial communities inhabiting mycorrhizospheres of pot birches and pot pines, as well as control pot substrate, were characterized with the high values of Sørensen index (0.756, 0.673, 0.729 respectively), suggesting high similarity of bacterial community diversity determined within these variants. At the same time, low values were calculated for most of the field variants (with the lowest 0.222 for field pine mycorrhizosphere and 0.242 for field pine bulk soil). The lowest values of the Jaccard index were determined for field pines (0.087 in mycorrhizosphere and 0.096 in BS), showing that bacterial communities shared a very low number of common species with each other. Jaccard index had the highest values in pot variants. The Bray-Curtis index calculated for pot variants had high values (from 0.614 in pot oak mycorrhizosphere to 0.814 in control pot substrate), characterizing bacterial communities as more similar. In contrast, the Bray-Curtis index determined for field oaks and pines had low values from 0.144 in field pine

mycorrhizospheres to 0.265 in field oak mycorrhizospheres, describing bacterial communities within these variants as less similar.

3.3.3. Plant and bacterial community association

3.3.3.1. Role of tree identity in the shaping of bacterial communities in mycorrhizosphere at the field

Trees of different species at the test field shaped mycorrhizospheres, similar in structure of bacterial communities (Figure 18), which was confirmed by ANOSIM (Tables S30-S31) and PERMANOVA (Tables S32-S33) tests. Pairwise comparisons of the most representative in field plants mycorrhizospheres bacterial classes revealed significant differences in representability of two classes: relative abundance of Acidobacteriia in field birches mycorrhizospheres significantly exceeded those in field oaks and field pines mycorrhizospheres, and relative abundance of Phycisphaerae inhabiting field birches mycorrhizospheres was significantly higher than in field oaks mycorrhizospheres (Table S34).

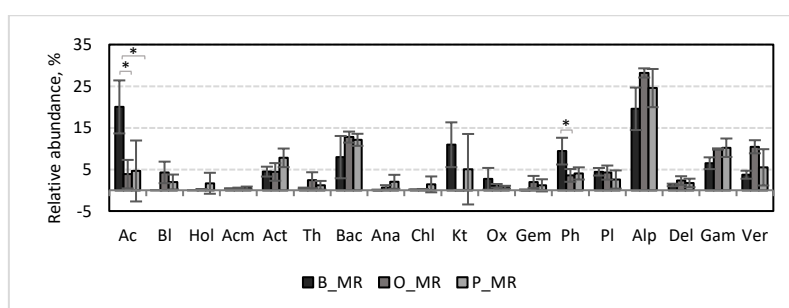


Figure 18. Comparison of the structure of bacterial communities inhabiting field plants mycorrhizospheres. Ac – Acidobacteriia, Acm – Acidimicrobiia, Act – Actinobacteria, Alp – Alphaproteobacteria, Ana – Anaerolineae, Bac – Bacteroidia, Bl – Blastocatellia, Chl – Chloroflexia, Del – Deltaproteobacteria, Gam – Gammaproteobacteria, Gem – Gemmatimonadetes, Hol – Holophagae, Kt – Ktedonobacteria, Ox – Oxyphotobacteria, Ph – Phycisphaerae, Pl – Planctomycetacea, Th – Thermoleophilia, Ver – Verrucomicrobiae. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant. Asterisks represent significant differences ($p < 0.05$).

SIMPER analysis showed that differences between field plants mycorrhizospheres were determined overall by Acidobacteriia, Ktedonobacteria and Alphaproteobacteria (Table S35).

ANOSIM (Tables S36-S37) and PERMANOVA (Tables S38-S39) tests did not demonstrate that bacterial communities, inhabiting bulk soil at different sampling sites, differed from each other; however, pairwise comparisons of relative abundance of the most abundant in bulk soil bacterial classes revealed that multiple taxa were distributed differently at the test field (Figure 19, Table S40).

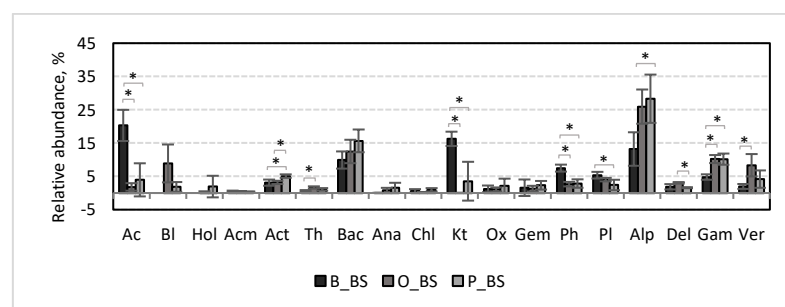


Figure 19. Comparison of the structure of bacterial communities inhabiting field plants bulk soil. Ac – Acidobacteriia, Acm – Acidimicrobiia, Act – Actinobacteria, Alp – Alphaproteobacteria, Ana – Anaerolineae, Bac – Bacteroidia, Bl – Blastocatellia, Chl – Chloroflexia, Del – Deltaproteobacteria, Gam – Gammaproteobacteria, Gem – Gemmatimonadetes, Hol – Holophagae, Kt – Ktedonobacteria, Ox – Oxyphotobacteria, Ph – Phycisphaerae, Pl – Planctomycetacea, Th – Thermoleophilia, Ver – Verrucomicrobiae. B – birch, O – oak, P – pine; BS – bulk soil. Asterisks represent significant differences ($p < 0.05$).

SIMPER analysis showed that the most contributing to differences between bulk soil at different sampling sites taxa were Acidobacteriia, Alphaproteobacteria and Ktedonobacteria (Table S41).

3.3.3.2. Role of tree presence in the shaping of bacterial communities in mycorrhizosphere at the field

Field plants did not show a rhizosphere effect: NMDS did not demonstrate separation of mycorrhizospheres and bulk soil of field trees, which was confirmed statistically (Figure 20, Tables S42-S45). The ordination of these variants was determined by site identity. Moreover, no significant differences in the relative abundance of the most representative bacterial classes between mycorrhizosphere and bulk soil for any tree species were observed (Tables S46-S48).

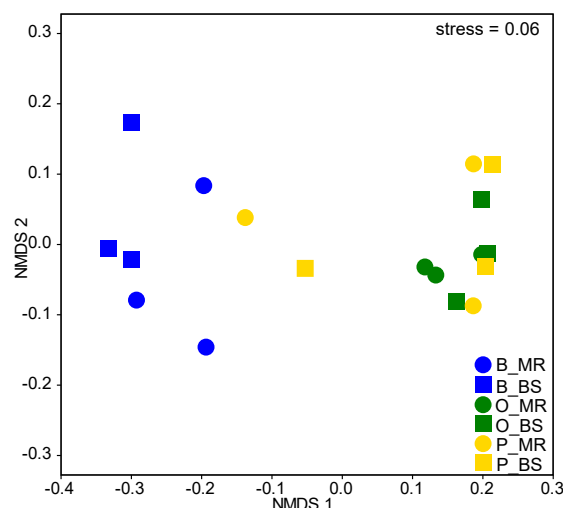
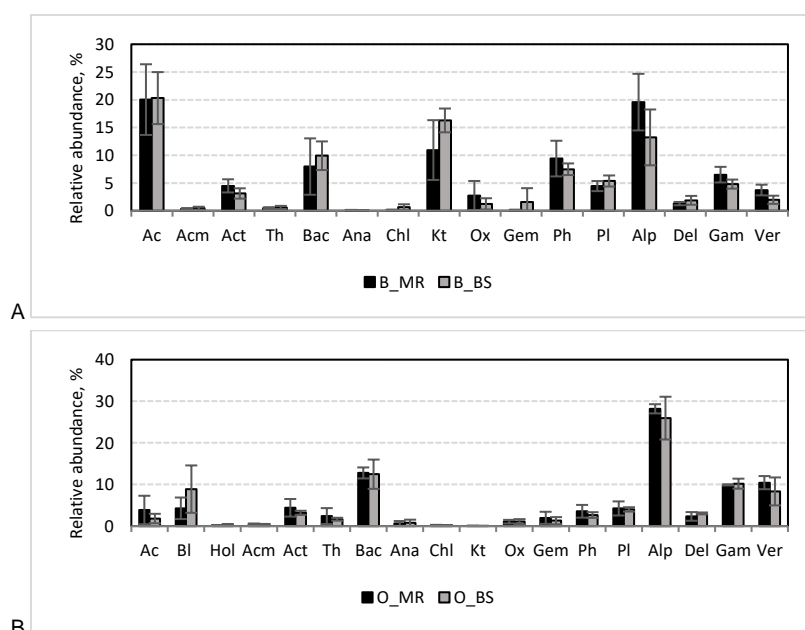


Figure 20. Non-metric dimensional scaling of field plants mycorrhizospheres and bulk soil based on the relative abundance of the most representative bacterial classes. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

SIMPER analysis showed that the most contributing to differences between field mycorrhizospheres and bulk soil taxa were Acidobacteriia, Alphaproteobacteria and Ktedonobacteria (Table S49).

The most abundant taxa found at the field birch sampling site were Acidobacteriia, Alphaproteobacteria and Ktedonobacteria (Figure 21A). The field oaks sampling site was inhabited mainly by Alphaproteobacteria and less by Bacteroidia, Gammaproteobacteria and Verrucomicrobiae (Figure 21B). The bacterial community in field pines variants was represented mainly by Alphaproteobacteria and Bacteroidia (Figure 21C).



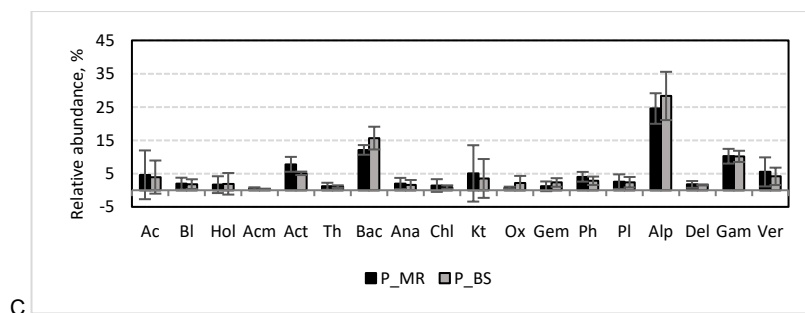


Figure 21. Comparison of the structure of bacterial communities inhabiting field plants mycorrhizospheres and bulk soil: **A** – field birches mycorrhizospheres and bulk soil, **B** – field oaks mycorrhizospheres and bulk soil, **C** – field pines mycorrhizospheres and bulk soil. Ac – Acidobacteriia, Acm – Acidimicrobiia, Act – Actinobacteria, Alp – Alphaproteobacteria, Ana – Anaerolineae, Bac – Bacteroidia, Bl – Blastocatellia, Chl – Chloroflexia, Del – Deltaproteobacteria, Gam – Gammaproteobacteria, Gem – Gemmatimonadetes, Hol – Holophagae, Kt – Ktedonobacteria, Ox – Oxyphotobacteria, Ph – Phycisphaerae, Pl – Planctomycetacea, Th – Thermoleophilia, Ver – Verrucomicrobiae. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil. Asterisks represent significant differences ($p < 0.05$).

3.3.3.3. Role of tree presence in the shaping of bacterial communities in mycorrhizosphere in pots

Pot plants established different from control pot substrate bacterial communities: NMDS demonstrated separation between control pot substrate and mycorrhizospheres of pot plants (Figure 22). Nevertheless, the statistical significance of this ordination was not confirmed (Tables S50-S53).

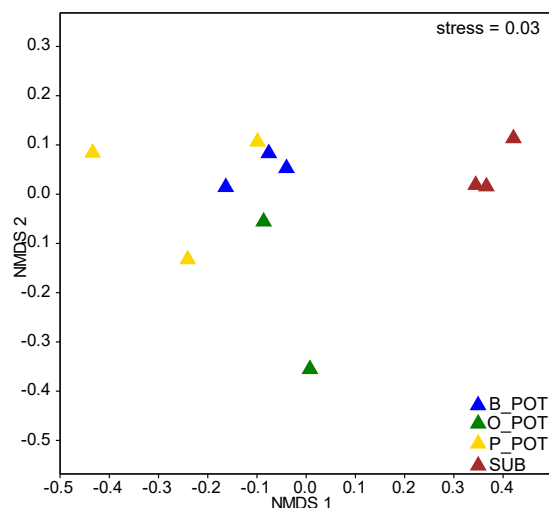


Figure 22. Non-metric dimensional scaling of pot plants mycorrhizospheres and control pot substrate based on the relative abundance of the most representative bacterial classes. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

SIMPER analysis showed that among the most contributing to differences between pot plants mycorrhizospheres and control pot substrate taxa were Acidobacteriia, Alphaproteobacteria, Bacteroidia and Ktedonobacteria (Table S54).

Pairwise comparisons revealed multiple significant differences in the relative abundance of the most representative bacterial classes between pot plants mycorrhizospheres and control pot substrate (Tables S55-S57). Relative abundance of Acidobacteriia, Bacteroidia, Gemmatimonadetes, Phycisphaerae and Gammaproteobacteria was significantly higher in pot birches mycorrhizospheres. In contrast, the relative abundance of Acidimicrobiia, Thermoleophilia, Chloroflexia and Ktedonobacteria was significantly higher in the control pot substrate (Figure 23A). Percentage of Actinobacteria, Bacteroidia, Phycisphaerae and Deltaproteobacteria was significantly higher in mycorrhizospheres of pot oaks compared to control pot substrate (Figure 23B). The bacterial community inhabiting pot pines mycorrhizospheres was characterized by the higher amount of

Acidobacteriia, Bacteroidia, Phycisphaerae, Delta- and Gammaproteobacteria compared to control pot substrate (Figure 23C).

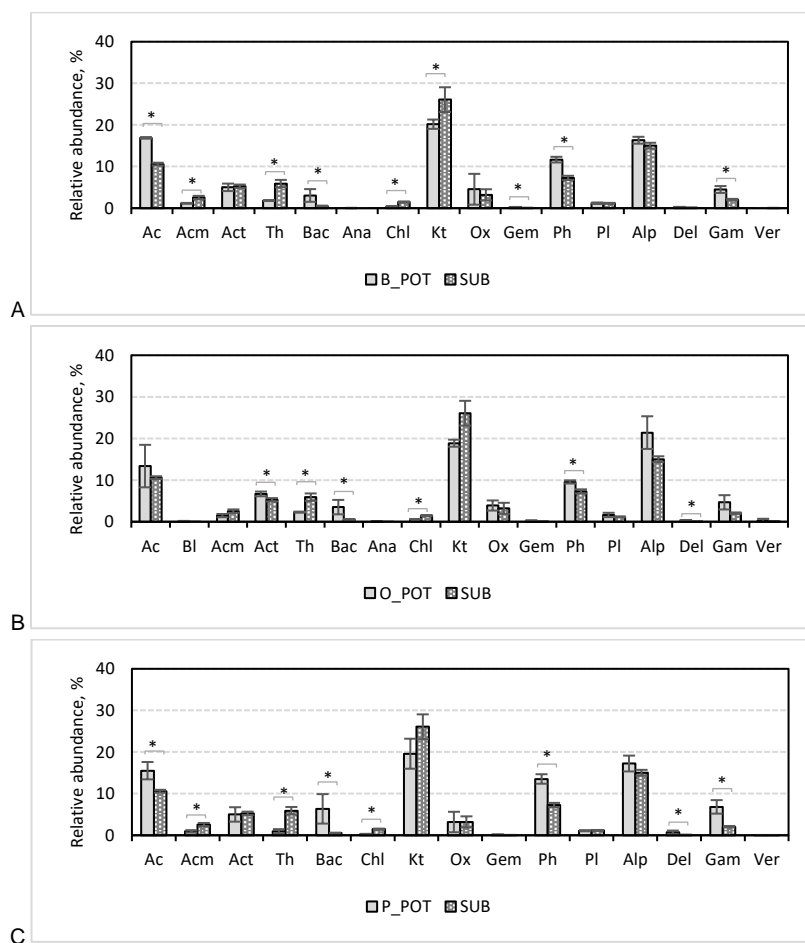


Figure 23. Comparison of the structure of bacterial communities inhabiting pot plants mycorrhizospheres and control pot substrate: **A** – pot birches mycorrhizospheres and control pot substrate, **B** – pot oaks mycorrhizospheres and control pot substrate, **C** – pot pines mycorrhizospheres and control pot substrate. Ac – Acidobacteriia, Acm – Acidimicrobiia, Act – Actinobacteria, Alp – Alphaproteobacteria, Ana – Anaerolineae, Bac – Bacteroidia, Bl – Blastocatellia, Chl – Chloroflexia, Del – Deltaproteobacteria, Gam – Gammaproteobacteria, Gem – Gemmatimonadetes, Kt – Ktedonobacteria, Ox – Oxyphotobacteria, Ph – Phycisphaerae, Pl – Planctomycetacea, Th – Thermoleophilia, Ver – Verrucomicrobiae. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant differences ($p < 0.05$).

3.3.4. Site identity and bacterial community association

NMDS demonstrated distinct ordination of two clusters: 1) cluster formed by field birches and field pine_2 sampling sites, 2) cluster formed by field oaks and field pines_1 and _3 sampling sites (Figure 24). Bacterial NMDS for variant “FIELD SITES” had a stress value of 0.06, representing a good method for ordination.

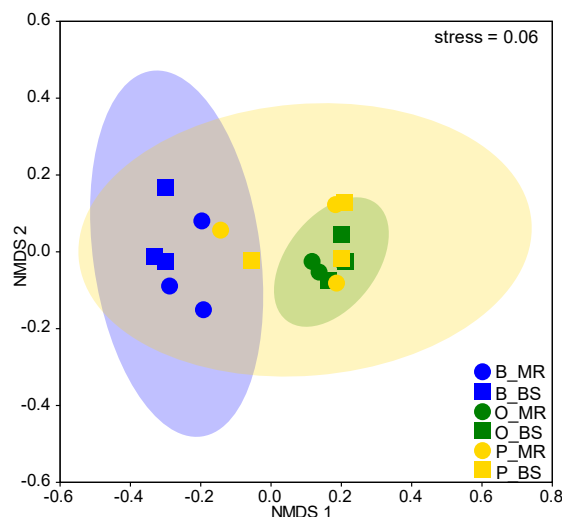


Figure 24. Non-metric dimension scaling of field sampling sites based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

ANOSIM test ($R = 0.69$, $p < 0.05$) confirmed the significance of ordination. ANOSIM R-value (Table 10) of pairwise comparison between field birches and field oaks sampling sites was 0.99, confirming high dissimilarity in bacterial community compositions between these sites. R-value between field birches and field pines was 0.76, showing relatively high dissimilarity in bacterial communities' structure with several common taxa. R-value of pairwise comparisons between field oaks and field pines sampling sites was low and constituted 0.23 revealing that bacterial communities of these sites were similar; however, ordination of these variants was not significant.

Table 10. Matrix table representing R-statistic of pairwise ANOSIM of field sampling sites based on the relative abundance of the most representative bacterial taxa. Asterisks represent significant differences ($p < 0.05$).

	Field Birches sampling site	Field Oaks sampling site	Field Pines sampling site
Field Birches sampling site			
Field Oaks sampling site	0.99*		
Field Pines sampling site	0.76*	0.23	

Similarly, the PERMANOVA test ($F = 11.57$, $p < 0.05$) showed the significance of ordination of field birches and field oaks, but not the ordination of field oaks and field pines (Table 11).

Table 11. PERMANOVA p-values of pairwise comparisons between field sampling sites based on the relative abundance of the most representative bacterial taxa. Asterisks represent significant differences ($p < 0.05$).

	Field Birches sampling site	Field Oaks sampling site	Field Pines sampling site
Field Birches sampling site			
Field Oaks sampling site	0.01*		
Field Pines sampling site	0.01*	0.22	

SIMPER analysis revealed that the most contributing to the dissimilarity between field sampling sites taxa were Acidobacteriia, Ktedonobacteria and Alphaproteobacteria (Table 12).

Table 12. SIMPER analysis demonstrating the contribution (%) of the most abundant bacterial classes to Bray-Curtis dissimilarity between field sampling sites. Only bacterial classes with a contribution higher than 10% are included in the table.

	Field Birches sampling site	Field Oaks sampling site	Field Pines sampling site
Field birches sampling site			
Field Oaks sampling site	Acidobacteriia (21.85%) Ktedonobacteria (17.15%) Alphaproteobacteria (13.89%)		
Field Pines sampling site	Acidobacteriia (22.04%) Alphaproteobacteria (14.87%) Ktedonobacteria (14.46%)	Alphaproteobacteria (12.2%) Verrucomicrobiae (11.27%) Blastocatellia (11.25%)	

3.3.5. Bacterial communities along the successional development of the mycorrhizosphere

NMDS showed distinct separation of three clusters: 1) a cluster formed by field oaks, field pine_1 and field pine_3, 2) a cluster formed by control pot substrate, 3) a cluster formed by field birches and all pot plants (Figure 25).

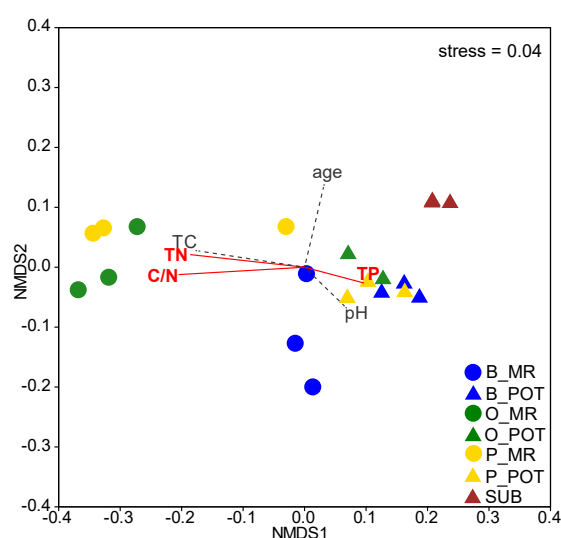


Figure 25. Non-metric dimensional scaling of bacterial communities along successional stages based on the relative abundance of the most representative bacterial classes. Vectors represent successional gradients. Parameters which had a significant effect shown in red colour B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

pH value and TP explained clustering of pot variants, while TC, TN and C/N ratio contributed to the distinction of field oaks and pines. Permutation analysis ($N = 999$, $p = 0.001$) showed significance of TN ($p = 0.028$), TP ($p = 0.017$) and C/N ($p = 0.001$) ratio, but not the stage of succession, in ordination of bacterial communities along the successional MR development.

ANOSIM (Tables S58-S59) and PERMANOVA (Tables S60-S61) tests did not confirm significance of ordination based on the successional gradients. ANOSIM R-coefficients showed that along simulated succession *control pot substrate* → *pot plant MR* → *field plant MR* bacterial communities associated with birches and pines tended to be less contrasting in structure. This trend was not observed for oaks, and associated with them bacterial communities had dissimilar compositions. Significance of these patterns was not confirmed (Table S59).

3.4. Fungal community analysis

Rarefaction curves showed that with increased sequencing depth, almost all samples reached or had a tendency to reach the plateau, suggesting that saturation in sequencing was attained (Figure 26). At a similar sequencing depth, the highest numbers of ASVs were revealed in pines bulk soil and control pot substrate samples. Conversely, the lowest numbers of ASVs were observed in field birches mycorrhizospheres and mycorrhizospheres of pot plants.

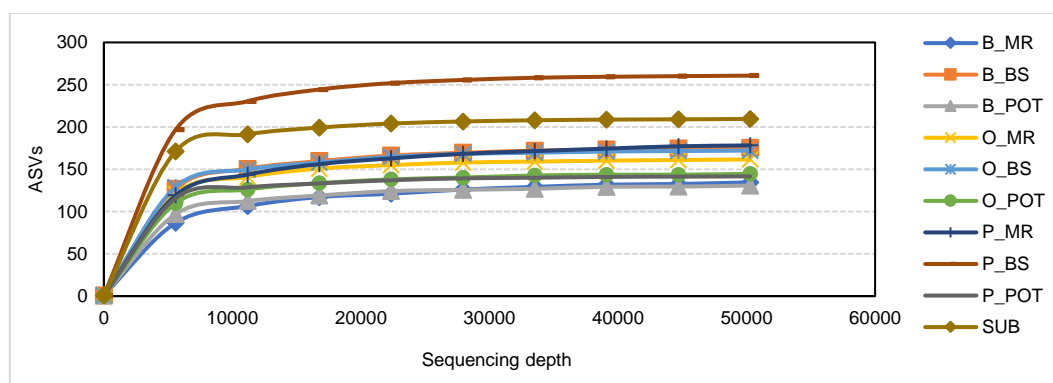


Figure 26. Rarefaction curves for fungal sequences. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

3.4.1. Fungal community structure

The most representative fungal families depending on the variant of the experiment were Thelephoraceae, Inocybaceae, Russulaceae, Leotiaceae and Herpotrichiellaceae. All together, they contributed from 35% (in control pot substrate) to 85% (in field birches mycorrhizospheres) of the fungal community (Figure 27).

Variants of the experiment differed in representability and relative abundance of fungal taxa. Only Thelephoraceae, Leotiaceae and Herpotrichiellaceae were found in all experiment variants with relative abundance higher than 1%. The highest proportion of these taxa was observed in pot birches mycorrhizospheres (44.0%), control pot substrate (22.6%) and field oaks mycorrhizospheres (22.1%). Atheliaceae and Pezizaceae were indicative for field pines variants and had a very low proportion. Hydnangiaceae was not found in field oaks variants, and its relative abundance in other variants did not exceed 6%. Serendipitaceae was observed in field oaks and pines variants and at a very low level in pot pines mycorrhizospheres and was absent in all birch variants.

The most representative fungal families were characterized at the genus level. The data are presented in Figure S9.

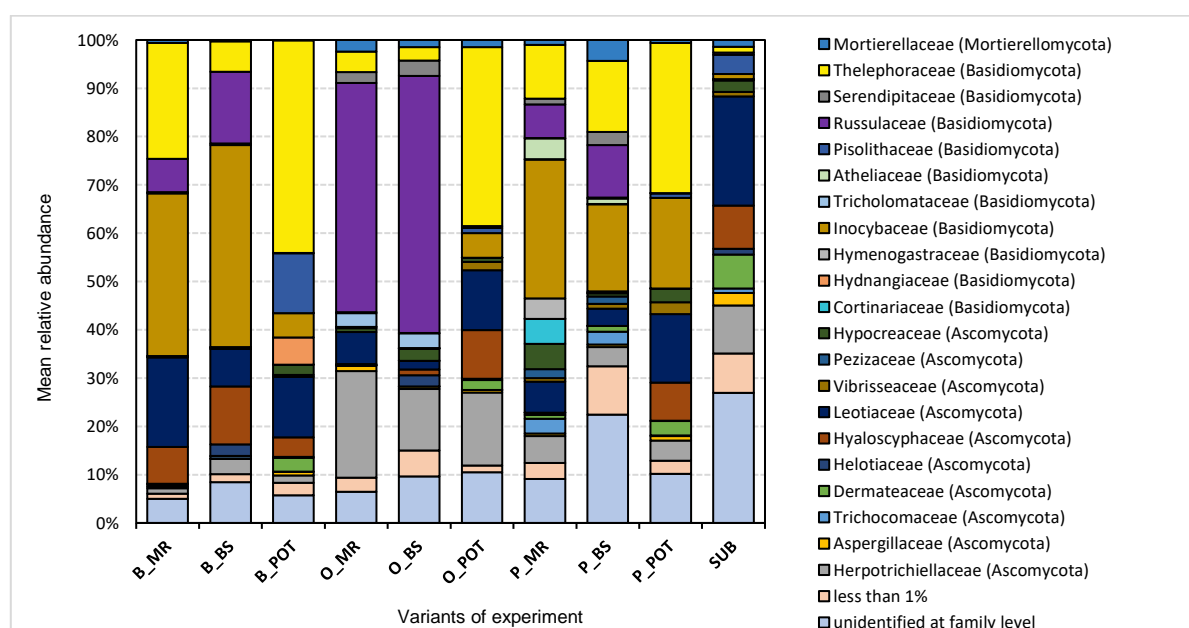


Figure 27. Structure of fungal communities in different variants of the experiment. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Fungal families with a relative abundance of more than 1% are shown.

3.4.2. Fungal community diversity

The lowest number of ASVs was determined for pot plants mycorrhizospheres; however, the number of ASVs in the control pot substrate was considerably higher (Table 13). The highest richness was defined for pines bulk soil. The highest diversity expressed as Gini-Simpson and Shannon indices was calculated for control pot substrate and pines bulk soil, whereas the lowest diversity was determined for field birches variants as well as for pot oaks. In contrast, Berger-Parker and Simpson dominance indices were the highest in these variants, showing lower evenness than other variants and emphasizing the significance of highly represented species.

Table 13. Diversity indices calculated for fungal communities in different variants of the experiment.

Variant	S	H_{SD}	H_{GS}	H_{SH}	H_{BP}
B_MR	138.67±49.41	0.25±0.06	0.75±0.06	1.95±0.49	0.38±0.09
B_BS	176.00±39.15	0.23±0.07	0.77±0.07	2.28±0.31	0.41±0.08
B_POT	133.00±11.14	0.24±0.25	0.76±0.25	2.47±0.88	0.36±0.33
O_MR	162.00±14.11	0.15±0.05	0.85±0.05	2.62±0.37	0.29±0.06
O_BS	172.33±64.40	0.21±0.12	0.79±0.12	2.51±0.41	0.38±0.16
O_POT	145.50±105.36	0.31±0.29	0.69±0.29	2.30±1.31	0.48±0.33
P_MR	182.67±24.54	0.17±0.08	0.83±0.08	2.61±0.34	0.33±0.16
P_BS	261.00±72.33	0.09±0.05	0.91±0.05	3.39±0.60	0.21±0.11
P_POT	142.00±11.14	0.14±0.03	0.86±0.03	2.83±0.12	0.27±0.08
SUB	210.00±17.78	0.04±0.00	0.96±0.00	3.87±0.06	0.11±0.01

S – richness, H_{SH} – Shannon diversity index, H_{GS} – Gini-Simpson index, H_{SD} – Simpson dominance index, H_{BP} – Berger-Parker index. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

ANOVA test revealed the significance of differences between groups of variants (Table S62).

No significant differences in diversity indices calculated for field plants mycorrhizospheres and corresponding bulk soil were found (Figure S10). Pairwise comparison of the variants revealed significant differences in all diversity indices only between control pot substrate and pot pines mycorrhizospheres (Figure S11). Fungal community in control pot substrate was more diverse (richness, Gini-Simpson and Shannon indices) than in pot pines mycorrhizospheres. At the same time, significantly higher values of Simpson dominance and Berger-Parker index calculated for pot pines showed the importance of the most abundant taxa for the diversity of pot plants.

Overall, fungal communities as replicates in different variants of the experiments were dissimilar (Table S63). Sørensen similarity had low values describing fungal communities within one variant as dissimilar. The highest values were determined for field birches bulk soil (0.267), pot pines mycorrhizospheres (0.225) as well as control pot substrate (0.214). Fungal communities within field pines variants (0.145 in mycorrhizosphere and 0.149 in bulk soil) and pot oaks mycorrhizospheres (0.124) were characterized with the lowest Sørensen coefficient values and, thus, were less similar to each. Similarly, the Jaccard coefficient was very low in all variants and ranged from 0.054 in field pines mycorrhizospheres to 0.108 in field birches bulk soil. The Bray-Curtis index described the fungal communities within variants of the experiment as dissimilar, with the highest value 0.509 calculated for field birches bulk soil and the lowest value 0.062 for field oaks mycorrhizospheres as well as pot birches mycorrhizospheres.

3.4.3. Plant and fungal community association

3.4.3.1. Role of tree identity in the shaping of fungal communities in mycorrhizosphere at the field

Trees of different species at the test field shaped mycorrhizospheres, similar in structure of fungal communities, which was confirmed with ANOSIM (Tables S64-S65) and PERMANOVA (Tables S66-S67) tests. Overall, the mycorrhizospheres of field plants were dominated by Inocybaceae, Russulaceae, Thelephoraceae, Leotiaceae and Herpotrichiellaceae (Figure 28). Relative abundance of Russulaceae and Herpotrichiellaceae was significantly higher in field oaks mycorrhizospheres compared to field birches and field pines mycorrhizospheres. The percentage of Hyaloscyphaceae determined in field birches mycorrhizospheres was significantly higher than in mycorrhizospheres of other field plants (Tables S68).

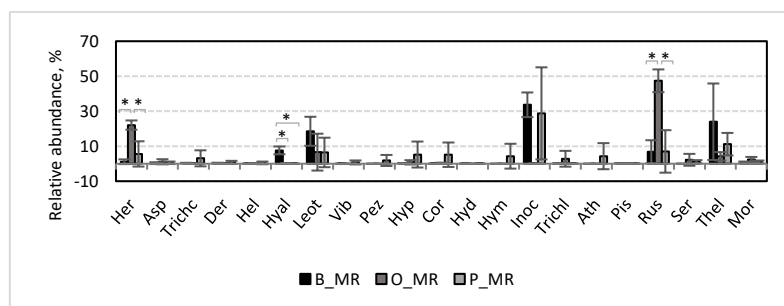


Figure 28. Comparison of the structure of fungal communities inhabiting field plants mycorrhizospheres. Asp – Aspergillaceae, Ath – Atheliaceae, Cor – Cortinariaceae, Der – Dermateaceae, Hel – Helotiaceae, Her – Herpotrichiellaceae, Hyal – Hyaloscyphaceae, Hyd – Hydnangiaceae, Hym – Hymenogastraceae, Hyp – Hypocreaceae, Inoc – Inocybaceae, Leot – Leotiaceae, Mor – Mortierellaceae, Pez – Pezizaceae, Pis – Pisolithaceae, Rus – Russulaceae, Ser – Serendipitaceae, Thel – Thelephoraceae, Trichc – Trichocomaceae, Trichl – Tricholomataceae, Vib – Vibrissaceae. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant. Asterisks represent significant differences ($p < 0.05$).

SIMPER analysis showed that the most contributing to differences between field plants mycorrhizospheres taxa were Russulaceae, Inocybaceae and Thelephoraceae (Table S69).

Similar to field plants mycorrhizospheres, fungal communities inhabiting bulk soil were dominated by Inocybaceae, Russulaceae, Thelephoraceae and Herpotrichiellaceae (Figure 29) and were similar to each other based on Bray-Curtis metric (Tables S70-S73).

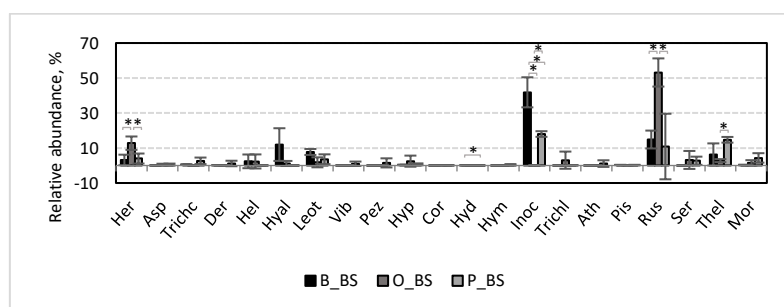


Figure 29. Comparison of the structure of fungal communities inhabiting field plants BSs. Asp – Aspergillaceae, Ath – Atheliaceae, Cor – Cortinariaceae, Der – Dermateaceae, Hel – Helotiaceae, Her – Herpotrichiellaceae, Hyal – Hyaloscyphaceae, Hyd – Hydnangiaceae, Hym – Hymenogastraceae, Hyp – Hypocreaceae, Inoc – Inocybaceae, Leot – Leotiaceae, Mor – Mortierellaceae, Pez – Pezizaceae, Pis – Pisolithaceae, Rus – Russulaceae, Ser – Serendipitaceae, Thel – Thelephoraceae, Trichc – Trichocomaceae, Trichl – Tricholomataceae, Vib – Vibrissaceae. B – birch, O – oak, P – pine; BS – bulk soil. Asterisks represent significant differences ($p < 0.05$).

Pairwise comparison of variants with each other revealed significant differences in the relative abundance of dominant taxa (Table S74).

SIMPER analysis showed that the most contributing to differences between bulk soil at different sampling sites taxa were Russulaceae, Inocybaceae and Thelephoraceae (Table S75).

3.4.3.2. Role of tree presence in the shaping of fungal communities in mycorrhizosphere at the field

Plants did not demonstrate rhizosphere effect for birches and oaks; however, NMDS showed that bulk soil samples and the mycorrhizosphere of pine_3 formed a distinct cluster (Figure 30). Nevertheless, ANOSIM (Tables S76-S77) and PERMANOVA (Tables S78-S79) tests did not confirm the significance of this separation.

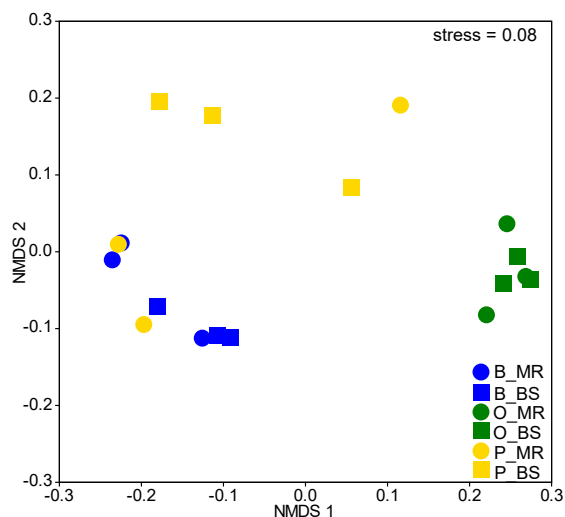
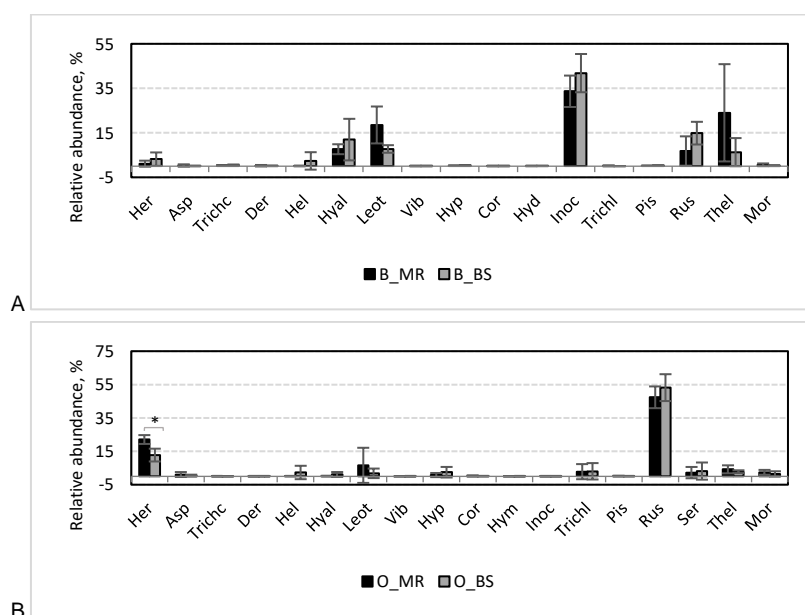


Figure 30. Non-metric dimensional scaling of field plants mycorrhizospheres and bulk soil based on the relative abundance of the most representative fungal families. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

SIMPER analysis showed that the most contributing to differences between field plants mycorrhizospheres and bulk soil taxa were Russulaceae, Inocybaceae and Thelephoraceae (Table S80).

The most representative fungal taxa at the field birches sampling site were Hyaloscyphaceae, Leotiaceae, Inocybaceae, Russulaceae and Thelephoraceae (Figure 31A, Table S81). The fungal community at the field oaks sampling site was inhabited predominantly by Russulaceae and Herpotrichiellaceae, and, to less extent, by Leotiaceae (Figure 31B). The difference in relative abundance between field oaks variants was determined only for Herpotrichiellaceae, whose percentage was significantly higher in the mycorrhizosphere compared to bulk soil (Table S82). Among the most representative fungal families at the field pines sampling site were Inocybaceae, Russulaceae and Thelephoraceae (Figure 31C, Table S83).



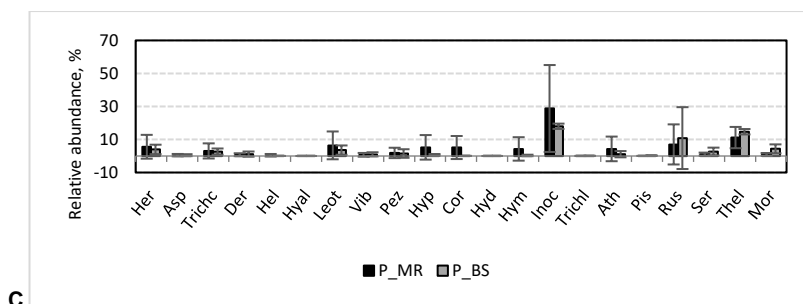


Figure 31. Comparison of the structure of fungal communities in field plants mycorrhizospheres and corresponding bulk soil: **A** – field birches mycorrhizospheres and bulk soil, **B** – field oaks mycorrhizospheres and bulk soil, **C** – field pines mycorrhizospheres and bulk soil. Asp – Aspergillaceae, Ath – Atheliaceae, Cor – Cortinariaceae, Der – Dermateaceae, Hel – Helotiaceae, Her – Herpotrichiellaceae, Hyal – Hyaloscyphaceae, Hyd – Hydnangiaceae, Hym – Hymenogastraceae, Hyp – Hypocreaceae, Inoc – Inocybaceae, Leot – Leotiaceae, Mor – Mortierellaceae, Pez – Pezizaceae, Pis – Pisolithaceae, Rus – Russulaceae, Ser – Serendipitaceae, Thel – Thelephoraceae, Trichc – Trichocomaceae, Trichl – Tricholomataceae, Vib – Vibrisseaceae. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil. Asterisks represent significant differences ($p < 0.05$).

3.4.3.3. Role of tree presence in the shaping of fungal communities in mycorrhizosphere in pots

Pot plants were associated with rather dissimilar to control pot substrate fungal communities; however, clustering of the samples was not so distinct as for bacterial communities (Figure 32, Tables S84-S87).

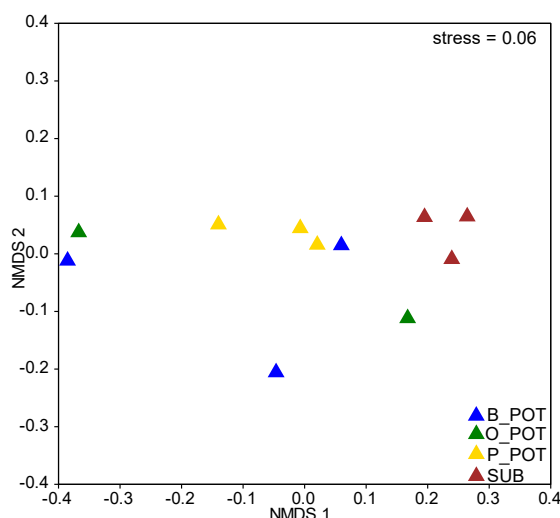


Figure 32. Non-metric dimensional scaling of pot plants mycorrhizosphere and control pot substrate based on the relative abundance of the most representative fungal families. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

SIMPER analysis showed that the most contributing to differences between pot plants mycorrhizospheres and control pot substrate taxa were Thelephoraceae, Leotiaceae, Inocybaceae, Herpotrichiellaceae (Table S88). At the same time, differences in fungal communities inhabiting pot plants mycorrhizospheres were mainly determined by Thelephoraceae, Inocybaceae, Herpotrichiellaceae and Pisolithaceae.

Pairwise comparisons revealed relatively contrasting fungal communities in pot birches and pot pines mycorrhizospheres compared to control pot substrate (Figure 33A, B, Tables S89, S91). In contrast, pot oaks mycorrhizospheres differed from control pot substrate only in the abundance of Pisolithaceae (Figure 33B, Table S90).

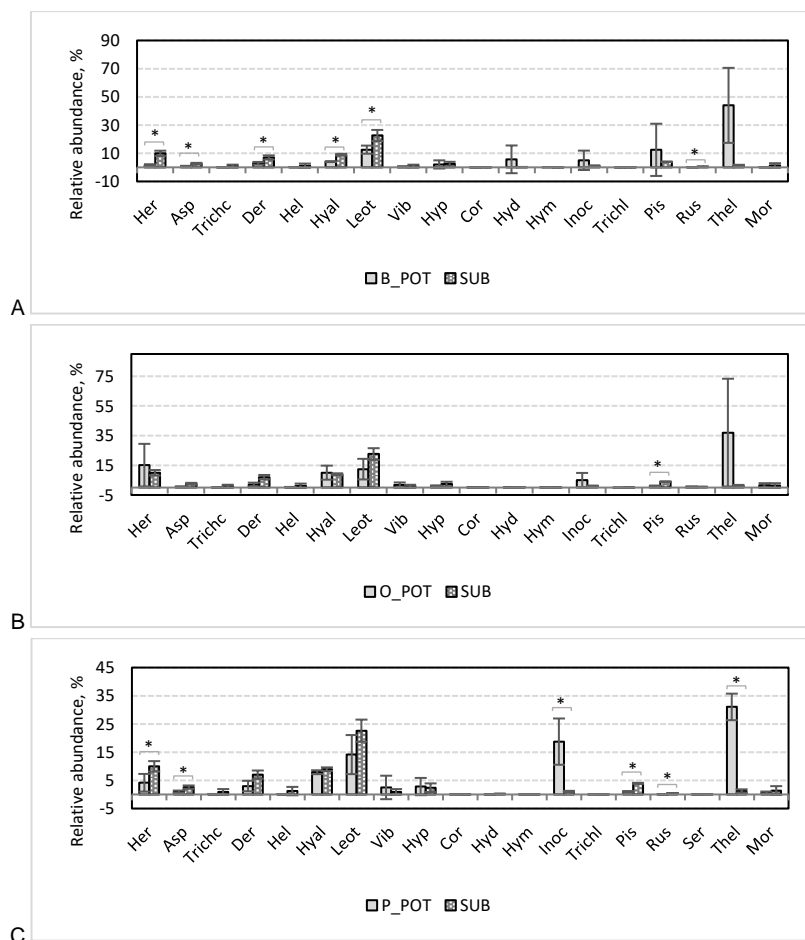


Figure 33. Comparison of the structure of fungal communities inhabiting pot plants mycorrhizospheres and control pot substrate: **A** – pot birches mycorrhizospheres and control pot substrate, **B** – pot oaks mycorrhizospheres and control pot substrate, **C** – pot pines mycorrhizospheres and control pot substrate. Asp – Aspergillaceae, Cor – Cortinariaceae, Der – Dermateaceae, Hel – Helotiaceae, Her – Herpotrichiellaceae, Hyal – Hyaloscyphaceae, Hyd – Hydnangiaceae, Hym – Hymenogastraceae, Hyp – Hypocreaceae, Inoc – Inocybaceae, Leot – Leotiaceae, Mor – Mortierellaceae, Pis – Pisolithaceae, Rus – Russulaceae, Ser – Serendipitaceae, Thel – Thelephoraceae, Trichc – Trichocomaceae, Trichl – Tricholomataceae, Vib – Vibrisseaceae. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant differences ($p < 0.05$).

3.4.4. Site identity and fungal community association

NMDS showed a distinct separation of fungal communities based on their site identity (Figure 34). Fungal NMDS had a stress value of 0.08, representing a fair method for ordination.

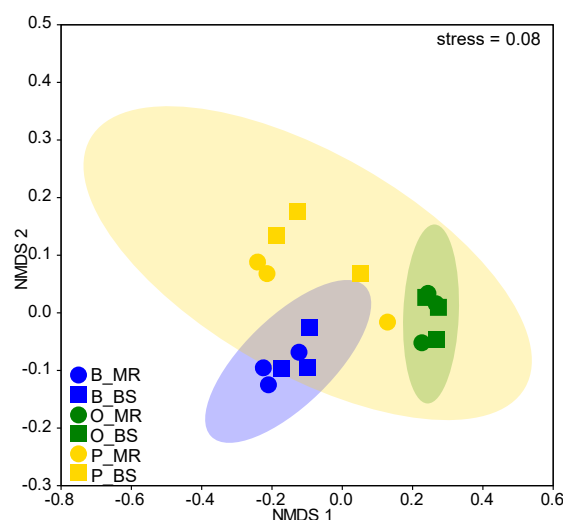


Figure 34. Non-metric dimension scaling of field sampling sites based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

ANOSIM test ($R = 0.79$, $p < 0.05$) confirmed that ordination of fungal communities was significant. ANOSIM R-value (Table 14) of pairwise comparison between field birches and field oaks sampling sites was 1.00, confirming dissimilarity in fungal community structure between these sites. R-value between field birches and field pines was 0.45, which showed a high similarity in fungal community composition. R-value of pairwise comparisons between field oaks and field pines sampling sites was high (0.79), revealing that, although fungal communities of these sites were dissimilar, they had several common taxa in composition.

Table 14. Matrix table representing R-statistic of pairwise ANOSIM of field sampling sites based on the relative abundance of the most representative fungal taxa. Asterisks represent significant differences ($p < 0.05$).

	Field Birches sampling sites	Field Oaks sampling sites	Field Pines sampling sites
Field Birches sampling sites			
Field Oaks sampling sites	1.00*		
Field Pines sampling sites	0.45*	0.79*	

PERMANOVA test ($F = 11.42$, $p < 0.05$) confirmed the significance of ordination of all sampling sites as well (Table 15).

Table 15. PERMANOVA p-values of pairwise comparisons between field sampling sites based on the relative abundance of the most representative fungal taxa. Asterisks represent significant differences ($p < 0.05$).

	Field Birches sampling sites	Field Oaks sampling sites	Field Pines sampling sites
Field Birches sampling sites			
Field Oaks sampling sites	0.01*		
Field Pines sampling sites	0.02*	0.01*	

SIMPER analysis revealed that the most contributing to the dissimilarity between field sampling sites taxa were Russulaceae, Inocybaceae, Thelephoraceae, Herpotrichiellaceae (Table 16).

Table 16. SIMPER analysis demonstrating the contribution (%) of the most abundant fungal families to Bray-Curtis dissimilarity between field sampling sites. Only fungal families with a contribution higher than 10% are included in the table.

	Field Birches sampling sites	Field Oaks sampling sites	Field Pines sampling sites
Field Birches sampling sites			
Field Oaks sampling sites	Russulaceae (27.5%) Inocybaceae (26.4%) Herpotrichiellaceae (10.6%)		
Field Pines sampling sites	Inocybaceae (19.9%) Thelephoraceae (13.1%) Russulaceae (12.7%)	Russulaceae (31.8%) Inocybaceae (17.7%) Herpotrichiellaceae (9.9%)	

3.4.5. Fungal communities along the successional development of the mycorrhizosphere

NMDS showed distinct separation of three clusters: 1) a cluster formed by pot plants and field birches and field pines mycorrhizospheres, 2) field oaks variants, 3) control pot substrate and pot oak_1 (Figure 35).

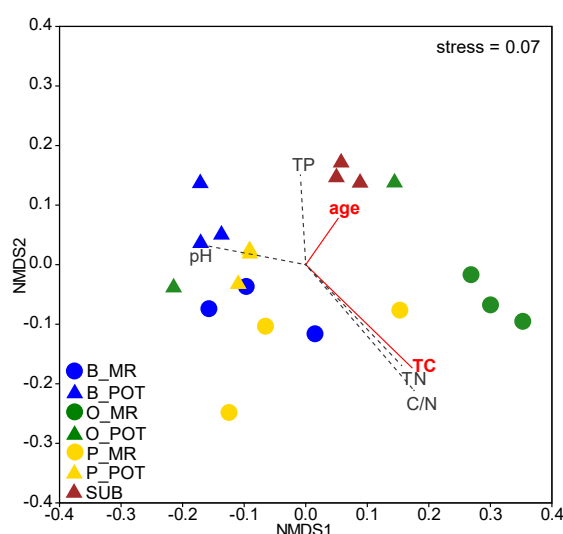


Figure 35. Non-metric dimensional scaling of fungal communities along successional stages based on the relative abundance of the most representative fungal families. Vectors represent successional gradients. Parameters which had a significant effect shown in red colour B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

TC, TN and C/N ratio determined the separation of field oaks; age and TP explained ordination of control pot variant, pH value defined ordination of cluster_1. Permutation analysis ($N = 999$, $p = 0.03$) showed that age ($p = 0.011$) and TC ($p = 0.025$) significantly determined ordination of fungal communities along the successional MR development

ANOSIM (Tables S92-S93) and PERMANOVA (Tables S94-S95) tests did not confirm the significance of the ordination. ANOSIM R-showed that along the simulated succession *control pot substrate* → *pot plant MR* → *field plant MR*, fungal communities tended to be less contrasting in structure. The significance of these patterns was not confirmed (Table S93).

3.5. Co-occurrence patterns in mycorrhizosphere

Field mycorrhizosphere co-occurrence network was formed by 99 nodes and 732 edges and consisted of two components: a small component formed by *Alatospora* (Leotiaceae) – n/d Acidimicrobiia, and a big component consisting of two sub-networks connected via 1) n/d Ktedonobacteria – *Pirellula* (Planctomycetes), and 2) a cluster *Burkholderia-Caballeronia-Paraburkholderia* (Gammaproteobacteria) – *Conexibacter* (Thermoleophilina) – *Mycobacterium* (Actinobacteria) – n/d Ascomycota (Figure 36). All species with a high node degree (from 31 to 35) were characterized with a

high closeness centrality value, suggesting their deep embedment into the network (Table S96). All those species were bacteria and represented Alpha- and Gammaproteobacteria, Vicinamibacteria, Holophagae, Planctomycetacia, Bacteroidia, Verrucomicrobiae. The highest betweenness centrality values (0.40) were determined for n/d Ktedonobacteria and *Pirellula* (Planctomycetacia) followed by n/d Acidobacteriia (0.09), n/d Saccharimonadia (0.08) and *Acidicapsa* (Acidobacteriia) (0.07). None of these taxa, except for Ktedonobacteria, were among the most abundant in the field. The highest value of betweenness centrality among fungi was determined for unidentified at a family level Ascomycota (0.05). At the same time, *Inocybe* (Inocybaceae), *Penicillium* (Aspergillaceae), *Metapochonia* (Clavicipitaceae) and *Lactarius* (Russulaceae) were characterized with the highest clustering coefficients (1.00).

The network based on the co-occurrence of generalists and specialists demonstrated a distinct separation of co-occurring specialists based on the tree species/sampling site preferences (Figure 37). 20.3% of bacteria were found in mycorrhizospheres of all field plants (generalists), and 52.7% revealed local preferences (specialists). 19% of fungi belonged to generalists, and 42.9% were characterized as specialists.

Pot plant mycorrhizospheres network consisted of 52 nodes and 85 edges and formed five components (Figure 38). The largest values of node degree, betweenness and closeness centrality were determined for n/d Ascomycota, *Exophiala* (Herpotrichiellaceae) and n/d Herpotrichiellaceae (Table S97). As the network consisted of several components, nodes forming small components were characterized with the highest closeness centrality: a cluster *Burkholderia-Caballeronia-Paraburkholderia* (Gammaproteobacteria), n/d Dermateaceae, *Pisolithus* (Pisolithaceae). In the big component *Exophiala* (Herpotrichiellaceae), n/d Hepotrichiellacea, n/d Ascomycota as well as *Nocardia* (Actinobacteria) and *Arthrobacter* (Actinobacteria) had the highest closeness centrality values.

Conexibacter (Thermoleophilia) and n/d Chloroflexi in a small component and *Mucilaginibacter* (Bacteroidia), *Streptomyces* (Actinobacteria), *Oidiodendron* (Myxotrichaceae) in the biggest component had the highest clustering coefficient value (1.00).

Network based on co-occurrence of generalists and specialists demonstrated that the vast majority of the species were generalists, and only a few demonstrated preference to a particular tree species (Figure 39).

Networks of co-occurring species constructed for each field sampling site (field plant mycorrhizosphere and corresponding bulk soil) and pot variants (pot plants mycorrhizosphere and control pot substrate) as well as network parameters can be found in Supplemental Material (Tables S98-S103, Figures S15-S20).

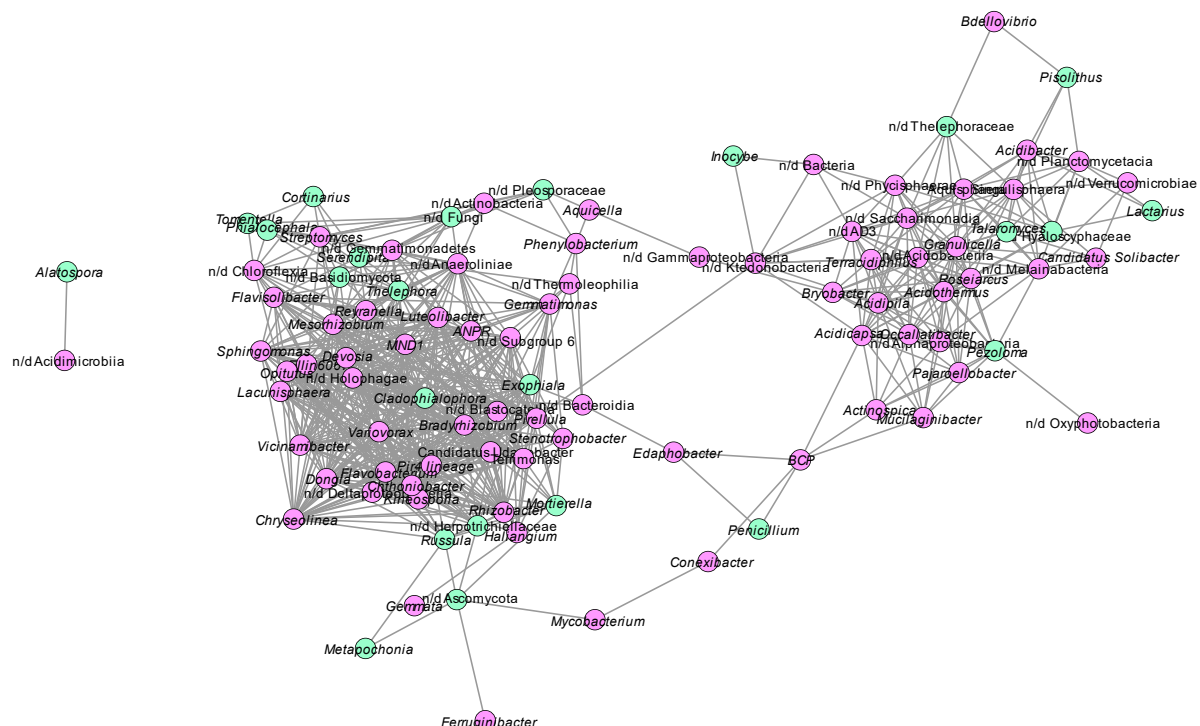


Figure 36. Field plants mycorrhizosphere interactions. Each node represents bacterial or fungal ASVs assigned to the genus level. Where ASVs could not be assigned to genus level, corresponding bacterial class or fungal family with the n/d were implemented. N/d Bacteria and n/d Fungi comprised all ASVs, which could not be assigned to class or family level, respectively. Here, the abbreviation ANPR is used for a cluster *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, BCP is used for a cluster *Burkholderia-Caballeronia-Paraburkholderia*. The colour of the node attributes assignment to bacteria (pink) or fungi (green).

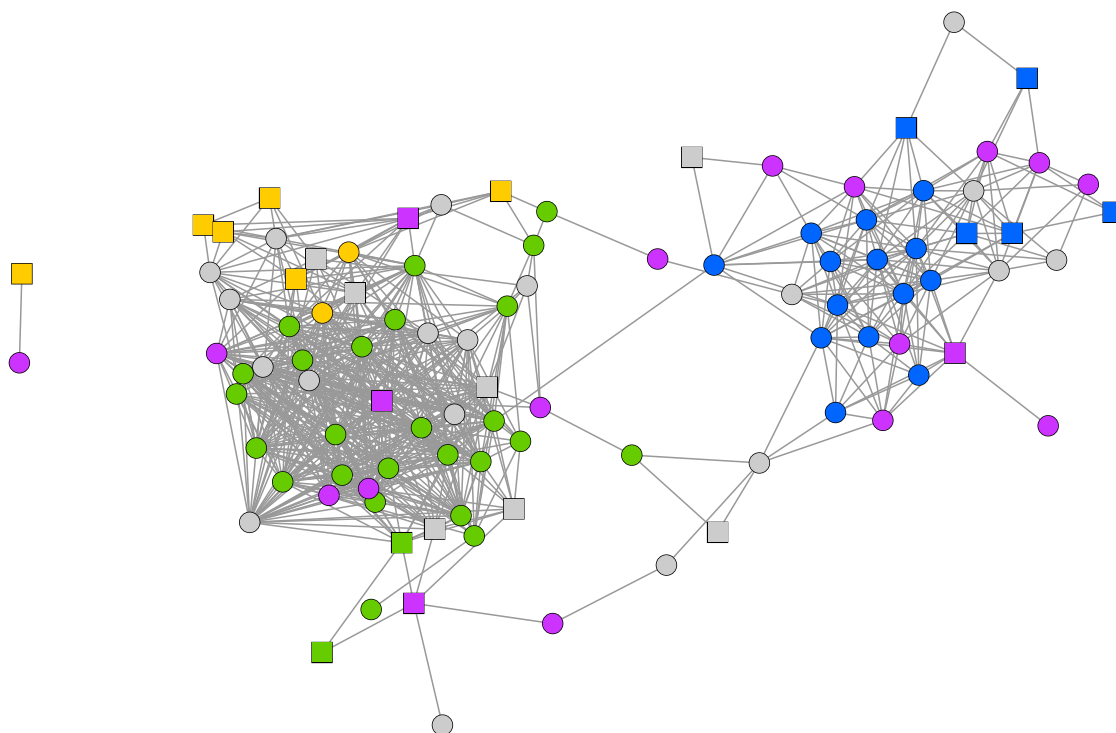


Figure 37. Network of co-occurring generalists and specialists in mycorrhizospheres of field plants. The shape of the node attributes assignment to bacteria (round) or fungi (square). The colour of the node attributes ASVs occupancy preference: generalists represented in mycorrhizospheres of all field trees (purple), specialists exhibited a preference to birch (blue), oak (green) or pine (yellow) mycorrhizospheres; grey colour depicts species represented in mycorrhizospheres of two tree species.

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3.6. Relationship between soil parameters and microbial community

3.6.1. Soil parameters and ECM community association

CCA result is shown as a biplot representing the correlation between soil characteristics and relative abundance of exploration types observed in all experiment variants (Figure 40). Contact exploration type correlated with the concentration of Al, Cu, Fe, Sr, U; short-distance exploration type associated with the concentration of Pb, Sr, Fe, U as well as with TP and C/N ratio; medium-distance exploration type correlated with the concentration of Cs and Mn; long-distance exploration type associated with the concentration of Zn, Ni, Co, Al, Cu and soil pH value.

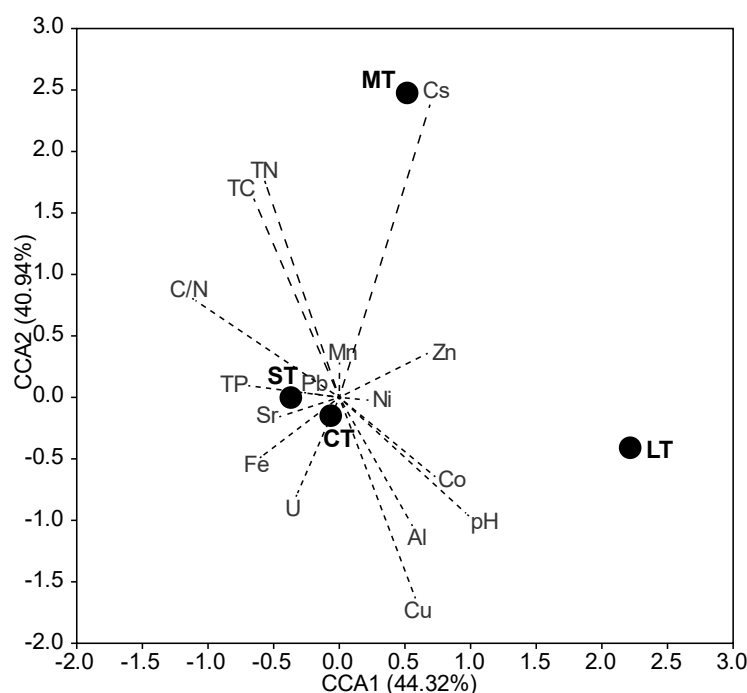


Figure 40. Canonical correspondence analysis biplot representing the correlation between soil parameters and exploration types of mycorrhiza. CT – contact exploration type, ST – short-distance exploration type, MT – medium-distance exploration type, LT – long-distance exploration type.

Although permutation test ($N = 999$, $p = 0.444$) did not reveal an overall significant association between soil parameters and exploration types, correlation analysis demonstrated significant positive correlations between short-distance exploration type and TC, TN and C/N ratio, medium-distance exploration type and concentration of Cs, and negative correlation between contact exploration type and TN (Table 17).

Table 17. Correlation coefficients between soil characteristics and exploration types of mycorrhiza.

Soil characteristics	Contact exploration type	Short-distance exploration type	Medium-distance exploration type	Long-distance exploration type
Al	0.27	-0.33	0.03	0.12
Co	0.00	-0.07	-0.17	0.29
Cu	0.12	-0.08	-0.38	0.20
Fe	0.35	-0.13	-0.30	-0.17
Mn	-0.33	0.31	-0.08	0.08
Ni	-0.36	0.38	-0.10	-0.03
Pb	-0.31	0.20	0.06	0.10
Sr	-0.36	0.35	-0.08	-0.02
Zn	0.03	-0.29	0.07	0.35
Cs	0.01	-0.25	0.40*	-0.03
U	-0.09	0.26	-0.25	0.01
TC	-0.33	0.52*	-0.11	-0.31
TN	-0.40*	0.52*	-0.04	-0.20
C/N	-0.27	0.49*	-0.13	-0.34
TP	0.38	-0.14	-0.03	-0.27
pH	0.18	-0.26	-0.13	0.25

Asterisks represent significant correlation ($p < 0.05$).

CCA biplot representing correlation of ECM taxa defined for field plants with soil characteristics (Figure 41) demonstrated that *Lactarius* sp. and *Mallocybe* sp. (both described for birches and forming contact exploration type mycorrhiza) positively correlated with the concentration of Fe, Al and Cu and negatively correlated with Mn, Ni, Pb, Sr and TC and C/N ratio. Not defined by sequence analysis, oak morphotype O_F_MT2 (contact exploration type) had a positive correlation with TC, TN, TP, and C/N ratio and negative correlation with the concentration of Al, U, and soil pH value. Pine morphotype formed by Thelephoraceae (contact exploration type) positively correlated with concentration of Mn, Pb, Sr, U and soil pH value and negatively correlated with the concentration of Fe and TP. Correlation analysis revealed that associations of all field morphotypes with contact exploration and soil characteristics were significant (Table S104). Oak morphotype formed by *Cortinarius* sp. (medium-distance exploration type) had a positive correlation with TN. *M. bicolor* and Helotiales (short-distance exploration type) described for oaks did not demonstrate an association with soil characteristics. Pine morphotypes formed by *T. argyraceum* and *R. mohelnensis* correlated with the concentration of Ni, Pb, Sr, Mn, U and soil pH value; however, correlation analysis did not confirm the significance of these correlations.

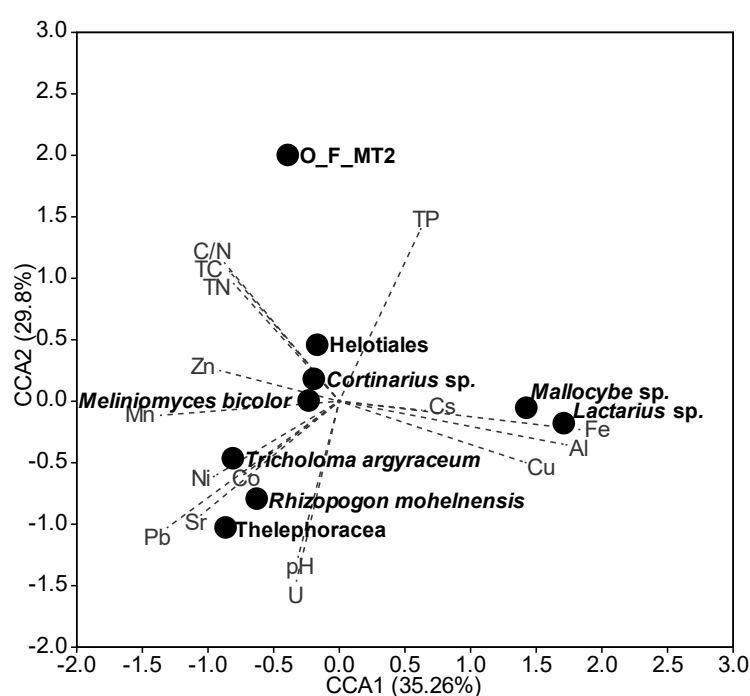
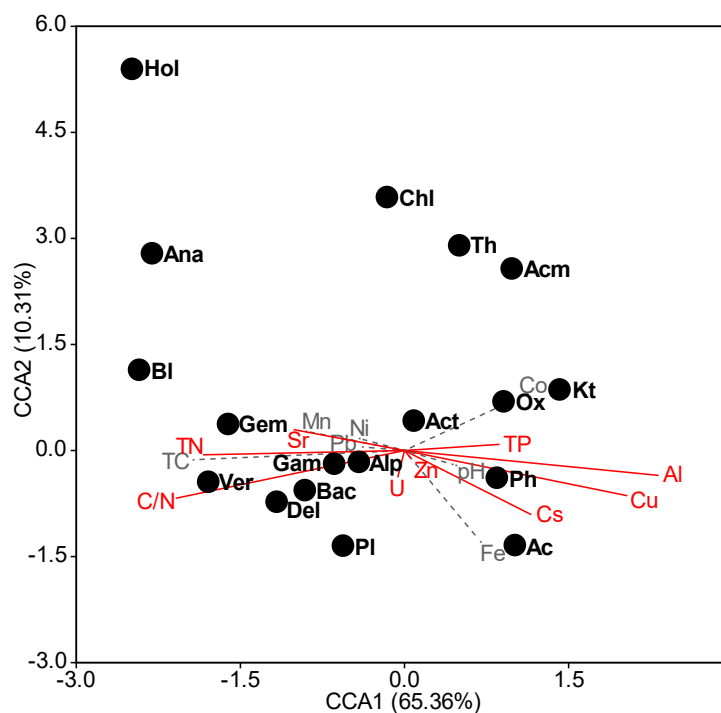


Figure 41. Canonical correspondence analysis biplot representing the correlation between soil parameters and mycorrhizal taxa described for field plants.

3.6.2. Soil parameters and bacterial community association



Correlation analysis demonstrated that the most representative bacterial classes were associated with particular soil characteristics (Table 18). Moreover, it supported results of PCA and NMDS for bacterial community: bacterial classes, which contributed to the dissimilarity between sampling sites the most, were significantly associated with soil characteristics typical for this site. For example, Acidobacteriia (with the highest relative abundance at the birch sampling site) had a strong negative correlation with Mn, Pb and Sr concentrations and a strong positive correlation with the content of Cu, Fe and Cs. Ktedonobacteria (with the highest relative abundance at the birch sampling site) correlated positively with the concentration of Al, Cu, Fe, and U. Alphaproteobacteria (with the highest relative abundance at the oak and pine sampling sites) formed a strong positive correlation with C/N and a moderate positive correlation with Mn. At the same time, this taxon had weak negative associations with the concentration of Al, Cu, Fe, which were not at high concentrations at the oak sampling site.

Table 18. Coefficients of correlation between soil characteristics and bacterial classes with relative abundance higher than 1%. Ac – Acidobacteriia, Act – Actinobacteria, Alp – Alphaproteobacteria, Ana – Anaerolineae, Bac – Bacteroidia, Bl – Blastocatellia, Chl – Chloroflexia, Del – Deltaproteobacteria, Gam – Gammaproteobacteria, Gem – Gemmatimonadetes, Hol – Holophagae, Kt – Ktedonobacteria, Ph – Phycisphaerae, Pl – Planctomycetacea, Th – Thermoleophilia, Ver – Verrucomicrobiae. Asterisks represent significant correlation ($p < 0.05$).

Soil characteristics	Ac	Bl	Hol	Act	Th	Bac	Ana	Chl	Kt	Gem	Ph	Pl	Alp	Del	Gam	Ver
Al	0.72	-0.89*	-0.63*	0.22	-0.65*	-0.43	-0.63*	-0.12	0.85*	-0.55*	0.81*	0.28	-0.59*	-0.72*	-0.63*	-0.75*
Co	0.17	0.19	0.10	0.21	0.49*	0.02	-0.01	-0.38	-0.13	-0.11	0.00	-0.50*	0.04	-0.26	0.17	-0.17
Cu	0.76*	-0.60*	-0.58*	-0.18	-0.25	-0.42	-0.77*	-0.27	0.66*	-0.57*	0.78*	0.15	-0.53*	-0.48*	-0.62*	-0.68*
Fe	0.76*	-0.71*	-0.64*	-0.14	-0.44	-0.49*	-0.84*	-0.58*	0.52*	-0.71*	0.79*	0.53*	-0.47*	-0.40	-0.74*	-0.40
Mn	-0.62*	0.63*	0.70*	0.46	0.68*	0.45	0.60*	0.04	-0.48*	0.20	-0.49*	-0.48*	0.48*	0.03	0.68*	0.42
Ni	-0.39	0.60*	0.63*	0.34	0.65*	0.26	0.56*	0.03	-0.49*	0.28	-0.48*	-0.76*	0.45	0.06	0.56*	0.18
Pb	-0.62*	0.43	0.55*	0.49*	0.53*	0.55*	0.62*	0.56*	-0.13	0.55*	-0.49*	-0.66*	0.44	-0.01	0.62*	0.04
Sr	-0.78*	0.52*	0.55*	0.46	0.45	0.51*	0.70*	0.49*	-0.28	0.49*	-0.59*	-0.61*	0.59*	0.06	0.76*	0.21
Zn	0.15	0.22	0.03	0.01	0.52*	0.23	-0.24	-0.35	0.01	-0.13	0.14	-0.34	-0.05	-0.28	0.03	-0.02
Cs	0.71*	-0.55*	-0.61*	0.06	-0.10	-0.14	-0.70*	-0.47	0.44	-0.30	0.57*	0.23	-0.43	-0.49*	-0.51*	-0.37
U	0.08	-0.46	-0.30	0.38	-0.35	-0.14	-0.07	0.45	0.60*	0.01	0.31	-0.16	-0.10	-0.38	-0.09	-0.60*
TC	-0.38	0.65*	0.32	-0.22	0.46	0.23	0.32	-0.02	-0.55*	0.43	-0.50*	-0.17	0.46	0.53*	0.08	0.52*
TN	-0.26	0.56*	0.21	-0.29	0.43	0.13	0.22	-0.11	-0.47*	0.34	-0.40	-0.02	0.29	0.52*	0.02	0.47*
C/N	-0.58*	0.63*	0.38	-0.06	0.29	0.34	0.46	0.11	-0.58*	0.41	-0.62*	-0.23	0.74*	0.38	0.30	0.60*
TP	0.26	0.09	-0.11	-0.60*	0.03	-0.29	-0.24	-0.40	-0.29	-0.12	-0.01	0.34	-0.04	0.29	-0.19	0.29
pH	0.04	-0.33	-0.27	0.58*	-0.13	0.13	-0.04	0.03	0.34	-0.15	0.13	-0.26	0.04	-0.63*	0.23	-0.42

CCA biplot represents the correlation between soil parameters and bacterial community diversity indices (Figure 43).

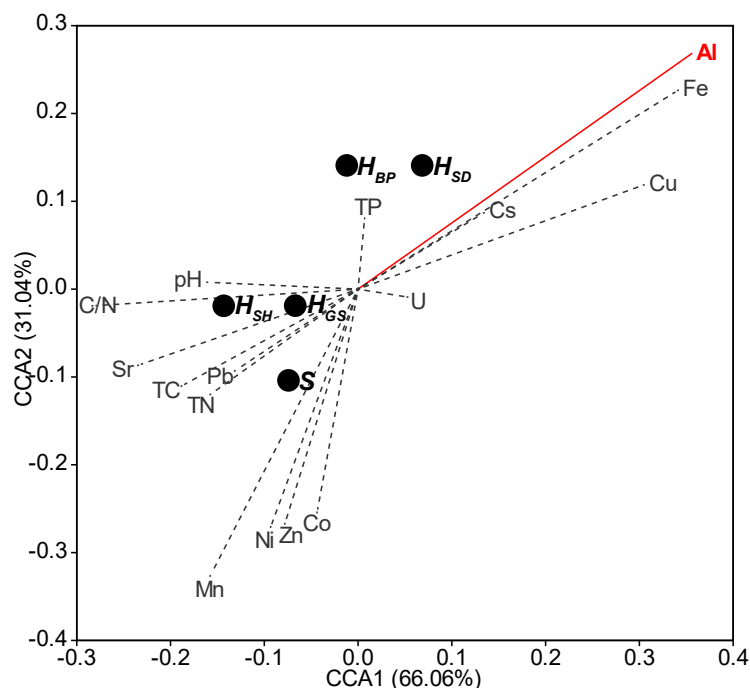


Figure 43. Canonical correspondence analysis biplot representing the correlation between soil parameters and bacterial community diversity indices. S – richness, H_{SH} – Shannon diversity index, H_{GS} – Gini-Simpson diversity, H_{SD} – Simpson dominance index, H_{BP} – Berger-Parker index. Soil parameters which had a significant effect shown in red colour.

Permutation analysis ($N = 999$, $p = 0.001$) revealed overall significance of Al content ($p = 0.04$). Correlation analysis confirmed significant associations of bacterial community diversity indices with particular soil parameters (Table 19). Al concentration, TC, and C/N ratio were the most influential soil parameters.

Table 19. Coefficients of correlation between soil characteristics and bacterial community diversity indices.

Soil characteristics	S	H_{SD}	H_{GS}	H_{SH}	H_{BP}
Al	-0.76*	0.51*	-0.51*	-0.74*	0.22
Co	-0.04	0.11	-0.11	0.00	0.10
Cu	-0.50*	0.38	-0.38	-0.46	0.12
Fe	-0.61*	0.36	-0.36	-0.52*	0.17
Mn	0.36	-0.35	0.35	0.44	-0.09
Ni	0.40	-0.29	0.29	0.43	-0.15
Pb	0.25	-0.30	0.30	0.28	-0.26
Sr	0.33	-0.24	0.24	0.30	-0.11
Zn	-0.03	-0.07	0.07	0.13	-0.03
Cs	-0.63*	0.35	-0.35	-0.51*	0.27
U	-0.40	0.39	-0.39	-0.52*	0.24
TC	0.54*	-0.50*	0.50*	0.61*	-0.44
TN	0.44	-0.35	0.35	0.48*	-0.31
C/N	0.58*	-0.65*	0.65*	0.65*	-0.56*
TP	0.13	-0.10	0.10	0.16	-0.16
pH	-0.38	0.36	-0.36	-0.44	0.39

S – richness, H_{SH} – Shannon diversity index, H_{GS} – Gini-Simpson index, H_{SD} – Simpson dominance index, H_{BP} – Berger-Parker index. Asterisks represent significant correlation ($p < 0.05$).

3.6.3. Soil parameters and fungal community association

CCA biplot represents the correlation between the most representative fungal families and soil characteristics (Figure 44). Permutation analysis ($N = 999$, $p = 0.009$) revealed overall significance of such soil characteristics as concentration of Al ($p = 0.003$) and Co ($p = 0.013$).

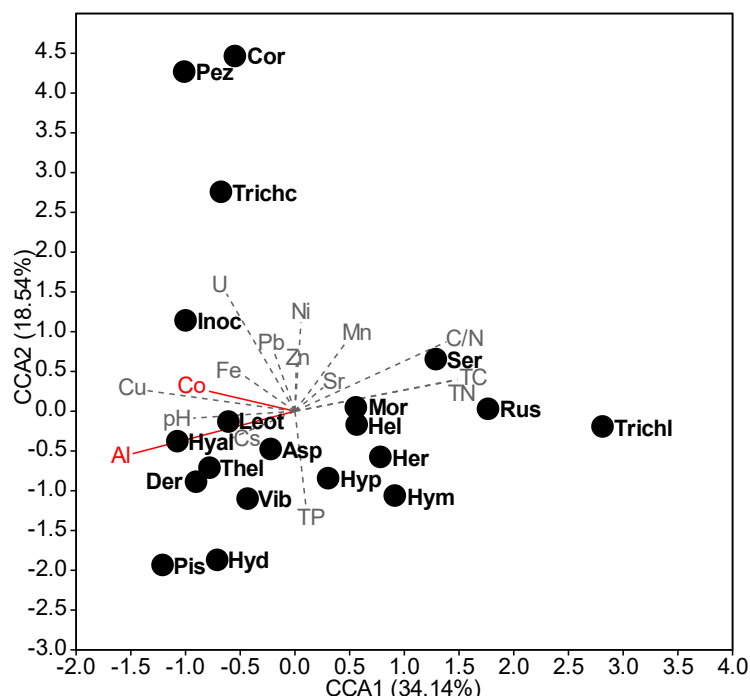


Figure 44. Canonical correspondence analysis biplot representing the correlation between soil parameters and the most representative fungal families. Asp – Aspergillaceae, Ath – Atheliaceae, Cor – Cortinariaceae, Der – Dermateaceae, Hel – Helotiaceae, Her – Herpotrichiellaceae, Hyal – Hyaloscyphaceae, Hyd – Hydnangiaceae, Hym – Hymenogastraceae, Hyp – Hypocreaceae, Inoc – Inocybaceae, Leot – Leotiaceae, Mor – Mortierellaceae, Pez – Pezizaceae, Pis – Pisolithaceae, Rus – Russulaceae, Ser – Serendipitaceae, The – Thelephoraceae, Trichc – Trichocomaceae, Trichl – Tricholomataceae, Vib – Vibrissaceae. Soil parameters which had a significant effect shown in red colour.

Correlation analysis showed associations of the most representative fungal families with particular soil parameters (Table 20). Furthermore, the results of correlation analysis demonstrated the link between the fungal taxa representability at a particular sampling site and soil parameters typical for this site. Thus, Thelephoraceae found in all variants of the experiment but with the highest relative abundance in pots positively correlated with the content of Al and soil pH value. Another fungal taxon found in all experiment variants was Herpotrichiellaceae (with the highest representability in field oaks variants), which negatively correlated with soil pH value and concentration of Al, Cu, U, and was positively associated with Mn content. Leotiaceae, highly presented at the birch sampling site and in all pot variants, demonstrated a positive correlation with the concentration of Al, Co, Cu, Fe, Cs and negative correlation with Pb, Sr, TC, TN and C/N ratio. Relative abundance of Inocybaceae was positively affected by the concentration of Al, Cu, Fe and U. At the same time, the negative correlation of this taxon with the content of Mn, Ni, Sr and TP was defined. Russulaceae, the most abundant fungal taxon at the field oak sampling site, had a positive correlation with TC, TN and C/N ratio and a negative correlation with concentration of Al, Co, Cu and soil pH value.

Table 20. Coefficients of correlation between soil characteristics and fungal families with relative abundance higher than 1%. Asp – Aspergillaceae, Cor – Cortinariaceae, Der – Dermateaceae, Hel – Helotiaceae, Her – Herpotrichiellaceae, Hyal – Hyaloscyphaceae, Hyd – Hydnangiaceae, Hym – Hymenogastraceae, Hyp – Hypocreaceae, Inoc – Inocybaceae, Leot – Leotiaceae, Mor – Mortierellaceae, Pez – Pezizaceae, Pis – Pisolithaceae, Rus – Russulaceae, Ser – Serendipitaceae, Thel – Thelephoraceae, Trichc – Trichocomaceae, Trichl – Tricholomataceae, Vib – Vibrissaceae. Asterisks represent significant correlation ($p < 0.05$).

Soil characteristics	Her	Asp	Trichc	Der	Hel	Hyal	Leot	Vib	Pez	Hyp	Cor	Hyd	Hym	Inoc	Trichl	Pis	Rus	Ser	Thel	Mor
Al	-0.46*	-0.02	0.14	0.50*	0.14	0.74*	0.63*	0.17	-0.17	-0.01	-0.49*	0.37*	-0.15	0.42*	-0.68*	0.53	-0.54*	-0.64*	0.36*	-0.51*
Co	-0.01	0.70*	0.13	0.77*	0.18	0.25	0.58*	0.24	0.17	0.14	-0.05	0.29	-0.29	-0.13	-0.33	0.40*	-0.55*	-0.24	0.02	-0.21
Cu	-0.45*	0.10	0.33	0.31	-0.04	0.57*	0.52*	-0.20	0.16	-0.24	-0.35	0.34	-0.50*	0.53*	-0.45*	0.13	-0.40*	-0.39*	0.21	-0.49*
Fe	-0.32	-0.04	0.22	0.16	0.28	0.72*	0.57*	-0.25	-0.17	-0.25	-0.40*	0.54*	-0.44*	0.41*	-0.30	0.32	-0.10	-0.60*	0.02	-0.48*
Mn	0.43*	0.43*	-0.12	-0.04	-0.15	-0.66*	-0.29	0.13	0.42*	0.22	0.53*	-0.23	0.23	-0.53*	0.33	-0.30	0.07	0.59*	-0.19	0.41*
Ni	0.17	0.20	-0.20	-0.12	-0.30	-0.64*	-0.31	-0.10	0.38*	-0.08	0.15	-0.41*	-0.03	-0.38*	0.12	-0.51*	-0.03	0.52*	0.08	0.26
Pb	0.04	0.02	0.02	-0.10	-0.46*	-0.84*	-0.45*	0.17	0.51*	0.01	0.24	-0.63*	0.34	-0.08	-0.04	-0.36	-0.15	0.54*	0.22	0.28
Sr	0.30	0.18	-0.11	-0.11	-0.31	-0.85*	-0.54*	0.26	0.51*	0.20	0.45*	-0.53*	0.41*	-0.39*	0.27	-0.41*	0.03	0.70*	0.05	0.43*
Zn	0.18	0.60*	-0.11	0.39*	-0.13	0.00	0.32	0.02	0.20	0.02	0.11	-0.09	-0.43*	-0.23	-0.15	0.14	-0.31	0.11	0.09	-0.03
Cs	-0.36	-0.20	-0.03	0.18	-0.04	0.44*	0.55*	-0.15	-0.24	-0.35	-0.42*	0.15	-0.40*	0.19	-0.57*	0.28	-0.19	-0.47*	0.31	-0.45*
U	-0.58*	-0.39*	0.59*	0.02	-0.31	-0.13	-0.02	0.20	0.53*	-0.15	-0.05	-0.04	0.29	0.70*	-0.41*	-0.19	-0.36	-0.03	0.32	-0.20
TC	0.20	-0.42*	-0.07	-0.73*	-0.37*	-0.58*	-0.57*	-0.47*	0.07	-0.32	0.13	-0.34	-0.01	-0.07	0.37	-0.74*	0.60*	0.57*	-0.18	0.32
TN	0.24	-0.42*	-0.04	-0.75*	-0.32	-0.50*	-0.52*	-0.49*	0.00	-0.30	0.17	-0.27	-0.02	-0.07	0.41*	-0.74*	0.67*	0.53*	-0.32	0.31
C/N	0.26	-0.36	-0.04	-0.72*	-0.38*	-0.63*	-0.64*	-0.33	0.18	-0.25	0.23	-0.38*	0.12	-0.13	0.47*	-0.72*	0.58*	0.65*	-0.13	0.42*
TP	0.11	0.23	-0.55*	0.19	0.41*	0.38*	0.12	-0.15	-0.53*	0.11	-0.18	0.25	-0.49*	-0.37*	0.13	0.36	0.00	-0.23	0.01	-0.27
pH	-0.42*	-0.04	0.18	0.23	-0.34	-0.19	0.03	0.33	0.38*	0.02	-0.08	-0.32	0.20	0.21	-0.42*	0.04	-0.50*	-0.02	0.61*	-0.17

CCA biplot represents the correlation between soil parameters and fungal community diversity indices (Figure 45).

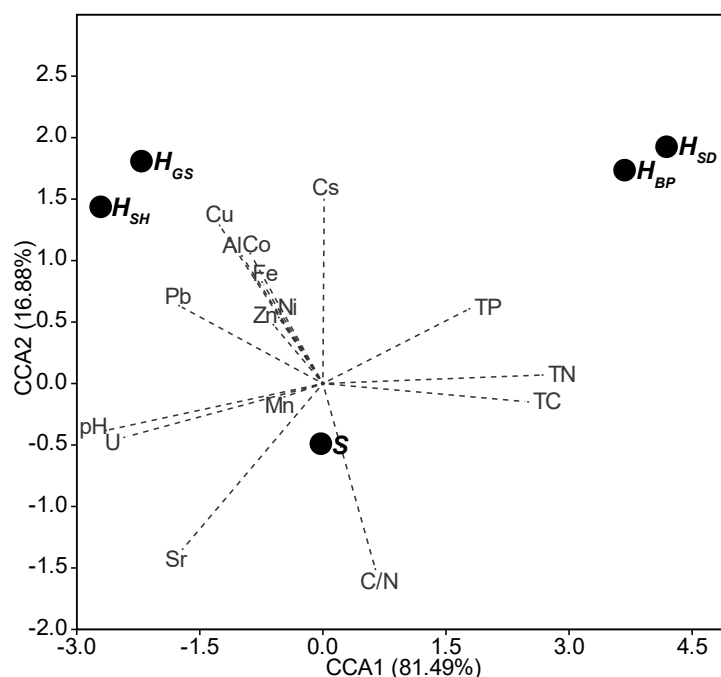


Figure 45. Canonical correspondence analysis biplot representing the correlation between soil parameters and fungal community diversity indices. S – richness, H_{SH} – Shannon diversity index, H_{GS} – Gini-Simpson index, H_{SD} – Simpson dominance index, H_{BP} – Berger-Parker index.

Permutation analysis ($N = 999$, $p = 0.729$) did not reveal the overall significant effect of soil parameters on fungal community diversity indices. However, correlation analysis showed a significant influence of particular soil characteristics on fungal community diversity (Table 21). The most affected by soil diversity index was richness, which was associated with the concentration of Pb, Sr, U.

Table 21. Coefficients of correlation between soil characteristics and fungal community diversity indices.

Soil characteristics	S	H_{SD}	H_{GS}	H_{SH}	H_{BP}
Al	-0.24	-0.22	0.22	-0.16	-0.35
Co	-0.24	-0.18	0.18	-0.08	-0.22
Cu	-0.04	-0.18	0.17	-0.01	-0.26
Fe	-0.21	0.01	0.00	-0.18	-0.15
Mn	0.26	-0.08	0.08	0.24	-0.01
Ni	0.19	-0.03	0.02	0.10	0.00
Pb	0.49*	-0.19	0.18	0.31	-0.12
Sr	0.44*	-0.23	0.22	0.38	-0.14
Zn	-0.06	-0.03	0.04	-0.09	-0.02
Cs	-0.47*	0.12	-0.11	-0.47*	0.00
U	0.42*	-0.37	0.35	0.34	-0.36
TC	0.26	0.25	-0.26	0.05	0.31
TN	0.19	0.37	-0.38	-0.04	0.43*
C/N	0.35	0.06	-0.07	0.25	0.12
TP	-0.54*	0.43*	-0.42*	-0.48*	0.34
pH	0.04	-0.55*	0.54*	0.29	-0.56*

S – richness, H_{SH} – Shannon diversity index, H_{GS} – Gini-Simpson index, H_{SD} – Simpson dominance index, H_{BP} – Berger-Parker index. Asterisks represent significant correlation ($p < 0.05$).

Although correlation analysis demonstrated the significance of the relationship between fungal diversity indices and soil, absolute values of correlation coefficients demonstrated weak to moderate strength of correlation.

3.7. Plant inoculation experiment

3.7.1. Plant growth parameters estimation

Tree species showed different performances related to a variant of the experiment: inoculated/non-inoculated (Table 22).

Table 22. Pot plants survival rate.

Tree species		Total	Plants with no leaves	Plants with leaves	Plant survival rate (%)
Birch	number of plants non-inoculated	12	4	8	66.7
	number of plants inoculated	14	12	2	14.3
Oak	number of plants non-inoculated	12	10	2	16.7
	number of plants inoculated	14	5	9	69.2
Pine	number of plants non-inoculated	10	2	8	80.0
	number of plants inoculated	10	4	6	60.0

Non-inoculated birches and pines demonstrated relatively high survival (66.7 and 80%, respectively), whereas non-inoculated oaks did not perform successfully, and only two oaks of 12 had leaves at the end of the experiment.

Inoculation of rhizosphere with the blend of ECM fungi resulted in the decrease of birches survival rate (14.3%) and the increase of oaks survival rate (69.2%). Although the number of alive inoculated pines at the end of the experiment was lower than the number of non-inoculated plants, the overall survival rate after inoculation remained relatively high (60.0%).

Analysis of plants growth dynamics showed a considerable decline in the number of alive birches after both inoculations (Figure 46).

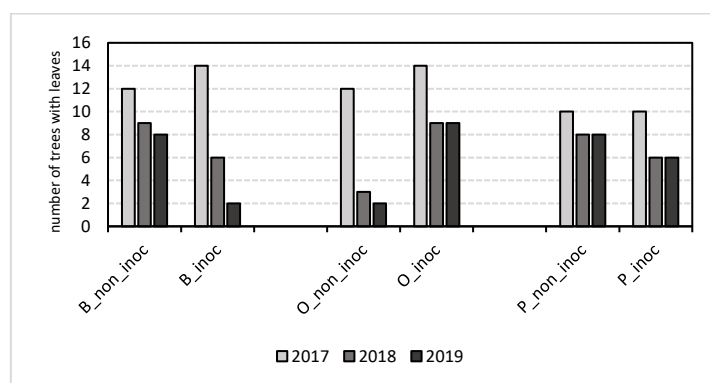


Figure 46. Plant growth dynamics during pot experiment. B – birch, O – oak, P – pine; non_inoc – non-inoculated variant of the experiment, inoc – inoculated variant of the experiment.

3.7.2. Metal content in the aboveground plant biomass

All tree species contained different concentrations of metals depending on the variant of the experiment. Overall, additional inoculation did not affect metal content in pot plants biomass. Although significant differences between field variants and one or both pot variants were observed, no particular trend in metal accumulation in plant aboveground biomass was determined (Figure S20).

4. Discussion

4.1. Kanigsberg represents an example of a unique post-mining site

Post-mining remediation activities performed at Kanigsberg resulted in drastic changes in the ecosystem. Relocation of waste rock materials to backfill open pits caused high spatial heterogeneity of Kanigsberg's substrate. As a consequence, the test field area undergoes a primary vegetation succession with sites at different stages of development.

All three test field sampling sites demonstrated distinct separation based on differences in soil chemical parameters. Birch sampling site illustrated a particularly early stage of primary succession with immature soil and total absence of litter and organic horizons. The soil contained a very low amount of total carbon, nitrogen and phosphorus and high concentrations of Al, Fe and Cu compared to other sites. Lack of grass vegetation might result in temporal loss of nutrients due to surface runoff caused by rainfalls. Moreover, this area was constantly exposed to solar radiation, fluctuations in temperature and water saturation and might undergo extensive physical and chemical weathering, which could lead to the production of new soil materials with modified physicochemical properties.

The oak sampling site was a part of a young forest stand where vegetation was represented by older alders and oaks, rare young birches. This area was characterized by the presence of an extensive grass cover, dense litter and a diffused organic layer. Compared to other sampling sites, the soil at the oaks area contained a relatively higher amount of total carbon, nitrogen and phosphorus. The presence of alders at the oak sampling site might explain the higher nitrogen content due to symbiosis with N₂-fixing actinobacteria *Frankia*. Moreover, it was shown that N₂-fixation could increase organic carbon (Chodak and Niklińska, 2010; Józefowska et al., 2019). The development of grass vegetation at the oak sampling site makes a significant input into the accumulation of organic carbon as well. Interestingly, increased concentration of Mn was typical for the oak sampling site.

The pine sampling site took a rather intermediate position between the birch and oak sites and shared common abiotic characteristics. Similarly to the oak sampling site, pines grew in a young forest stand; however, no grass vegetation was observed here. Soil chemical analysis demonstrated exceptionally high variability between soil samples for the pine sampling site. The soil was characterized by higher pH values than other sites; however, statistical tests did not confirm the significance of the differences. This result contradicts the known fact that coniferous trees acidify soils compared to deciduous trees, which is related to the higher saturation of soils with hydrogen cations, less saturation with Al, and lower alkaline cations (Józefowska et al., 2019). Early remediation steps at Kanigsberg included liming at particular sites to increase pH of topsoil layer and, in this way, reduce the rate of soil acidification and toxic metals' mobilization (M. Riefenstahl, Institute of Geosciences, Friedrich Schiller University; personal communication). Unfortunately, as there are no reliable document sources describing which remediation techniques were applied in particular areas, it can be only hypothesized that the pine sampling site was affected by local liming, which could explain higher pH values.

4.2. Tree species growing at Kanigsberg revealed the potential for phytoremediation

The success of vegetation establishment depends in many respects on adaptations of the tree species to newly created topsoil at post-mining areas (Pietrzykowski, 2019). Among the typical woody pioneer species which can naturally colonize post-mining areas are birches. They possess a high level of adaptability and tolerance to unfavourable conditions (drought, low nutrients content, heavy metals). Moreover, this species can occupy forest gaps and, therefore, facilitates soil functioning and biodiversity. On the other hand, birches are known to be particularly sensitive to intraspecific competition (Dubois et al., 2020). Indeed, similar growth patterns were observed at Kanigsberg. Young birch seedlings exclusively represented vegetation at the birch sampling site with scarce grasses. At the same time, only rare birch seedlings were found at the oak sampling site, where vegetation formed a relatively dense canopy. Furthermore, birches developed superficial horizontally distributed root systems, which might be explained by extensive inclusions of coarse material (Mauer and Palátová,

2003) and general low soil fertility (Rosenvald et al., 2011). Numerous studies confirmed that birches not only tolerate increased concentrations of heavy metals but also can accumulate heavy metals in biomass with particularly high affinity to Zn (Eltrop et al., 1991; Utriainen et al., 1997; Dmuchowski et al., 2010; Dmuchowski et al., 2014; Szwalec et al., 2018; Desai et al., 2019).

PCA demonstrated that the content of bioavailable Al was one of the abiotic factors distinguishing the birch sampling site at Kanigsberg. Moreover, this study revealed numerous significant correlations between taxa typical for birch sampling area and the content of Al, suggesting a considerable effect of this metal on not only vegetation but also on the structure of associated microbial populations. Birches, in general, can tolerate elevated concentrations of Al in soil (Jonczak et al., 2020). At the same time, fluctuations in the soil acidity might increase the content of bioavailable Al^{3+} and, therefore, pose an obstacle for the successful establishment of other vegetation in affected areas (Roy et al., 1988). Clegg and Gobran (1995) reported that the growth of birch seedlings was suppressed at a concentration of 3 mM Al, which is much higher than it was measured in Kanigsberg soil. Unfortunately, there is no defined threshold at which Al becomes toxic to plants under natural conditions, as it depends on the complex of local abiotic conditions (pH, soil mineral composition, water content etc.).

Analysis of metal content in green biomass demonstrated that pot birches accumulated only Mn and Cd compared to field birches. On the other hand, the concentration of Fe, Cu, Sr, Pb and U in field birches significantly exceeded those in pot variants. This observation demonstrates that as birches grew, they excluded some metals from the biomass. Altogether, birches can be considered as species with the high potential for phytoremediation of post-mining areas at early stages of succession and can be used for heavy metal contaminated soils with minimal risk of soil re-contamination.

In the temperate climate zone of central Europe, oak is attributed to climax or late successional-stage species (Pietrzykowski, 2019). Application of climax tree species at post-mining areas might lead to long-term ecosystem stability (Borišev et al., 2018). Oak represents a valuable target species in the reforestation of disturbed areas, especially when mineral fertilizers (NPK) are applied (Heinsdorf, 1996; Pietrzykowski, 2019). At Kanigsberg, particular sites where soil had better quality and contained more nutrients could harbour late-stage oaks. Frouz et al. (2015) reported that oak seedlings demonstrated better growth at the unreclaimed site of a post-mining site than the reclaimed site where alders were artificially planted. This observation confirms that naturally successional woody vegetation can create good conditions for late-stage oaks.

Oaks are known to accumulate heavy metals in their biomass. For example, Placek et al. (2016) observed significant accumulation of Cd, Zn and Pb in green biomass of oaks, especially after the addition of fertilizers. After transfer in pots, oaks accumulated a great amount of Mn in green biomass compared to field oaks and other tree species. One of the explanations of this result might be a stress response of oaks to disturbance effect and, consequently, a change in plant physiology. Pot substrate contained a significantly lower amount of phosphorus compared to the field oak sampling site's soil. Plants are known to exude more organic acids to obtain limited in soils phosphorus (Hocking, 2001). As a consequence, an increase in the production of organic acids might intensify the mobilization of Mn and cause its accumulation in green biomass. Moreover, an increase of water saturation of pot substrate might result in the establishment of microhabitats with reductive conditions. A decrease in oxygen content caused the dissolution of Mn oxides, intensified Mn^{2+} mobilization and led to Mn accumulation in plant tissues (Carretero and Kruse, 2015; Luzati et al., 2016; Zhang et al., 2020). Besides, organic matter in the soil at the field oak sampling site might form stable Mn-organic compounds and, in this way, prevent Mn mobilization (Carretero and Kruse, 2015). As oaks are broadleaved species, it was not recommended to use them for phytoremediation because of the high risk of soil re-contamination after the seasonal fall of leaves (Placek et al., 2016). In addition, the high mortality level of non-inoculated plants displayed low adaptability of oaks to disturbance. On the other hand, inoculated oaks demonstrated better performance than non-inoculated oaks in general. Therefore, the application of oaks for phytoremediation purposes might be suitable for disturbed ecosystems with relatively low metal

contamination levels; the implementation of fertilizers or mycorrhizal blends might considerably improve the growth of oaks.

Pines represent typical pioneer woody species. They are widely used in the reclamation of post-mining areas due to high tolerance and adaptability to unfavourable environmental conditions (Baumann et al., 2006; Chodak and Niklińska, 2010; Pietrzykowski and Daniels, 2014; Pietrzykowski, 2019). Fertilization of pines with sewage sludge as a source of organic matter improved their growth in polluted soils (Placek et al., 2016). Besides, site-adapted NPK fertilization resulted in the establishment of well-growing pine plantations at post-mining areas poor in nutrients (Heinsdorf, 1996). Pines can accumulate heavy metals in their tissues and, therefore, can be effective in phytoextraction (Placek et al., 2016; Saladin, 2015). Both field and pot pines contained a prominently higher amount of Al compared to birches and oaks. Reimann et al. (2001) reported that pines could accumulate up to 10 times higher Al amounts in green biomass than deciduous birch and willow. Pot experiment showed that pines were the least affected by disturbance tree species, regardless if they were non- or inoculated with the ECM blend. Transfer in pots led to the accumulation of Mn, Co and Cu in green biomass, while Cr, Fe, Ni and Sr were excluded. Overall, as a turnover of aboveground biomass in coniferous tree stands is markedly slower than in deciduous tree stands, pines can be applied to the remediation of soils contaminated with heavy metals.

4.3. Characterization of ECM community patterns

4.3.1. The low diversity of the ECM community reflected a state of Kanigsberg's ecosystem development

The classic Jaccard and Sørensen coefficients widely used to describe similarity/dissimilarity between assemblages are sensitive to sample size, especially for communities with rare species. This might lead to an overall underestimation of true similarity between compared communities. A probabilistic approach developed by Chao et al. (2005) and applied in this study incorporates the effect of unseen shared species and, thus, lessens the sample-size bias of similarity indices. Similarity indices based on presence/absence (Jaccard, Sørensen) and abundance of morphotypes (Bray-Curtis) have the highest values 1, when compared communities have the same composition, and the lowest values 0, in the case when communities do not share species (Hao et al., 2019). The Jaccard coefficient counts the presence of the unique species, while the Sørensen coefficient counts shared species in compared communities. High values of similarity indices in this study characterized ECM communities of trees of one species as very similar. The lowest values of the Jaccard coefficient (0.500) were calculated for field oaks and pines, suggesting that ECM communities of one/or several trees comprised unique morphotypes which were not described for other tree(s). Bray-Curtis similarity considers the relative abundance of morphotypes and in this study described ECM communities of all trees as highly similar as well.

All similarity indices calculated for birches ECM communities had the lowest values for non-inoculated variants, whereas similarity indices determined for ECM communities of oaks and pines were the highest for non-inoculated plants. Dynamics of similarity indices between variants can be related to changes in environmental conditions. The higher number of unique species at the field oaks and pines sampling sites might be related to their original soil heterogeneity determined the presence of microhabitats in the soil where unique species could be beneficial for the host plant in terms of nutrients uptake. Non-inoculated pot birches developed a voluminous root system after two years in the pot experiment. The growth of plant roots resulted in new habitats for exploration by already associated ECM fungi. It led to the establishment of symbiotic relationships with new ECM species that existed as propagules in pot soil substrate, which might increase the overall dissimilarity of described ECM communities.

The number of ECM species described in heavy metal contaminated areas is different in published studies and determined by many factors, including the trees' age, vegetation succession stage, and contamination level. What is common for these works is that compared to reference sites or soils with a relatively low level of contamination, ECM communities in metal-polluted soils are almost always characterized by low diversity and rate of mycorrhization and the dominance of several taxa. Host plants select symbionts that either alleviate metal-induced stress by decreasing uptake of toxic metals or/and improve the supply of essential nutrients to their host under unfavourable conditions (Hartley et al., 1997; Urban et al., 2008).

A very low number of ECM morphotypes in all variants of the experiment was observed. For the test field area, in sum, only 11 morphotypes were described. These observations correspond with previous studies of ECM communities at the Kanigsberg area. In his thesis, Märten (2017) described two morphotypes for birches growing close to the birch sampling site in the given study. Gherghel (2009) described five morphotypes for oaks populating Kanigsberg. ECM communities of pines naturally growing at Kanigsberg were characterized first time in this work.

Similar trends were observed for the plants growing in other metal contaminated areas. Staudenrausch et al. (2005) characterized overall 23 different morphotypes for birches growing at three experimental sites that were parts of a former uranium mining area and differed in the content of heavy metals and presence/absence of organic horizon. Lower mycorrhization rate and reduced richness and ECM community diversity were observed at a bare heap compared to the reference site and a heap site with an organic horizon. Additionally, the dominance of four morphotypes at both heap sites was determined. The authors explained the reduction of ECM community diversity by faster turnover of ectomycorrhiza under heavy metal stress and by selecting ECM species tolerant to unfavourable conditions. Rudawska et al. (2011) reported that the total number of mycorrhizal roots of Scots pine per 1 g of root dry weight was significantly lower in metal contaminated soil compared to slightly contaminated site and a control non-polluted area. Interestingly, the number of ECM species observed in metal influenced soil was higher compared to control soil. At the same time, the Shannon index value was the lowest, and the dominance index was the highest, suggesting the prevalence of particular taxa in the ECM community. Katanić et al. (2015) observed only four ECM species on roots of poplars growing in copper-affected soil. The most representative morphotype formed by *Thelephora terrestris* contributed to 89% of overall diversity. Hryniewicz et al. (2008) determined that five morphotypes formed by Thelephoraceae constituted from 53 to 91% of the mycorrhizal roots of *Salix caprea*, growing at a former ore mining site. An unidentified morphotype described as *Pinirhiza arenosa* contributed up to 70% of all ectomycorrhiza observed in two zinc wastes (Mleczko, 2004). Experiments on the influence of applied heavy metals on ECM formation confirmed that the increase of metal content reduced in general mycorrhization rate and the number of mycorrhizal tips (Dixon, 1988; Dixon and Buschena, 1988; Chappelka et al., 1991; Hartley-Whitaker et al., 2000; Duñabeitia et al., 2004).

The site's age seems to be crucial for ECM community diversity and might explain the low number of ECM species described for field plants. Numerous works demonstrated that mycorrhizal colonization rate, ECM community diversity and the number of morphotypes were the lowest at young tree stands and increased along the chronosequence (Helm et al., 1996; Rao et al., 1997; Peter et al., 2001; Palfner et al., 2005; Gebhardt et al., 2007). Moreover, Peter et al. (2001) speculated that colonization of the seedlings by ECM spores and propagules at early successional stages was more significant than by mycelium due to sparse vegetation. Besides, metal-polluted soils generally contain a lower number of fungal spores, which, together with low fine roots density typical for younger trees, might lead to the decreased rate of mycorrhization and community diversity (Leyval et al., 1997).

4.3.2. Molecular identification and characteristics of ECM fungi

It was impossible to compare different variants of the experiment based on the ECM taxonomy as sequence analysis of ECM morphotypes observed for pot plants was not successful in general. Although PCR is a powerful molecular tool widely used in mycorrhizal identification, it is a very sensitive method at the same time. Application of the modified method of direct PCR of mycorrhiza showed satisfactory results when field ECM communities were analyzed, and from 11 morphotypes, only one remained unidentified. Sequence analysis for pot plants, however, failed. Several reasons might explain this. First, pot plants mycorrhiza looked very dried. Although only healthy-looking short roots were described and sampled for further molecular identification, one cannot exclude that some of the mycorrhizal roots were not viable, and DNA extrication might have a very low yield. Another problem is the co-extraction of DNA from several fungal species, which can also prohibit successful sequence analysis (Kaldorf et al., 2004; Iotti and Zambonelli, 2006). Additionally, phenolic compounds and melanins can restrict the activity of Taq polymerase. Eckhart et al. (2000) showed that Taq polymerase formed a distinct complex with melanins that inhibited PCR. Soil complex compounds such as humic and fulvic acids can strongly inhibit PCR as well: humic acids were shown to be able to interact with Taq polymerase, affect primers annealing process, chelate magnesium ions which are necessary for Taq polymerase (Tsai and Olson, 1992a, b; Kreader, 1996). Heavy metals can negatively affect the molecular identification of ectomycorrhiza. Positively charged metal ions can form chemical bonds with negatively charged DNA (termed as M-DNA complexes), inhibiting in this way access of Taq polymerase to DNA during PCR. Such divalent metal ions as Zn^{2+} , Sn^{2+} , Cu^{2+} , Fe^{2+} , Ni^{2+} were shown to have the highest affinity to DNA molecule and, thus, the greatest PCR inhibitory effect (Sagripanti et al., 1991; Tsai and Olson, 1992a; Hartzell and McCord, 2005; Kuffel et al., 2021). Pot substrate contained high amounts of Al, Cu and Fe. It was noticed that while the overall abundance of dark-coloured mycorrhiza in pots decreased compared with field variants, most of the mycorrhiza and plant roots had a reddish to bright brownish colour. The high original content of iron-containing compounds could lead to precipitation and binding of Fe ions to plant roots.

Basidiomycota was the most diverse among the field morphotypes and included Russulaceae, Inocybaceae, Cortinariaceae, Thelephoraceae, Rhizopogonaceae, Tricholomataceae. Ascomycota was represented by *M. bicolor* and an oak morphotype *Pinirhiza bicolorata* defined as Helotiales by sequence analysis. All identified field ECM species were previously described as ubiquitous in different ecosystems, including metal affected soils. All field morphotypes, except for *M. bicolor*, occurred only at a particular sampling site. *M. bicolor* was the only species common for all test field sampling sites and among the most representative ones.

Meliniomyces bicolor

M. bicolor belongs to *Hymenoscyphus ericae* aggregate and is known for the ability to form both ericoid mycorrhizal symbiosis with ericaceous species and ectomycorrhizal symbiosis with temperate forest trees (Grelet et al., 2010; Martino et al., 2018). *M. bicolor* is a mycobiont of morphotype *Piceirhiza bicolorata* found on *Picea* sp., *Pinus* sp., *Betula* sp., *Populus* sp., *Quercus* sp. and *Salix* sp. (Vrålstad et al., 2000; Hambleton and Sigler, 2005). Dark-coloured mycorrhiza described for field plants in this experiment was formed exclusively by *M. bicolor*. The presence and dominance of this morphotype at the field can be an ECM community adaptation to unfavourable local conditions and might be related to the incorporation of melanins in the cell wall. Melanins are dark-coloured hydrophobic pigments found in plants, animals, fungi and bacteria. In fungi, they are located in cell walls and/or can be released as extracellular compounds (Fogarty and Tobin, 1996). Melanins are known to retain an enormous amount of water and, thus, can be considered as antidesiccants (Butler and Day, 1998). Ubiquitous ascomycete *Cenococcum geophilum*, which forms abundant melanized mycorrhiza, is known for the ability to tolerate low soil water availability (Mexal and Reid, 1973; Pigott, 1982; Coleman et al., 1989; Jany et al., 2003; Di Pietro et al., 2007; Querejeta et al., 2009; Herzog et al., 2013; Rossi et al., 2015; Carcaillet

et al., 2020). The ability of fungal melanins to bind metals is of particular interest. Melanins comprise various functional groups (carboxyl, phenolic, hydroxyl, and amino- groups), which provide ion-exchange sites for metals (Fogarty and Tobin, 1996). The particularly high affinity of fungal melanins to copper (Gadd and De Rome, 1988) and iron (Senesi et al., 1987) was shown.

Unfortunately, there are no studies on the tolerance of *M. bicolor* to metal-induced stress conditions. However, taking into account the high abundance of this fungus in field samples, one can assume that *M. bicolor* could tolerate elevated concentrations of toxic metals as well as fluctuations in water availability under natural conditions. Additional experiments to prove the high environmental stress resistance of *M. bicolor* should be performed.

Dark-coloured *C. geophilum* was reported to form ectomycorrhiza with plants at Kanigsberg (Gherghel, 2009; Märten, 2017). In contrast, none of the morphotypes in the given study was sequenced as *C. geophilum*. Several authors mentioned that *M. bicolor* could be misidentified as *C. geophilum* (Douhan et al., 2007; Villarreal-Ruiz and Neri-Luna, 2017). Grelet et al. (2010) pointed out that the abundance of *C. geophilum* in soils was highly likely overestimated at the expense of dark-coloured *H. ericae*-like representatives. Thus, the given study emphasizes the importance of molecular analysis in ECM identification.

Lactarius sp.

Association of *Lactarius* sp. with the field birches might be related to the high tolerance of this fungus to the unfavourable local conditions, particularly to elevated concentrations of aluminium. Numerous studies showed that ECM fungi could tolerate different levels of aluminium *in vitro* both in monoculture and in symbiosis with the host plant. Egerton-Warburton (2015) revealed that *Eucalyptus* in symbiosis with three different ecotypes of *Pisolithus* accumulated lower aluminium amounts than non-mycorrhizal seedlings after exposure to aluminium. An interesting finding was that a *Pisolithus* ecotype isolated from the mine site reduced the level of Al within the seedlings more efficiently compared to ecotypes isolated from forest soil. This observation was explained by the binding of Al to the fungal cell walls and, therefore, limiting the accumulation of Al into plant tissues. Moyer-Henry et al. (2005) examined Al tolerance in non-mycorrhizal and mycorrhizal (with *P. tinctorius*) pine seedlings. With the help of the lumogallion staining and confocal microscopy, the authors observed considerable accumulation of Al by extraradical mycelium and hyphal mantle after mycorrhizal seedlings were exposed to Al providing another evidence that ECM can restrain Al penetration into the plant. Gu et al. (2021) compared Al-accumulation and Al-tolerance in *P. tinctorius* and *Lactarius deliciosus*, both isolated from Southwest China's forest. The authors showed that *L. deliciosus* expressed higher tolerance to elevated aluminium concentrations and higher accumulation of the metal in mycelium biomass compared to *P. tinctorius*. Among the main mechanisms involved in Al-tolerance were bioconcentration, a high number of active sites for Al^{3+} , hyphal mantle thickness, cation exchange capacity, and immobilization on the cell wall. Unfortunately, there is still a lack of field studies on the symbiotic relationship between tolerant ECM species with host plants in soils containing high aluminium concentrations and, therefore, they represent high interest.

Mallocybe sp.

At the test field, *Mallocybe* was exclusively identified on birches roots. *Mallocybe* is a subgenus of *Inocybe* genus and was previously observed on *Salix*, *Betula* and *Dryas* (Cripps et al., 2010) as well as in mixed stands with adult *P. sylvestris* (Vauras and Larsson, 2011). Phylogenetically closely related *Inocybe* sp. is ubiquitous in soil and was found in metal affected soils (Mleczko, 2004; Krpata et al., 2008; Moser et al., 2008). Interestingly, in this study *Mallocybe* sp. was observed on pines in both pot variants, but not at the field. Thus, one can speculate that this species could be preserved in pot substrate as spores and could later infect pine roots.

Cortinarius sp. and *Tricholoma* sp.

Both species have very similar ecological strategies and are known to form extensive hydrophobic rhizomorphs. Lilleskov et al. (2011) suggested that both species were well-adjusted to explore surrounding substrate for organic nitrogen under N-limited conditions. *Cortinarius* sp. was solely observed at the field oaks sampling site. This ECM species was reported to produce Mn-peroxidases – enzymes that enable oxidative decomposition of humus and, thus, mobilize organically bound nitrogen (Bödeker et al., 2014). This study revealed a significant correlation of *Cortinarius* sp. with the content of total nitrogen. Therefore, the presence of *Cortinarius* sp. at this field site might be indicative of the content of recalcitrant organic matter in the soil. Both species were previously observed in contaminated areas; however, they were not among the most representative species (Mleczko, 2004; Staudenrausch et al., 2005; Hryniewicz et al., 2008; Krpata et al., 2008; Bierza et al., 2020).

Rhizopogon mohlensis

R. mohlensis was identified on field pines and inoculated pot pines. It seems that this species was persistent to the disturbance caused by the transfer of the plants to the pots and changes in abiotic conditions. The high ability of *Rhizopogon* spores to maintain viability during a long period of time was discussed previously (Torres and Honrubia, 1994; Horton et al., 1998). Moreover, it was mentioned that *Rhizopogon* could successfully colonize small disturbed habitats because of its ability to germinate from spores very fast (Bruns, 1995).

Other morphotypes

Telephoraceae is a common component of the ECM belowground fungal community and is especially widely distributed in coniferous forests (Köljalg et al., 2000) and often found in heavy metal contaminated areas (Hryniewicz et al., 2008; Krpata et al., 2008). The brown colour of telephoroid morphotypes, observed in the given study, might be related to the incorporation of melanins in the cell walls and can be an adaptive mechanism to soil drought, attack of antagonistic fungi (Köljalg et al., 2000) as well as heavy metal pollution (Hryniewicz et al., 2008).

Order Helotiales includes species with a wide range of lifestyles: from saprobes and plant pathogens to ericoid mycorrhizal and ectomycorrhizal symbionts. Several melanized representatives isolated from *Piceirhiza bicolorata* roots of trees growing at Cu-mine spoils belonged to *Helotiales* (Vrålstad et al., 2002).

Hyaloscyphaceae belonging to Helotiales formed several pot morphotypes. Hyaloscyphaceae includes ubiquitous soil species with a predominantly saprotroph lifestyle (Luis et al., 2017).

4.3.3. Field ECM fungi preferred contact and short-distance exploration strategies

Field morphotypes formed mycorrhiza preferentially with contact and short-distance exploration types. This observation is consistent with results previously received for undisturbed forest stands. Rosinger et al. (2018) analyzed numerous studies on ECM communities on root systems of *Fagus sylvatica*, *Picea abies*, and *Pinus sylvestris* throughout Europe. They revealed that contact and short-distance exploration types had a higher mean abundance in undisturbed ecosystems compared to medium and long-distance exploration types.

The ratio of ECM exploration types in anthropogenically affected soils is of particular interest. Hryniewicz et al. (2008) observed six morphotypes with contact, five morphotypes with short-distance and three morphotypes with medium-distance exploration types in three metal contaminated soils. Contact exploration type was dominant at all sites. The authors attributed morphological characteristics of contact exploration types to the ability to tolerate unfavourable conditions through the decrease of the surface area and, therefore, smaller exposure to the contaminated environment. Rudawska et al. (2011) compared three sites different in level of contamination (non-contaminated soil, slightly contaminated located area near former chemical plant, and heavy metal-influenced site at copper

smelter). While the control site was dominated by the contact exploration type, contact and short-distance exploration types prevailed at the slightly contaminated area. Interestingly, that metal-affected area was characterized by a high abundance of morphotypes with medium-distance exploration type. Authors speculated that abundant emanating hyphae and ramifying rhizomorphs typical for medium-distance exploration type functioned as a filter hindering the entry of heavy metals into the host plant cells. Bierza et al. (2020) observed distinctive dominance of mycorrhiza with long-distance exploration in heavy-metal contaminated soils. The abundance of this exploration type was significantly related to the toxicity index of the total concentration of heavy metals. Authors mentioned that far-reaching rhizomorphs, on the one hand, can explore and absorb nutrients from immense soil volume and, on the other hand, can function as a natural filter hampering the transport of heavy metal to host plants. In the given study, permutation analysis did not reveal the significant correspondence between any toxic metals and exploration types of mycorrhiza. Therefore, it can be assumed that abiotic parameters not considered in this study could explain the distribution of exploration types of mycorrhiza.

4.3.4. Re-planting affected trees performance and functional diversity of associated ECM community

Transfer of the plants from the test field to pots affected tree species differently. The survival rate of plants in non-inoculated variants indicated the different ability of the plants to tolerate considerable changes in abiotic conditions and, thus, affect associated fungal symbionts. In non-inoculated pot variants, the number of ECM morphotypes increased for birches, decreased for oaks and did not change for pines compared to field variants. The survival rate of non-inoculated oaks was the lowest compared to other non-inoculated tree species and comprised only 16.7%. Changed soil parameters in pot variants could explain the decrease in the number of observed ECM species as well as the low performance of oaks not adapted to these unfavourable conditions. At the same time, pot substrate was sampled near the birches sampling site. Therefore, newly established conditions in pots were similar to field conditions for birches. Coarse material at the test field soil can create a spatial disconnection between root systems of different plants as well as between plant roots and ECM mycelium and spores. Removal of coarse material during the preparation of pot substrate could eliminate this dispersion, create new habitats and, thus, enhance the chances for successful colonization of birches roots by adapted to these conditions ECM species.

Morphotyping revealed that pot birches, to less extent pot oaks, extensively enlarged their root system by forming new roots. At the same time, the original roots of all pot plants looked very dried, and some even non-viable. The decrease in the number of live roots could lead to the loss of extramatrical mycelium connected to these roots.

It was assumed that additional inoculation of pot plants should contribute to the overall pool of ECM spores in soil and increase root colonization. However, pot plants reacted to inoculation differently. Oaks were the only tree species for which additional inoculation had a positive effect on plant growth. Moreover, one morphotype on inoculated oak was identified as *Pisolithus sp.* and was not observed for other oaks variants. Therefore, it can be the only example of successful infection of pot plants from the ECM blend, which contained *P. tinctorum* spores/hyphae.

ECM communities responded differently to the transfer of the host plants from the field to pot substrate. The functional diversity of ECM communities described for non-inoculated birches did not change and was characterized by the almost equal presence of contact and short-distance exploration types. The formation of rhizomorphs cost the plant carbon (Agerer, 2001). Therefore, the abundance of contact and short-distance exploration types might reflect the adaptation of birches to retain and use carbon for their growth rather than to invest it into soil exploration. Moreover, birches developed extensive root systems in both pot variants, and, therefore, the development of rhizomorphs was unnecessary as an overall increase of root surface could provide the plant with water and nutrients.

In non-inoculated oaks and pines abundance of ectomycorrhiza with rhizomorphs increased significantly. The development of rhizomorphs could facilitate the extensive exploration of newly created niches. For oaks, the significant decline in the content of total nitrogen and total phosphorus in pot substrate compared to original field soil might cause a considerable shift in functional traits of ECM community toward expansion of medium-distance rhizomorphs. On the other hand, Baldrian (2009) proposed that when the host plant is under stress, ECM fungi can start to degrade the host plant's roots to use them as nutrients and support the development of extraradical mycelium to search for new non-mycorrhizal roots. As non-inoculated plants' roots looked damaged, the significant increase of rhizomorphs observed in this study for pot oaks and pines might support the proposed idea. Besides, the hydrophobicity of rhizomorphs can indirectly control the access of water into mycelium. Compared to natural precipitation fluctuations at the field, where dry soil conditions might last for several days, the water regime in pots, in contrast, was improved and kept stable. Thus, extensive development of hydrophobic rhizomorphs in non-inoculated pots might be the strategy to control water intake and prohibit the uncontrolled inflow of water. Moreover, for oaks and pines development of hydrophobic rhizomorphs might be a strategy for effective nutrients transport as hydrophobic properties will likely prevent the loss of solutes during the medium and long-distance transfer to the host plant (Lilleskov et al., 2011).

Additional inoculation of pot plants led to the loss of rhizomorphs. All pot ECM formed mycorrhiza with contact and shortdistance exploration types. An interesting observation was made for inoculated pines. They formed mycorrhiza with woolly silver mantle with infrequent emanating hyphae without rhizomorphs, later identified as *Rhizopogon mohelnensis*. The same species was observed on field pines roots, where it formed distinctive rhizomorphs. One possible reason to explain the observed changed exploration strategy might be that the addition of the ECM blend caused a change in water content. The first inoculum contained peat as a carrier of strains. It is known that peat is characterized by high wettability and water absorbency (Michel, 2010). Therefore, the ECM blend could accumulate and retain the additional water in the pot plant root zone. It was reported that the production of hydrophobic mycelium and rhizomorphs could have ecological importance in relatively dry conditions (Unestam, 1991; Unestam and Sun, 1995). Similarly, Bakker et al. (2006) observed dominance of hydrophilic contact exploration type on pines roots growing at the humid site, attributed to the more efficient strategy of substrate exploration in the organic horizon. Changes noticed between pot variants support this observation. Hence, the application of the ECM blend could contribute to the improvement of the water regime in pots and might lead to the loss of rhizomorphs by ECM fungi as more carbon-cost and unnecessary under these conditions.

Supplement of non-sterile blend could increase the quantity of nutrients and, therefore, intensify the growth of saprotrophs. Under conditions of increased resource availability, saprotrophic fungi could outcompete ECM fungi and prohibit the establishment of new symbioses and lead to the death of already existing ectomycorrhizal roots. Besides, Lindahl et al. (2010) suggested that physical disturbance of microbial community might result in transformation of disturbance-sensitive ECM fungi into a resource for saprotrophs. Extensive damage of mycorrhizal roots observed in the pot variants might contribute to the nutrient pool and, therefore, enhance the growth of free-living saprotrophic fungi. Another explanation of the loss of rhizomorphs by pot plants is based on Bruns's (1995) review that the spatial position of short roots might determine their infection by ECM fungi. He specified that short roots near the stem get more carbohydrates, while the most distant short roots receive "what is left". In this concern, lack of host carbohydrates limits infection by carbon-demanding fungi ("late-stage") as well as the formation of rhizomorphs. Most of the roots observed next to the stem, where the ECM blend was placed, looked dried and damaged, and only viable-looking short roots found on the distance from the stem were included in morphotyping. Consistent with the above review, one could expect that insufficient or even disrupted flow of carbohydrates from the host plant to far-distant roots might hamper the development of rhizomorphs.

4.3.5. ECM community demonstrated weak associations with soil parameters

Field ECM species with contact distance exploration type were the most affected by soil characteristics. However, the patterns of these correlations with abiotic factors differed among the trees. As field sampling sites differed from each other in the content of several toxic metals as well as the content of total carbon, nitrogen and phosphorus, the relative abundance of exploration types was associated with the soil characteristics specific for a particular sampling site. At the test field, dark-coloured morphotypes with short-distance exploration type (*M. bicolor* and Helotiales) did not depend on soil chemical parameters. Moreover, they took the central position on the CCA plot, which indicates their widespread distribution and probably high tolerance to unfavourable conditions at Kanigsberg. Interestingly, medium- and long-distance exploration types observed at the pine sampling site were not explained by soil chemistry as well. At the oak sampling site, medium fringe exploration type positively correlated with the content of total nitrogen. Lilleskov et al. (2011) discussed different exploration types in terms of the ability of ECM fungi to explore soil differently as a response to nitrogen deposition. It was shown that ECM fungi with hydrophobic branching rhizomorphs were highly represented under N-limited conditions and were adapted to explore substrate for organic nitrogen. Moreover, it was hypothesized that ECM fungi with these functional traits would produce a wide range of hydrolytic enzymes. Observations made in the given study support this hypothesis. Indeed, ECM fungi with a medium-distance fringe subtype (*Cortinarius* sp. and *T. argyraceum*) and long-distance exploration types (*R. mohelnensis*) were found only at oaks and pines sampling sites which were parts of more developed tree stands. It can be assumed that under conditions typical for the test field, several ECM taxa had the advantage to explore the soil to get nitrogen from complex organic compounds. On the other hand, ECM fungi with contact and short-distance exploration types rely on nutrient uptake via their hydrophilic mantles. Therefore, these ECM fungi are of lower carbon cost to their host, and they tend to explore the soil for labile nitrogen forms (Hobbie and Agerer, 2010; Lilleskov et al., 2011). Overall, it can be concluded that field plants promoted ecological filtering toward selecting specific ECM species with particular exploration types, which would contribute to plants tolerance to abiotic conditions specific at each sampling site.

4.4. Characterization of microbial community patterns in primary succession

Most of the studies on the structure of microbial communities during the primary succession are related mainly to alpine landscapes, recently deglaciated terrains and areas disturbed by fires and deforestation. However, ecosystems re-established after mining activities receive attention as well. At the same time, landscapes resulted from natural plant colonization pose a particular interest. Understanding which microorganisms are recruited by trees naturally growing at former mines might facilitate remediation techniques applied in a specific area.

4.4.1. Tree presence influence: site identity conceals the rhizosphere effect

The rhizosphere represents a so-called “hot spot” and usually harbours a higher abundance of microorganisms than surrounding bulk soil. Plants growing at a bare substrate might be considered as cores or centres of colonization by bacteria and fungi. The most striking result in this study was that no significant differences between mycorrhizosphere and bulk soil were found related to any tree species at the test field.

Previous studies demonstrated rather contradictory results on the rhizosphere effect of plants at early stages of succession. Miniaci et al. (2007) demonstrated interesting results on the rhizosphere effect of the pioneer plant *Leucanthemopsis alpine* on microbial community structure and microbial activity at different distances from the plant. Despite the observed significant increase in total and active cells counts and enzymatic activity in the proximity to the plant, richness and Shannon index values did not change at the distance from the plant. The authors suggested that sampled bulk soil could have arisen

from the extended area of *L. alpine* influence, and, therefore, no rhizosphere effect was observed. In the present work, bulk soil was sampled from 15 to 20 cm from the plant stem, where no root system was observed. At the field birch sampling site, trees tended to form a dense belowground root network. One can assume that some ECM fungi associated with fine roots could extend the zone of plant influence further than 20 cm from the stem. At the oak sampling site, the roots of the test trees were interconnected with grass roots, so that surrounding vegetation might have lessened the oaks' selective effect on microorganisms in the rhizosphere.

Tscherko et al. (2004) reported that a pioneer plant *Poa alpina* influenced soil microbial communities differently along the chronosequence in the alpine ecosystem. The rhizosphere did not differ from bulk soil at early successional stages, indicating that the plant did not select specific microorganisms. Interestingly, this plant created a physicochemical gradient for microorganisms at subsequent mature stages, which could be related to the increase of soil and root exudates heterogeneity. As a consequence, an established wide range of distinct habitats was colonized by various microorganisms. Tscherko et al. (2005) showed that vegetation formed by different plant species at the early successional stage did not affect the microbial biomass, phospholipid fatty acids or enzyme activity, and no differences in microbial communities between rhizosphere and bulk soil were observed. Authors speculated that plants under unfavourable conditions, typical for the early successional stage, did not select specific microorganisms in the rhizosphere, and soil parameters determined microbial community composition.

In contrast to discussed above works, Ciccazzo et al. (2014a) showed that 12 pioneer plants species from the high mountain environment selected specific rhizobacterial taxa compared to bulk soil. At the same time, the authors reported that five of twelve species were clustered together according to NMDS based on the Bray-Curtis distance, explaining this observation by the local occasional highly-disturbing processes, so that the random abiotic conditions can be co-drivers of the rhizobacterial community structure. Sun et al. (2018) demonstrated that three different pioneer plants growing at copper mine tailings shaped significantly different in structure rhizosphere microbiomes compared to their bulk soils. Interestingly, *Alnus cremastogyne* (perennial woody plant) differed from *Pennisetum perpureum* and *Typha angustifolia* (both annual types of grass) in the structure of rhizosphere microbial communities, suggesting that vegetation type and related to this type quality of litter and the composition of root exudates might play an important role in the structuring of the bacterial community.

As microbial community associated with trees at the field could be affected not only by a plant but also by site identity, only comparisons of the pot plants mycorrhizospheres might elucidate the role of tree presence and identity.

It was previously mentioned that post-mining areas are generally characterized by high soil heterogeneity. On the ecosystem scale, it creates a wide range of microhabitats with different abiotic and biotic conditions, which, in the end, facilitates higher biological diversity and ecosystem stability and resilience in general. However, from the practical point of view, the coarse structure might complicate the microbial community characterization. Pot experiment enabled to remove macro-scale variations and equalize abiotic conditions for the plants and microorganisms, representing a simplified way to model processes occurring in nature.

Pot plants were associated with microbial communities different in structure from those in control pot substrate, suggesting a rhizosphere effect. This result, on the one hand, contradicted observations obtained for field plants, mycorrhizospheres of which did not differ from surrounding bulk soil, on the other hand, supported the assumption that site identity might define microbial community structure. Interestingly, the pot plants effect was more pronounced for the bacterial community than for associated fungi. Transfer to the pots could considerably affect plants, as indicated by their survival rates. Therefore, it can be expected that depending on host plant physiology, ECM fungi might also experience disturbance caused by re-planting. This study assumed that homogenization of pot substrate and

related decrease in the number of habitats with contrasting abiotic conditions should lead to the high similarity of microbial communities in this environment. Indeed, the transfer of the plants to pots led to the increase of similarity of bacterial communities between related tree species. On the contrary, fungal communities associated with pot plants revealed an opposite trend, indicating a disrupted link between the host plant and the associated fungal community. Besides, a considerable shift in fungal community structure from the dominance of ECM fungi to the prevalence of saprotrophs could lessen the dependence of the fungal community on the plants and, therefore, decrease the rhizosphere effect for fungi in general.

As pot plants did not form litter in the glasshouse experiment, root exudation might explain the rhizosphere effect. An extensive root development observed for pot plants might be associated with the increased pool of root exudates, as Badri and Vivanco (2009) discussed, contributing to the selection of microorganisms. In addition, changes in the quality of root exudates due to the shift in abiotic conditions might also enhance the rhizosphere effect. Control pot substrate contained the increased concentration of Al compared to field oaks and pines sampling sites and the decreased content of total nitrogen and phosphorus compared to the field oak sampling site. Previously published studies demonstrated that the high aluminium content in soils and nutrient deficiency led to the changes in root exudates composition, confirming the importance of abiotic parameters in root exudation (Ma, 2000; Liao et al., 2006; Neumann and Römheld, 2007; Liang et al., 2013).

Moreover, association with fungi is known to change the extent and composition of root exudates. Meier et al. (2013) demonstrated that loblolly pine seedlings associated with pathogenic, saprotrophic and ECM fungi released more exudates than pines associated only with ECM fungi. Infection with pathogens increases the rate of exudation to communicate with rhizobacteria and ECM fungi in order to produce defence compounds (Jousset et al., 2010). Although non-significantly, the diversity of fungal communities in pot plants mycorrhizospheres increased due to the higher percentage of saprotrophs and, probably, pathogens. Hence, it can be assumed that a shift in fungal community structure and exploration strategy could facilitate changes in exudation by pot plants and lead to the pronounced for bacteria rhizosphere effect in pots.

4.4.2. Tree identity influence: tree species does not drive microbial community composition

Multivariate analysis of microbial community structure dataset demonstrated that field trees shaped rather contrasting in composition mycorrhizospheres. Associated with field birches bacterial communities distinctly differed from those associated with field oaks and pines. Furthermore, field oaks contrasted with field birches and pines in the structure of fungal communities. Mixed vegetation at the oak stand might contribute to the development of distinctive fungal communities in oaks mycorrhizospheres. In contrast, birches and pine stands represented by isolated trees could form root-fungal associations similar in composition. Interestingly, two different types of vegetation represented by deciduous (birch) and coniferous (pine) trees comprised a united fungal cluster. This result suggests that factors other than tree species determined the structure of fungal communities at the field.

Numerous previously published studies support the result on the species-specific influence of the plant on microbial community structure during primary succession. Ciccazzo et al. (2014a) demonstrated a strong effect of plant identity on the rhizobacterial community in a high mountain ecosystem during an early primary succession, suggesting the capability of pioneer plants belonging to different species to harbour a specific rhizobacterial community under highly oligotrophic conditions. Kuske et al. (2002) showed that bacterial communities in rhizospheres of different plant species growing at high-elevation arid grasslands were dissimilar. Reported differences were explained by species-specific root growth patterns, which could modify local soil conditions and cause changes in bacterial communities. Knelman et al. (2012) reported that vegetation type (alder vs spruce) correlated with bacterial community structure in a glacier forefield. Plant-specific differences in bacterial community composition were

determined by plant-mediated acidification of soil (pH value), root exudates' chemical composition, and litter.

Miniaci et al. (2007) suggested that shifts in microbial community composition in the rhizosphere of pioneer plants growing at a glacier forefield might be related to the plant-species variation of rhizodeposition patterns. Unfortunately, exudate profiles of pioneer plants under natural conditions are not studied very well, and it is challenging to estimate the input of root exudates into the structuring of associated microbial communities. Nevertheless, there are several works based on the study of the chemical composition of plant root exudates and their direct and/or indirect impact on rhizosphere microbial community structure. For example, Boldt-Burisch et al. (2019) observed that the quantity and composition of organic acids were significantly different among three pioneer plants at the post-mining area. Released organic acids were probably involved in obtaining plant-available phosphorous. Grayston et al. (1998) demonstrated in a pot experiment that enrichment of soil with sucrose in order to mimic carbon inputs into the soil resulted in dissimilarities between the carbon sources (mainly carbohydrates, carboxylic acids and amino acids) utilized by microbial communities from the different plant rhizospheres. This result suggests that plants can differ in the exudation of these compounds and, thus, shape microbial communities, distinctive in structure. Abiotic factors can affect the quality and quantity of root exudates. For example, Westover et al. (1997) demonstrated that plant-species related differences between microbial communities' structures were partially explained by the influence of environmental factors (such as temperature and soil moisture) on root exudations. Besides, Dietz et al. (2020) showed that under field conditions, the composition of polar root exudates depended on local abiotic conditions (soil moisture, texture), while the pattern of semi-polar metabolites was influenced mainly by plant species (grass or forb vegetation). In contrast, in their review, Dennis et al. (2010) questioned the importance of root exudates' impact on the structure of the microbial community. The authors hypothesized that based on the fact that root exudates were released mainly at root apices and rapidly mineralized, the overall time of their influence on the local microbial community would be insignificantly short, which in turn might explain the absence of the differences between rhizosphere and bulk soil microbial community structure.

The chemical composition of plant litter is considered as one of the factors driving the structure of the rhizosphere microbial community. Most of the works evaluating the input of plant species on microbial community structure demonstrated that soil under different vegetation stands varied in chemical parameters determined mainly by pH values and content and quality of organic matter (Frouz and Nováková, 2005; Tschierko et al., 2005; Knelman et al., 2012; Šnajdr et al., 2013; Chodak et al., 2015). At birch and pine sampling sites, where young trees did not produce an extensive amount of foliage, the leaf/needle litter sink was insignificant. Therefore, the quality of litter would have a minor effect on soil chemical parameters at these sites. At the same time, PCA demonstrated that the content of total carbon and total nitrogen contributed considerably to the distinction of the oak sampling site, where the litter horizon was observed. Based on these results, it can be suggested that the developed litter horizon at Kanigsberg might have a more significant effect on soil and associated microbial community compared to the species-specific chemical composition of root exudates. These conclusions were supported by published studies as well. For example, Chodak et al. (2015) demonstrated significant differences in physiological profiles of microbial communities under different trees only in litter horizon but not in mineral soil. This observation was explained by the uniformity of organic matter in mineral soil, whereas organic matter in the litter was more related to the chemical composition of plant cover. Šourková et al. (2005) explained the highest microbial biomass under oaks and alder stand compared to pines by the litter quality, namely C/N ratio and the low content of phenolics in leaves compared to needles, suggesting that vegetation type had a great effect on the microbial community. Urbanová et al. (2015) showed that the quality of the litter (coniferous or broadleaf) rather than soil parameters influenced bacterial and fungal community structure. Moreover, tree species was an important predictor

of microbial community composition, as 37% of the fungi and 28% of bacteria displayed a significant preference for one particular litter.

Glasshouse experiment demonstrated that two years were not enough to establish plant species-specific mycorrhizospheres. Ordination plots did not show a tendency to distinguish either bacterial or fungal communities based on their associations with particular tree species. This result supports an assumption that observed at the test field differences in microbial communities, initially attributed to the tree species, were determined predominantly by site identity. Several studies demonstrated similar observations and showed that different plant species might shape identical in the structure microbial communities at the early stages of ecosystem development. Arctic environment plants belonging to various species were associated with similar fungal communities suggesting that symbiosis with fungi-generalists may facilitate more efficient colonization of new habitats (Botnen et al., 2014). Nunan et al. (2005) demonstrated that plant species did not impact bacterial community composition in grassland soil. In contrast, environmental factors were crucial for explaining bacterial community structure in the rhizoplane of grasses. Moreover, Tscherko et al. (2005) observed plant species' effect on the composition and activity of rhizosphere microorganisms only after 43 years of succession in a recently deglaciated alpine terrain.

Although the statistical tests did not confirm the significance of all discussed above patterns, the received results can be considered as expected trends. High spatial heterogeneity typical for post-mining areas could lead to high inter-variant variability and explain why the comparison of the variants did not attain statistical significance. On the other hand, rarefaction curves reached plateau both for bacterial and fungal communities, suggesting that microbial populations of each subsample were fully represented, and the most abundant as well as rare species were covered by sequence. The increase of sampling can overcome this inconclusiveness related to the statistical insignificance of results. Thus, characteristics of community diversity based on larger sampling should theoretically approach those characteristics as estimated for the entire population (Bolyen et al., 2019). On the other hand, one should consider the fact that former mines are highly variable environments; therefore, the increase in sampling might not necessarily improve their representativeness.

4.4.3. Site identity shapes bacterial and fungal communities in different ways

It is widely discussed that during the early stage of succession microbial community structure in rhizospheres of pioneer plants is determined predominantly by abiotic conditions. The low content of nutrients, first of all, nitrogen and phosphorus, lack of organic matter, low pH values and presence of toxic metals are among the most affecting abiotic factors at post-mining areas (Pietrzykowski, 2019). Field sampling sites at Kanigsberg were established approximately at the same time. Nevertheless, the development of sampling sites went on different trajectories, and they acquired extremely contrasting properties and were colonized by contrasting microbial communities. The difference between field sampling sites was determined by the content of several toxic metals as well as such soil parameters as the content of total carbon, total nitrogen, total phosphorus, C/N ratio and pH values. Site identity, therefore, is defined by soil quality and state of soil development, which is inseparably linked to vegetation succession.

Combination of taxonomic data and the results of soil chemical analysis defined for mycorrhizosphere and corresponding bulk soil for each tree species allowed to assess the impact of site influence on microbial community patterns. Thus, it was shown that contrasting abiotic conditions at the birch sampling site determined a distinct separation of bacterial community from field oaks and pines bacterial communities. Along the simulated succession *control pot substrate* → *pot plant MR* → *field plant MR*, bacterial community structure was affected by the content of total nitrogen, total phosphorus and C/N

ratio, but not by the stage of succession. This result confirms that the bacterial community's composition was rather subjected to ecological filtering and weakly linked with vegetation succession. CCA demonstrated a significant correlation of bacterial community structure with multiple soil chemical parameters. As a result of their unicellularity and small cell size, bacteria occupy microhabitats which, on the one hand, might be separated from the zone of plant root influence, and, on the other hand, can be exposed to the effect of a larger number of abiotic conditions (Urbanová et al., 2015). The Al concentration was among the most affecting soil parameters. The high content of this metal was apparently related to low pH values, since in the soils with pH values lower than 5.0 Al is solubilized (Kinraide, 1991; Piña and Cervantes, 1996). Moreover, the content of organic matter, which provides binding sites for Al-ions and can immobilize this metal, was low at the birch sampling site (Piña and Cervantes, 1996). Al has no biological function and is toxic at high concentrations to plants and soil microorganisms (Jaiswal et al., 2018). The high content of Al negatively affected the abundance of the most representative bacterial taxa and reduced the richness and diversity of the bacterial community. Although not confirmed statistically, diversity indices had the lowest values in the field birch variants. These results confirm most of the published data reported on the toxicity of Al for microorganisms (Yang et al., 2012; Lian et al., 2019; Niu et al., 2020; Shi et al., 2020). Nevertheless, several taxa positively correlated with the high content of Al. Among them were Ktedonobacteria and Phycisphaerae. Unfortunately, the ecological functions of both taxa are not characterized. Shi et al. (2020) reported that several OTUs attributed to Ktedonobacteria were particularly enriched in rhizospheres at high Al concentrations. Therefore, the present study confirms the potential of these bacteria to tolerate the high content of Al. Microorganisms inhabiting the rhizosphere of plants growing in acidic soils have developed a range of mechanisms to tolerate high concentrations of Al and, as a consequence, can decrease the toxicity of Al to plants. Among them are the release of siderophores (Hu and Boyer, 1996; Mora et al., 2017), production of oxalic acid (Hamel et al., 1999), surface and intracellular binding (Guida et al., 1991).

Although field oaks and pines sampling sites had overlapping chemical parameters, the fungal communities in the mycorrhizospheres of these plants had rather different structures. Comparison of fungal communities along the simulated succession *control pot substrate* → *pot plant MR* → *field plant MR* revealed the significance of successional stage: more developed field oak sampling site was separated from younger field birch and pine sampling sites as well as all pot variants which modelled early stage of succession.

The correlation of fungal community with soil parameters was determined by the content of Al and Co. Although particular fungal taxa were associated with soil parameters, these correlations were not as extensive as for bacterial communities. These results confirmed the (3) *hypothesis* proposing that bacterial community structure was more dependent on soil chemistry than fungal community. Similar conclusions were made by Poll et al. (2006), who showed that fungi assimilated carbon from the litter while bacteria took up nutrients from the soil. Low associations of fungal taxa with the soil might reflect their dependence on plants. Harantová et al. (2017) demonstrated a close association between fungi and vegetation, suggesting that fungal community structure was determined by successional stage while bacterial community reflected soil chemical parameters. Similarly, Kolaříková et al. (2017) reported that none of the considered soil chemical parameters significantly affected the ECM fungal community, suggesting that the fungal community structure was determined rather by host plant-fungus interactions than abiotic conditions. Martínez-García et al. (2015) demonstrated that the AM fungal community underwent a significant shift in the structure defined by plant community changes rather than by the considerable alterations of soil chemical parameters during the ecosystem development.

Content of total carbon, total nitrogen, and C/N ratio considerably impacted the distinction of field oak sampling site from other sites, suggesting that this area represented a more advanced successional stage and had more favourable conditions for the development of microbial populations. CCA

demonstrated that total carbon and nitrogen content and C/N ratio significantly influenced bacterial community structure. In contrast, fungal community structure was almost not associated with these parameters, and only certain taxa were affected. Although bacterial communities did not correlate with the stage of vegetation succession, significant association with the content of total carbon and nitrogen and C/N ratio might reflect the indirect effect of vegetation on bacterial communities through the input of organic matter. Moreover, the pot experiment demonstrated a noticeable rhizosphere effect on the bacterial community. This result suggests that the plant presence might affect bacterial community structure; however, this effect was not pronounced at the field, apparently due to strong environmental selection. Similarly, Ciccazzo et al. (2014b) observed that carbon and nitrogen content gradients between sites at different development stages determined bacterial community profiles. In the cited study, differences among the sites, based on the amount of carbon and nitrogen, were explained, mainly by the occurrence and density of a plant cover.

Sánchez-Marañón et al. (2017) used a pedological approach to explain distribution patterns of bacteria in the soil. Although the authors did not consider the influence of the plant on bacterial communities, they showed that bacteria adapted to the soil they colonized. The authors concluded that despite the postulate “Everything is everywhere, but the environment selects”, bacterial community structures might change significantly along a pedogenic gradient. It was demonstrated, for example, that the abundance of Acidobacteria (oligotrophs) decreased and the abundance of Actinobacteria, Proteobacteria and Bacteroidetes (all copiotrophs) increased when soil quality parameters improved. Likewise, the relative abundance of Acidobacteria was significantly higher at the birch sampling site, where soil contained a low amount of total carbon and nitrogen compared to the oak sampling site. Moreover, the percentage of Actinobacteria, Bacteroidetes, and Alpha- and Deltaproteobacteria was higher at oak and pine sampling sites than at the birch sampling site.

Several bacterial taxa were indicative for particular sampling sites, and the local abiotic factors significantly determined their distribution patterns. As the bacterial community was not subjected to a direct tree-species effect, certain bacterial taxa can be attributed to tolerant or sensitive to particular metals microorganisms.

The given study did not demonstrate the overall effect of soil pH on the microbial community; however, particular taxa correlated with this parameter. These results contradict most published works that showed that pH value significantly affected microbial community, especially bacterial population (Chodak and Niklińska, 2010; Knelman et al., 2012; Kaiser et al., 2016; Gagnon et al., 2020a). Soil acidity might indirectly affect the microbial community through the solubility of different elements. It has been shown that in acidic soils, mobilization and, consequently, the bioavailability of Al, Fe, Mn, Cu and Zn increased (Lammel et al., 2018). Moreover, in soils with low pH value, phosphorus in the forms of phosphate tends to precipitate with Fe, Al and Mn and, therefore, become unavailable (Lammel et al., 2018). On the one hand, highly diverse in chemical conditions microhabitats at the field due to high soil heterogeneity might reduce the overall effect of pH on the microbial community. On the other hand, additional correlation analysis based on soil chemical parameters demonstrated that pH value significantly affected the content of Al, Cu, Ni, Pb, Sr, Zn, Cs and U (data are not shown). Several previously published works demonstrated a direct correlation between soil acidity and mobilization and the increased bioavailability of Al under these conditions (Piña and Cervantes, 1996; Niu et al., 2020). Therefore, the results of the given study indicate that soil acidity might be related to the mobility of several toxic metals and, therefore, might indirectly affect microbial community structure.

4.4.4. Several taxa have the potential to be beneficial in the mycorrhizosphere during colonization of unvegetated substrate

Plants need a microbial symbiont for the successful colonizing of a newly established substrate. At the early stage of succession, plants might enrich their mycorrhizospheres with particular bacterial and

fungus taxa, which might be beneficial under harsh environmental conditions. Therefore, microorganisms that were more abundant in pot plants mycorrhizospheres than control pot substrate might be potentially beneficial. Moreover, maintenance of original field taxa in pot plants mycorrhizospheres at a relatively high level compared to control pot substrate might also indicate the importance of these taxa for plant performance.

All pot plants mycorrhizospheres contained a higher amount of Acidobacteriia, Bacteroidia, Phycisphaerae, Oxyphotobacteria, Alphaproteobacteria, and Gammaproteobacteria compared to control pot substrate.

Acidobacteriia is one of the most ubiquitous bacterial classes and comprises species with highly diverse nutrient preferences and ecological roles (Barns et al., 2007; Kielak et al., 2016a; Oshkin et al., 2019; Kalam et al., 2020). Several previously published studies demonstrated associations of some Acidobacteriia with plants. In *in vitro* experiment presence of Acidobacteriia had a positive effect on the growth of *Arabidopsis thaliana* (Kielak et al., 2016b). Moreover, the ability of tested strains to produce IAA and siderophores was detected. Based on these results, it can be concluded that some members of class Acidobacteriia might possess properties of PGPB and can facilitate the growth of the plants under unfavourable conditions. Acidobacteriia is often found in environments with extreme conditions, including highly acidic substrates and soils with high levels of toxic metals (Barns et al., 2007; Zhang et al., 2007; Wakelin et al., 2012). Eichorst et al. (2011) showed that the proportion of Acidobacteriia was the highest in soils containing less carbon. Correlation analysis demonstrated that Acidobacteriia was significantly negatively affected by the C/N ratio and non-significantly by the content of total carbon and nitrogen. Although it seems that the content of several toxic metals mainly explained the distribution of Acidobacteriia, the relative abundance of this taxon was the highest in soils containing a low amount of carbon (field birches and pines sampling sites as well as pot variants) which might reflect Acidobacteriia preferences to soils with oligotrophic conditions.

In pot plants mycorrhizospheres Acidobacteriia was mainly represented by genera *Granulicella* and *Bryobacter*. Interestingly, *Granulicella* was observed at all field sampling sites and pot variants; however, the highest relative abundance was defined only in soil under the field birches stand and in pot variants. Furthermore, correlation analysis demonstrated that *Granulicella* was positively associated with the content of Al (data are not shown), which corresponds with the study of Lian et al. (2019), who reported for the first time this genus as Al-tolerant. Therefore, this species might be considered as indicative for the mycorrhizosphere of the plants growing in soils with a high content of Al.

Bacteroidia was one of the most contributing to the bacterial diversity class. Pot plants mycorrhizospheres were enriched with *Mucilaginibacter* compared to its low relative abundance in control pot substrate, suggesting that pot plants selected this genus.

Mucilaginibacter has been found in different habitats: soils (Urai et al., 2008; Jing et al., 2016; Liu et al., 2017; Huq et al., 2019), freshwater (Joung et al., 2015), wetlands and peat bogs (Pankratov et al., 2007; Baik et al., 2010). Moreover, this genus is a typical inhabitant of the rhizosphere (Kim et al., 2010; Madhaiyan et al., 2010; Wei et al., 2017). *Mucilaginibacter pedocola* TBZ30T isolated from a heavy metal contaminated paddy field demonstrated tolerance to multiple heavy metals and could adsorb Zn^{2+} and Cd^{2+} during cultivation and produced a great amount of exopolysaccharides (Tang et al., 2016; Fan et al., 2018). Similarly, *Mucilaginibacter kameinonensis* and *Mucilaginibacter rubeus* isolated from a gold/copper mine exhibited high resistance to a wide range of heavy metals (Li et al., 2018). Correlation analysis did not demonstrate strong associations of *Mucilaginibacter* with any toxic metals chosen for the given research; therefore, it can be assumed that the distribution of this genus in different variants of the experiment might be determined mainly by a presence of the plant, and, thus, could be considered as potentially PGPB.

It is believed that N₂-fixation by free-living microorganisms is the most crucial process launching natural succession, both for microbial communities and vegetation (Ogle and Redente, 1988). Similarly, Harantová et al. (2017) suggested that poor-nutrient conditions typical for unvegetated early stage of succession at post-mining areas would facilitate autotrophic succession and promote the abundance and activity of autotrophic and N₂-fixing bacteria. In terms of asymbiotic N₂-fixation, the distribution of class Oxyphotobacteria might be of interest. Oxyphotobacteria belongs to the phylum Cyanobacteria and includes cyanobacteria capable of photosynthesis (Monchamp et al., 2019). Many cyanobacteria fix nitrogen under anoxic or micro-oxic conditions as well as aerobically (Flores et al., 2015). Schmidt et al. (2008) found a diverse community of cyanobacteria which markedly contributed to the successional development of recently deglaciated unvegetated soils via photosynthesis and N₂-fixation. The relative abundance of Oxyphotobacteria was higher in pot variants. During the maintenance of the glasshouse experiment, the formation of greenish algae-like patches on the surface of the pot substrate was noticed (Figure S21). Interestingly, Schütte et al. (2009) mentioned that recently deglaciated sites had a high proportion of coarse gravel with patchy cyanobacterial crusts and mosses. Probably, algae-like formations observed in the pot experiment comprised cyanobacteria as well. Although the overall percentage of Oxyphotobacteria in the bacterial community was very low, it can be assumed that these bacteria might play an important role at the initial stages of succession and contribute to the pool of soil nitrogen and carbon.

The relative abundance of Phycisphaerae in pot plants mycorrhizospheres was higher than in field plants mycorrhizospheres and in control pot substrate. This observation suggests that plants had a selective pressure on the representativeness of these bacteria in pot plants mycorrhizospheres. Phycisphaerae belongs to the phylum Planctomycetes and includes three genera altogether: *Algisphaera* and *Phycisphaera*, isolated from a marine alga (Fukunaga et al., 2009; Yoon et al., 2014), and *Tepidisphaera*, found in terrestrial hot springs (Kovaleva et al., 2015). Phycisphaerae strongly correlated with the content of Al, Cu and Fe. It is known that members of phylum Planctomycetes dominate surface biofilms, habitats created by micro- and macroalgae, rocky particles, microbial mats, where they often co-occur with phototrophs. In Wiegand et al. (2018) “algal scavenging” lifestyle of Planctomycetes was hypothesized. According to this work, Planctomycetes can be plentiful in habitats of accumulated carbon sources in an oligotrophic water body. Despite their slow growth in carbon enriched habitats, they can produce antimicrobial secondary metabolites to outcompete faster-growing heterotrophic bacteria or algicides to scavenge algae. Authors summarized that Planctomycetes might play a key role as degraders of complex carbon compounds in different types of habitats.

The distribution of Phycisphaerae had a pattern similar to that described for Oxyphotobacteria, suggesting that “algal scavenging” lifestyle concept could be applied to Phycisphaerae observed in the given work. Moreover, for the first time, this study showed that Phycisphaerae might be specifically selected by plants growing under unfavourable conditions. Altogether, it can be hypothesized that Phycisphaerae might play an important role for plants at a primary succession.

One of the core bacterial taxa in pot variants was represented by a cluster *Burkholderia-Caballeronia-Paraburkholderia* (Gammaproteobacteria). Its relative abundance was higher in pot plants mycorrhizospheres than corresponding field plants mycorrhizospheres and control pot substrate, indicating that plants in pots selected these particular bacteria in mycorrhizospheres. *Burkholderia* group comprises both PGPB and plant pathogens (Coenye and Vandamme, 2003; Mannaa et al., 2018). *Burkholderia* is often associated with N₂-fixation and, therefore, might play a crucial role for plants at primary succession (Ciccazzo et al., 2016; Puri et al., 2020). *Burkholderia* is a constant inhabitant of plants rhizospheres (Bevivino et al., 1998; Richardson et al., 2002; Compant et al., 2008; Zeng et al., 2018). Uroz et al. (2016) observed enrichment of Norway spruce and beech rhizospheres with *Burkholderia*, highlighting the importance of this genus in solubilizing minerals and, thus, facilitating plant health and nutrition. Moreover, *Burkholderia* produces phytohormones and siderophores

(Caballero-Mellado et al., 2007; Suárez-Moreno et al., 2012; Mannaa et al., 2018). Several plant-associated *Paraburkholderia* were able to solubilize phosphorus (Hsu et al., 2018; Mannaa et al., 2018). Enrichment of pot plants mycorrhizospheres with bacteria from *Burkholderia-Caballeronia-Paraburkholderia* cluster might help plants tolerate unfavourable conditions in pots and promote their growth.

Alphaproteobacteria was one of the most abundant bacterial taxa in pot plants mycorrhizospheres. Within this class, *Sphingomonas* was a core bacterial genus in pot plants mycorrhizospheres. *Sphingomonas* is considered as a ubiquitous genus and has been previously isolated from a wide range of environments, including soils contaminated with organic pollutants and heavy metals (Vanbroekhoven et al., 2004; Xie et al., 2010; Kim et al., 2014b; Sheu et al., 2015; Zhou et al., 2016). *Sphingomonas* uses different mechanisms to cope with heavy metal-induced stress: biosorption, exclusion, intra- or extracellular precipitation and/or bioaccumulation by microbial chelators or bacterial siderophores, active efflux transport, and enzymatic detoxification (Asaf et al., 2020). *Sphingomonas* is attributed to PGPB due to its ability to produce phytohormones (Khan et al., 2014; Yang et al., 2014), fix nitrogen (Yang et al., 2014; Lowman et al., 2016), release organic acids (Khan et al., 2017). Luo et al. (2019) reported that *Sphingomonas* sp. Cra20 stimulated the development of root structure in *A. thaliana* under drought. Therefore, the presence of *Sphingomonas* might be beneficial for the plants in terms of their growth under unfavourable abiotic conditions.

Actinobacteria were equally represented at the field and in pots; however, the abundance of genera within this class varied among experiment variants. Thus, the percentage of genus *Actinospica* was higher in all pot plants variants compared to pot substrate. It was previously reported that rhizospheres of different plants might be enriched with this bacterial genus (Colin et al., 2017; Zeng et al., 2018; Valadares et al., 2020). *Actinospica* was shown to be an obligate acidophile (Golinska et al., 2015). Correlation analysis demonstrated a strong positive correlation of *Actinospica* with the content of Al and Co, suggesting that these bacteria might be able to tolerate increased concentrations of toxic metals. Thus, the putative beneficial role of *Actinospica* for plants under unfavourable conditions might be proposed.

Ktedonobacteria was one of the most abundant classes, particularly highly represented in pot variants. It is necessary to mention that the relative abundance of Ktedonobacteria might be overrepresented because of the high number of 16S rRNA genes copies across the genome (Chang et al., 2011). Ktedonobacteria comprises bacteria with an actinomycetes-like morphology and belongs to the phylum Chloroflexi. Ktedonobacteria is widely distributed in nature, including extreme environments, and is predominant under oligotrophic conditions (Yabe et al., 2017; Zheng et al., 2019; Rachmania et al., 2020). It has been assumed that Ktedonobacteria might tolerate high concentrations of heavy metals. For example, Epelde et al. (2015) compared several sites at an abandoned Pb-Zn mine with different metal contamination levels. They described the higher relative abundance of Ktedonobacteria in more polluted soils, suggesting that these bacteria might tolerate the increased content of metal contaminants. Correlation analysis revealed strong positive associations between Ktedonobacteria and the content of Al, Cu and U, and weak negative correlations with the content of total carbon, total nitrogen, and C/N ratio. Fan et al. (2019) reported that Ktedonobacteria were among bacteria negatively affected by the content of organic matter, suggesting their low competitiveness under nutrient-rich conditions. Although the role of Ktedonobacteria in the soil is unknown, a large genome might imply a vast potential to produce various secondary metabolites, and the high relative abundance of these bacteria in nutrient-poor soil might suggest their importance in performed functions.

Differences in fungal community structure between pot plants mycorrhizospheres and unvegetated control pot substrate were expectedly determined mainly by the dynamics of ECM fungi. Moreover, the control pot substrate represented a pool of unidentified Ascomycota and *Pezoloma*.

Unidentified at a genus level Thelephoraceae contributed the most to the fungal diversity. Its relative abundance was particularly high in pot plants mycorrhizospheres as well as in the mycorrhizosphere of field birches. Correlation analysis revealed that only pH value had a strong positive effect on the presence of Thelephoraceae. The majority of Thelephoraceae members belongs to pioneer fungi and contributes to an ECM-resistant propagule bank in disturbed soils (Kałucka and Jagodziński, 2017). A very low amount of Thelephoraceae characterized the original pot substrate. This observation suggests that Thelephoraceae members in pot plants mycorrhizospheres might originate from pre-existing plant roots fungal community. Moreover, the increase in relative abundance and maintenance of Thelephoraceae at a high quantitative level in pot variants might reflect high tolerance of these fungi to disturbance effect as well as high competition with saprotrophs. Altogether, Thelephoraceae might be considered as beneficial for plants at primary succession, where local disturbance occurs.

Pezoloma belongs to the Leotiaceae family and is known to form ericoid mycorrhiza (ERM) with plants from the Ericaceae family. The establishment of ericoid mycorrhiza is considered as essential for the survival of ericaceous plants as they colonize acidic nutrient-poor soils (Midgley et al., 2017). ERM fungi do not produce extensive mycelial and, thus, they cannot explore surrounding substrate for nutrients. Ericaceous plants benefit from ERM symbiosis through the ability of ERM fungi to produce a wide range of extracellular phenol-oxidizing enzymes degrading organic sources of nitrogen and phosphorus in soil (Cairney and Burke, 1998). It has been suggested that some ERM fungi might not only exhibit a symbiotic lifestyle but also live as saprotrophs in habitats rich in organic matter (Martino et al., 2018). Interestingly, the highest relative abundance of *Pezoloma* was observed in the control pot substrate where no plants were introduced. On the one hand, it can be suggested that *Pezoloma* might represent mainly dormant fungal pool, originated from fungal communities that preserved in the substrate after the mining activity. On the other hand, it was shown that the genome of *Pezoloma ericae* contained a high number of genes coding carbohydrate-active-enzymes (CAZ) involved in chitin degradation (Martino et al., 2018). Control pot substrate comprised a great amount of unidentified fungi, which might represent a large pool of fungal propagules, dormant spores, mycelium, which might use the trace organic matter as a nutrients source (plant residues, wood material), or establish a symbiotic partnership with algae in the form of lichens. This reservoir of fungi might serve as a source of chitin in the control pot substrate. Therefore, it can be assumed that *Pezoloma* in unvegetated soil might switch to a saprotrophic lifestyle and use fungal biomass as a source of nutrients.

Enrichment with *Pezoloma* was also observed in pot plants mycorrhizospheres in comparison with field plants. Most of the original roots of pot plants at the end of the glasshouse experiment looked severely damaged or non-viable and, thus, could represent a source of organic matter for saprotrophs. Therefore, it can be assumed that the high percentage of *Pezoloma* in pot plants mycorrhizospheres might indicate that members of this genus could reveal a saprotrophic lifestyle.

4.4.5. The most representative taxa have the potential to be beneficial for plants in terms of tolerance to field unfavourable environmental conditions

The vast majority of bacterial taxa were represented at all sampling variants and can be attributed to “generalists”. This group includes microorganisms adapted to a wide range of environmental conditions. Several taxa were found exclusively at a particular sampling site and/or habitat, suggesting their adaptations to certain soil conditions, and can be considered as so-called “specialists”. Characteristics of several generalists and specialists and their dynamics in different variants of the experiment are discussed further.

Alphaproteobacteria was the most abundant and one of the most diverse bacterial classes in all experiment variants. The relative abundance of this taxon was higher (non-significantly) in field plants mycorrhizospheres. Correlation analysis displayed a positive effect of the C/N ratio on the relative abundance of these bacteria. It has been previously reported that Alphaproteobacteria are generally found in nutrient-rich habitats, including rhizosphere (Goldfarb et al., 2011; Fierer et al., 2012; Mendes et al., 2013; Kim et al., 2014a; Fernández-Gómez et al., 2019). Without a doubt, Alphaproteobacteria can be considered a core taxon in Kanigsberg's soil microbial community. Despite the drastic differences in soil quality, its high relative abundance and presence at all sampling sites might characterize Alphaproteobacteria as a species with the high potential to tolerate and adapt to harsh environmental conditions.

Among Alphaproteobacteria, two N₂-fixing genera *Mesorhizobium* and *Bradyrhizobium* as well as a cluster *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* were exclusively found at field oaks and pines sampling sites and in several pot oaks and pines samples replicates with a very low relative abundance. The distribution of these genera positively correlated with the content of total carbon and total nitrogen and was strongly affected by the content of Al, which seems to be toxic for these bacteria (data are not shown). It has been reported that low soil phosphorus availability limited N₂-fixation (Uliassi and Ruess, 2002). In the given study, only the content of total phosphorus was measured; therefore, it is difficult to make a conclusion about the availability of phosphorus to plants at different sites. Moreover, it can be assumed that aluminium at the birch sampling site and in the control pot substrate might immobilize phosphorus. Gessa et al. (2005) showed that the presence of Al in a soil-root interface model hampered the phosphate flux at pH 4.00 and 4.50, suggesting that Al can inhibit the phosphate uptake by plants. This observation might explain the low abundance of N₂-fixing Alphaproteobacteria in pots and at the field birch sampling site. The presence of bacteria that form a symbiosis with legumes in oaks samples might be related to the presence of grass vegetation at the field oak sampling site. Besides, the association of *Bradyrhizobium* species with non-legumes and their ability to act as PGPB has been demonstrated before (Lian et al., 2002; Wagner et al., 2019).

Gammaproteobacteria

Field oak and pine sampling sites were characterized by the high diversity of Gammaproteobacteria. The most representative genus was *Acidibacter*, with the highest relative abundance in field birches and oaks variants and the lowest in field pines variants. Correlation analysis did not demonstrate any associations between *Acidibacter* and soil parameters, suggesting that the distribution of this taxon was determined mainly by plant presence. This genus has been previously found in rhizobioms of different plants (Zeng et al., 2018; Zi et al., 2020) as well as at a post-mining area (Ezeokoli et al., 2020) and heavy metal contaminated soil (Böhmer et al., 2020). Falagán and Johnson (2014) described a novel acidophilic genus *Acidibacter ferrireducens* isolated from a pit lake at an abandoned metal mine, which was able to tolerate high concentrations of either iron or aluminium in the medium. Although little is known about the functions of *Acidibacter* in soil, its high tolerance to several toxic metals might be beneficial for plants growing at metal contaminated areas.

Bacteroidia

Bacteroidia was one of the most diverse and abundant classes at the field. Field birch and pine sampling sites were mainly represented by *Mucilaginibacter*, while *Terrimonas* prevailed at the oak sampling site. *Terrimonas* has been isolated from rhizosphere soil (Han et al., 2017; Kim et al., 2017). Correlation analysis demonstrated that Al, Fe and Cu had a strong negative effect on the relative abundance of *Terrimonas*, and its distribution mainly correlated with the content of total carbon, total nitrogen and C/N ratio. Based on these results, it can be assumed that *Terrimonas* found at Kanigsberg might be sensitive to particular metal pollutants and prefer habitats enriched with organic matter.

Several taxa were indicative for particular sampling sites. Blastocatellia and Holophagae were strongly inhibited at the birch sampling site and in all pot variants, including control pot substrate. Correlation analysis revealed strong negative associations with the content of Al, Cu and Fe, and strong positive associations with the content of Mn and Ni. At the same time, Blastocatellia positively correlated with the content of total carbon and total nitrogen as well as the C/N ratio. This class was particularly enriched in the bulk soil of field oaks. In contrast to the observed results, members of the class Blastocatellia have been reported to prefer oligotrophic conditions and were tolerant to drought (Wüst et al., 2016; Huber et al., 2017). Moreover, Ivanova et al. (2020) reported that Blastocatellia in tundra soils negatively correlated with carbon and nitrogen availability. Unfortunately, Blastocatellia was represented solely by uncultured bacteria, or bacteria, not identified at the genus level. With that, it can be assumed that pool of unidentified Blastocatellia could contain bacteria with diverse lifestyles, which could explain a discrepancy between the results in this study and cited above works. Although Holophagae was not among the most abundant taxa, it was indicative for the field oak and pine sampling sites. This class comprises four described representatives, three of which are strict anaerobes (Ivanova et al., 2020). Even though sampling for sequencing was performed from the upper layers, where aerobic conditions should theoretically prevail, it cannot be excluded that some sequences were retrieved from microhabitats with anaerobic conditions. Blastocatellia and Holophagae identified at Kanigsberg might belong to bacteria with “yet-unknown” functions different from already reported growth patterns (Ivanova et al., 2020).

Anaerolineae demonstrated similar to Blastocatellia and Holophagae patterns of representativeness in soil, except for the birch sampling site, where Anaerolineae was represented at a low level, and Blastocatellia and Holophagae were not found at all. Although Anaerolineae is rather a ubiquitous taxon found in different ecosystems, its environmental functions remain unclear (Blazejak and Schippers, 2010). Interestingly, it has been reported that Anaerolineae, found in marine sediments, incorporated acetate and glucose under anaerobic sulfate-reducing conditions (Blazejak and Schippers, 2010) and was believed to belong to the core microbial populations in anaerobic digesters (Xia et al., 2016). Anaerolineae, found in paddy fields, positively correlated with total carbon content (Lopes et al., 2015). Moreover, it was shown that this taxon was involved in the degradation of N-containing compounds. Correlation analysis in this study did not reveal the association of Anaerolineae with the content of total carbon, total nitrogen or C/N ratio, and only toxic metals had a strong effect. Thus, it can be assumed that Anaerolineae found at Kanigsberg might represent bacteria with environmental preferences not yet described.

Verrucomicrobiae

Members of the phylum Verrucomicrobia contribute approximately to 1–10% of the total bacterial 16S rRNA in soil (Navarrete et al., 2015). Verrucomicrobiae is widely distributed in the environment and has been previously found in aquatic and terrestrial systems (Buckley and Schmidt, 2001); however, still little is known about ecological functions performed by these bacteria. Transfer of the plants in pots had a strong negative effect on Verrucomicrobiae, suggesting their low tolerance to disturbance. Buckley and Schmidt (2001) received rather inconclusive results on the influence of soil management history on the verrucomicrobial rRNA relative abundance, showing that the amount of verrucomicrobial rRNA was lower in the cultivated field than in fields that had never been cultivated. Interestingly, in the same work, the weak positive correlation between soil moisture and the abundance of Verrucomicrobiae was revealed, suggesting that this parameter can be an important predictor of verrucomicrobial rRNA relative abundance in soil. Although differences in the content of water between field sampling sites and pot variants were not measured, it can be stated that pot substrate was more saturated than soil at the test field. Therefore, in contrast to the cited above study, it can be assumed that the increase in soil moisture in pots could eliminate Verrucomicrobiae under these conditions. Navarrete et al. (2015) cited many studies, which showed that Verrucomicrobia phylum is likely to be particularly sensitive to

changes in the environment. Verrucomicrobiae represents an oligotrophic lifestyle and might be dependent on sources of available carbon (Bergmann et al., 2011; Navarrete et al., 2015). It can be speculated that after transfer in pots, there was a temporal increase in the amount of labile carbon due to the rise of root exudates released by plants as a response to disturbance, which, in turn, could lead to the elimination of slow-growing sensitive taxa from pot substrate. Moreover, correlation analysis demonstrated that the content of Al, Cu and U had a strong negative effect and the C/N ratio had a strong positive effect on the relative abundance of Verrucomicrobiae. These observations could characterize Verrucomicrobiae as rather sensitive to disturbance and support results, reported previously.

Fungal community structure was mainly determined by ECM fungi, suggesting their exceptional importance at early stages of succession at the post-mining area. The most contributing fungi were unidentified at a genus level Thelephoraceae, *Russula*, *Inocybe* and *Lactarius*. *Lactarius* sp. and Thelephoraceae were discussed before, and the characteristics of these taxa here are omitted.

The distribution of *Russula* in different experiment variants demonstrated its preference for a more advanced successional habitat at the field oak sampling site. Similar results were obtained by Kolaříková et al. (2017), who observed sequential changes in the ECM community: from the dominance of Pezizales at early stages of succession on a mine spoil to the prevalence of *Russula* at later successional sites. Russulales were rare at early stages of succession at the post-mining area, but their relative abundance increased at later stages (Harantová et al., 2017). Correlation analysis revealed strong associations between Russulaceae and the content of total carbon and total nitrogen, which confirms that the dynamics of *Russula* was determined by vegetation succession. As a representative of late-successional fungi, *Russula* occupies mainly undisturbed habitats, has high carbohydrate requirements and obtains nutrients predominantly from the organic pool (Kaľucka and Jagodziński, 2017). Kyaschenko et al. (2017) showed that *Cortinarius* and *Russula*, dominated in older stands of *P. sylvestris*, correlated with enzymes involved in the mobilization of nitrogen and/or phosphorus from organic matter, suggesting the importance of these fungi in the decomposition of complex organic matter. There was a considerable decrease in the relative abundance of *Russula* in all pot variants, suggesting that the transfer of the plants in pots had a substantial disturbance effect on this fungus.

Inocybe successfully colonizes nonmycorrhizal habitats and is usually attributed to early-successional fungi (Ishida et al., 2008). *Inocybe* was one of the dominant genera at field birch and pines sampling sites. Interestingly, *Inocybe* sequences were not defined for field oaks variants; however, this genus was observed for pot oaks. Although the pool of *Inocybe* in control pot substrate was very low, it seems that transfer of oaks to pots stimulated colonization of oak roots with *Inocybe*. It has been reported that the spores of early-successional fungi, including *Inocybe*, were able to easily germinate, especially in the presence of host roots (Ishida et al., 2008; Kaľucka and Jagodziński, 2017). Although *Inocybe* is a rather ubiquitous species, little is known about its ability to tolerate heavy metals (Esteve-Raventós et al., 2018). Bell et al. (2015) reported that willows, the rhizosphere of which was dominated by *Inocybe*, displayed efficient bioaccumulation of total Zn in shoots. This result might suggest the ability of *Inocybe* to increase mobilization and bioavailability of metals.

The distribution of saprotrophic *Exophiala* (Herpotrichiellaceae) at the field is of interest. *Exophiala* was mainly represented at the field oak sampling site and, to less extent, at the field pine sampling site. One of the most studied representatives of *Exophiala* genus is a dark septate endophyte *Exophiala pisciphila*, which is widely distributed in different environments, including usual soil (Wang et al., 2011) and heavy metal contaminated areas (Zhan et al., 2015a). A series of studies showed that *E. pisciphila* tolerated the presence of Cd via the considerable increase in antioxidant defence and accumulated metal in cell walls in the forms of Cd-phosphate complexes (Zhan et al., 2015a; 2015b). Moreover, the

ability of *E. pisciphila* to decrease the toxicity of heavy metals for maize by limiting the translocation of metal ions from roots to shoots was demonstrated (Li et al., 2011). These results indicate the ability of this species to tolerate metal-induced stress. Species *Exophiala radialis* has been reported to be widely distributed in the northern hemisphere and is frequently associated with the roots of living plants (Macia-Vicente et al., 2016). *Exophiala* had no strong associations with any soil parameters. This observation might indicate *Exophiala*'s preferences for habitats associated with diverse vegetation cover and probably dependence on the labile carbon source.

4.5. Different levels of succession determine distinct patterns of microbial interactions

Most of the works on the structure of microbial communities focus mainly on the relative abundance of particular taxa as well as the description of α - and β -diversity. Understanding interactions between taxa co-existing in different habitats might give insight into the functional roles of these taxa. Field plants mycorrhizosphere network was characterized by considerably higher complexity than pot plants mycorrhizosphere network, suggesting that field plants shaped more stable and less dynamic mycorrhizospheres.

Bacteria played an important role in the establishment of the network in field plants mycorrhizospheres. The most contributing to co-occurrence patterns bacteria were represented by a wide range of typical soil members: Alpha- and Gammaproteobacteria, Actinobacteria, Bacteroidia which are commonly considered as copiotrophs and might be particularly abundant in mycorrhizospheres, and Acidimicrobiia, Verrucomicrobiae, Oxyphotobacteria, Phycisphaerae and Planctomycetacia comprising the group of soil oligotrophs. Ho et al. (2017) discussed that soil physicochemical status, mainly carbon and nitrogen availability, might determine a life strategy in the environment. Thus, the inclusion of bacteria with diverse substrate affinity into the field mycorrhizosphere network might reflect the co-existence of highly diverse in conditions microhabitats. More importantly, most bacteria with a strong influence and control over the network were previously reported as PGPB. For example, several strains of N_2 -fixing symbiotic with legumes *Mesorhizobium* were capable of producing IAA (Menéndez et al., 2020) and promoted the growth of non-legumes. *Flavobacterium*, which is often found in the rhizosphere community, possesses a wide range of PGPB features, namely, the ability to promote plant defence against pathogens (Sang and Kim, 2012; Kolton et al., 2014), solubilize phosphorus (Sharma et al., 2013; Lidbury et al., 2019) and produce IAA (Sang and Kim, 2012). The ACC deaminase-containing rhizobacterium *Variovorax paradoxus* 5C-2 was reported to promote plant growth under soil water deficit by decreasing ethylene production in plants which is known to inhibit the growth of the plants in dry soils (Dodd et al., 2009; Jiang et al., 2012).

Interestingly, fungi had a minor impact on the field mycorrhizosphere network. At the same time, ECM *Inocybe* and *Lactarius* (a birch specialist) as well as *Penicillium* and *Metapochonia* were characterized by the highest clustering coefficient values (1.00). An important role of ECM fungi *Inocybe* and *Lactarius* in the local communication within the network might indicate their ability to form a niche for interactions with bacteria and other fungi and, therefore, can be an example of ectomycorrhizosphere communication.

Transfer of plants to pots had a rather negative impact on the co-existence of microorganisms. In the pot plants mycorrhizosphere network the diversity of influential bacteria reduced to Gammaproteobacteria, Actinobacteria, Acidobacteriia, Bacteroidia and Thermoleophilia, which could probably be related to the homogenization of pot substrate and, therefore, a decrease in variability of microhabitat conditions. Several bacteria with PGPB features were key organisms in pot plants mycorrhizospheres, for example, two bacterial clusters *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Burkholderia-Caballeronia-Paraburkholderia*. The most contributing to the network fungi were represented mainly by soil saprotrophs (Herpotrichiellaceae, Dermateaceae, Mortierellaceae, Myxotrichaceae) and ectomycorrhizal Pisolithaceae and Inocybaceae. In their review, Ballhausen and

de Boer (2016) summarized the results of several works which demonstrated that saprotrophic fungi in the rhizosphere were able to incorporate recently fixed plant ^{13}C faster than mycorrhizal fungi, suggesting that fungal saprotrophs could be major consumers of root exudates. By analogy with the term “mycorrhizosphere”, the authors further proposed the term “sapro-rhizosphere” to define the zone affected by saprotrophic fungi growing on root exudates. On the other hand, the incorporation of saprotrophs in the pot plants mycorrhizosphere network might indicate their involvement in the decomposition of non-viable and damaged plant roots. The number of genes involved in the decomposition of organic matter is lower in ECM fungi compared to free-living fungi (Martino et al., 2018). Therefore, a considerable shift in the fungal community due to the elimination of the most abundant ECM species in pot plants mycorrhizospheres, an increase of organic matter pool due to extensive damage of original plant roots as well as considerable development of new roots in pots might create niches for saprotrophic fungi and make them more important components of the network. Moreover, it has been shown that several strains of a saprotrophic genus *Mortierella* belonged to plant growth-promoting fungi and were able to facilitate the acquisition of P and Fe, produce ACC deaminase and protect plants from pathogens (Ozimek and Hanaka, 2021).

Co-occurrence of generalists and specialists in the field network reflected contrasting abiotic conditions between sampling sites. In the pot network a higher number of specialists was attributed to pot oaks. At the same time, pot oaks specialists belonged mainly to generalists in the field mycorrhizosphere network. This observation suggests the capability of oaks to preserve the members of the original field mycorrhizosphere microbial community. Moreover, several pot oak specialists possess PGP features (*Streptomyces* and bacteria from a cluster *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*). *Singulisphaera* was previously found among the most abundant bacteria in mycosphere of *Russula griseocarnosa* and was proposed as mycorrhiza helper bacteria (Yu et al., 2020). On the other hand, *Candidatus Xiphinematobacter* is known as an endosymbiont of plant-pathogenic nematodes *Xiphinema* group, which are ectoparasites of roots in a wide range of plants (Mobasserri et al., 2019). The presence of the nematode bacterial endosymbionts in pot oaks mycorrhizospheres and their great impact on the local communication within the network (clustering coefficient 0.83) might indicate that pot oaks were subjected to infection with nematodes, which might be one of the explanations of their low survival in the pot experiment.

Networks of pot birches and pines formed two non-connected components consisting of the host plant network and the unvegetated substrate's microbial community network. Interestingly, the networks of both plants tended to get compartmentalized and become more complex compared to corresponding field plants networks. This observation, together with a high plants survival rate, might not only indicate great adaptability of birches and pines to considerable changes in abiotic conditions but also suggest that these plants derived benefit from newly established networks. In contrast, the pot oak network became less connected compared to the field oak and, therefore, less stable. Thus, considerable changes in abiotic conditions in pot variants compared to the natural field environment might be a reason that caused disruption of the oak network.

4.6. Outlook

Kanigsberg represents a unique environment. Techniques applied for remediation here led to the establishment of areas with contrasting abiotic conditions and, as a result, the development of sites with different stages of plant succession. Differences between sampling sites were determined mainly by the content of several toxic metals as well as the content of total carbon (mainly determined by organic carbon fraction) and total nitrogen. The vegetation cover and its composition considerably contribute to soil development, as root exudates and plant litter are the primary sources of organic matter at the early stages of succession. Tree presence did not affect microbial community structure at the field, as no significant differences between mycorrhizosphere and bulk soil were observed. This observation rejects

the (1) *hypothesis*. Several reasons could explain the given result. First, young trees might not expose a selective force and, thus, microbial communities in their mycorrhizospheres could be mainly determined by abiotic conditions. Second, the absence of differences between mycorrhizosphere and bulk soil could arise from imperfect soil sampling where bulk soil samples could be collected from the zone of the plant influence. This assumption can be confirmed by the fact that ECM fungi, abundant at the field plants, may grow from the rhizosphere into bulk soil and, thus, expand the zone of plant influence and minimize the differences between mycorrhizosphere and bulk soil.

Field birches, oaks and pines grew at sites with different levels of soil development and shaped dissimilar in structure mycorrhizospheres. Although this observation confirmed the (2) *hypothesis*, the differences at a site level, determined by abiotic and biotic conditions, might affect the structure of microbial communities associated with different tree species. Therefore, it was impossible to distinguish unambiguously between the impact of plant or soil parameters on microbial populations in mycorrhizospheres.

Bringing trees at the same successional stage and maintaining them in soil substrate with the equal quality might resolve the problem of variation in natural conditions. A two-years-glasshouse experiment demonstrated that plants exhibited rhizosphere effect and were associated with microbial communities, different in structure from control pot substrate, confirming the (1) *hypothesis*. This result contradicted the observation made for the field plants. Differences between pot plants mycorrhizospheres and unvegetated soil might be explained by changes in plant physiology as a response to the disturbance effect. Although the chemical composition of root exudates was not studied in this work, the performance of the pot plants indirectly indicated that plants underwent stress reactions of different strengths. Resulted from disturbance the significant shift in the structure of the ECM community, confirmed by the sequence analysis and morphotyping, contributed to the differences between pot plants mycorrhizospheres and control pot substrate. Plant identity did not determine differences in the structure of microbial communities, and pot birches, oaks and pines shaped similar mycorrhizospheres. Although this result contradicts the observation made for field plants, it confirms the assumption that the level of site successional development affects the structure of the microbial community in mycorrhizospheres. Comparison of microbial communities along the chronosequence *control pot substrate* → *pot plant MR* → *field plant MR* confirmed that abiotic conditions mainly influenced bacterial community structure. In contrast, fungal community structure was related to the successional stage, driven predominantly by vegetation, supporting the (3) *hypothesis*.

The network analysis results demonstrated that to be an important component of microbial community does not necessarily mean to be a core taxon or to be among the most abundant species. Most of the taxa with large control and importance over the network possessed features promoting plant growth or demonstrated high tolerance and adaptability to unfavourable environmental conditions. Moreover, several ECM fungi at the field revealed a potential to be a core of ectomycorrhizosphere communication. Pot experiment demonstrated that plants had different capabilities to establish or expand networks in the new environment. Based on the plant performance in the pot experiment, it can be assumed that plants and microbial interactions had a reciprocal influence on each other. In terms of succession and ecosystem development, it can be concluded that as the ecosystem develops, communication between microorganisms becomes more complex. Number of generalists at the field decreased, compared to pot variants, probably, due to the increase in natural field soil complexity, which resulted in the establishment of new habitats with diverse abiotic conditions. In this highly complex environment, bacteria were more important in microbial communication within the rhizosphere.

The presence of sites at different stages of development found reflection in the distribution of ECM taxa with particular functional traits within Kanigsberg. A complex of factors including the age of trees, level of soil maturity (presence/absence of litter, the content of carbon and total nitrogen) and, apparently, water content determined the presence of certain taxa as well as their exploration strategies. Moreover, plants could select ECM with a particular tolerance level to toxic metals and water saturation-drying fluctuations. Additional experiments with non-contaminated soil as a reference substrate might give an

insight into ecological filtering processes at Kanigsberg and should be considered for future experiments.

Birches adapted to pot substrate qualities performed well in the pot experiment without inoculation. In contrast, birches and their indigenous ECM communities did not tolerate the addition of the ECM blend. In opposite to birches, oaks were not adapted to pot substrate, and considerable changes in soil chemistry resulted in high mortality of non-inoculated oaks. Besides, changes in soil parameters led to a shift in functional traits of the oak indigenous ECM community toward medium-distance exploration in pot substrate. Inoculation of oaks resulted in a relatively high survival rate compared to other trees. Oaks revealed the only example of successful inoculation from the ECM blend by *P. tinctorius*. Pines exhibited an intermediate position among the trees in this experiment. They were able to tolerate pot substrate qualities. Similar to oaks, the transfer of pines into the new substrate resulted in the significant development of rhizomorphs. In general, original ECM communities of oaks and pines demonstrated the capacity to respond to changes in soil chemical parameters. Glasshouse experiment provided results, rejecting the (4) *hypothesis*, and demonstrated that it is very important to “search” for the appropriate ECM inoculum composition suitable for particular tree species and local environmental conditions. There might be no “universal” blend, especially in terms of metal-polluted soils. Because of unsuccessful sequence analysis, it cannot be defined if a tree filtering process toward particular ECM species took place. Nevertheless, changes observed in both pot variants could be a good example of succession in ECM functional traits in disturbed ecosystems.

Results of this study emphasize the importance of site-specific management of post-mining areas. This should consider the site development stage, local contamination with particular heavy metals, type and diversity of vegetation. Reasonableness of fertilizer amendments as well as additional inoculation with mycorrhizal or bacterial blends should be tested in advance. The data on relative abundance, importance in microbial interactions, and functional strategies of bacteria and fungi are of great importance and must be implemented into phytoremediation practice. In the case of microbial-assisted phytoremediation isolation of site-adapted bacteria and fungi, screening for PGP features as well as their efficiency in heavy metal immobilization in soils or enhancement of metal uptake by plants is recommended.

5. References

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Declaration of honour (Ehrenwörtliche Erklärung)

I, Olga Bogdanova, hereby confirm that I am familiar with the valid doctoral examination regulations. I declare that I have composed and written this dissertation "Microbial processes in mycorrhizosphere of plants growing at a former uranium mining site" on my own, and that I neither did use any text passages from third parties nor my own previous final theses without citing those. In addition, I also confirm that I cited the tools, personal communication, and sources having been used as well as that I provided names of the persons who assisted in selecting and analysing materials, and supported me in writing the manuscript. I confirm that I did not receive any assistance from specialized consultants, and that any third party did not receive either direct or indirect financial benefits from me for work connected to this dissertation. I declare that I have not submitted this dissertation as my final thesis for a state examination or other scientific examination and that the same, a substantially similar, or another scientific paper to any other institution of higher education or to any other faculty as a doctoral thesis.

Hiermit bestätige ich, Olga Bogdanova, dass mir die geltende Promotionsordnung der Fakultät bekannt ist. Ich erkläre, dass ich diese Dissertation "Microbial processes in mycorrhizosphere of plants growing at a former uranium mining site" selbst angefertigt habe, keine Textabschnitte eines Dritten oder eigener Prüfungsarbeiten ohne Kennzeichnung übernommen und alle von mir benutzten Hilfsmittel, persönliche Mitteilungen und Quellen in der Arbeit einschließlich aller Personen, die mich bei der Auswahl und Auswertung der Materialien sowie bei der Herstellung des Manuskripts unterstützten, angegeben habe. Ich bestätige, dass die Hilfe einer kommerziellen Promotionsvermittlung nicht in Anspruch genommen wurde und dass Dritte weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen. Ich erkläre, dass ich diese Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe und dass die gleiche, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung bei einer anderen Hochschule oder anderen Fakultät als Dissertation eingereicht wurde.

Olga Bogdanova

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Curriculum vitae

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Education

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Lomonosov Moscow State University, Moscow, Russia
Soil Science Faculty, Soil Biology Department September 2005 – June 2010
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Professional experience

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Research Assistant

Diagnostic and Prevention Research Institute Moscow, Russia
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Scholarships

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Teaching experience

Friedrich Schiller University Jena, Germany
Practical course supervisor 2019

Practical course "Cell biology and communication in basidiomycetes"

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MSc. student project advisor 2018-2019
Master thesis "Characterization of potential hydrophobin8 overexpressing transformants from the ectomycorrhizal fungus *Tricholoma vaccinum*" (MSc. Anna Bonrath)

Friedrich Schiller University Jena, Germany
Practical course supervisor 2018

Practical course "Cell biology and communication in basidiomycetes"

Friedrich Schiller University Jena, Germany
BSc. student project advisor 2018

Bachelor thesis "Interaction between *Tricholoma vaccinum*, *Picea abies* and *Streptomyces* under the influence of nickel" (BSc. Markus Salbreiter)

Conferences

Talks

- Microbial community structure across the ectomycorrhizosphere and bulk soil of trees growing at a former uranium mining area
Bio-Geo-Colloquium (Oral presentation)
Jena, Germany, 2021 (online)
- Microbial interactions in ectomycorrhizosphere under the metal stress
18th Symposium on remediation (Oral presentation)
Jena, Germany, 2019

Posters

- “How do mycorrhizal fungi cope with metal stress?”
18th Congress of European Mycologists (Poster session)
Warsaw, Poland, 2019
- “Microbial interactions within ectomycorrhizosphere in heavy metal contaminated soils”
17th Symposium on remediation (Poster session)
Jena, Germany, 2018
- “Microbial interactions within ectomycorrhizosphere in heavy metal contaminated soils”
11th International Mycological Congress (Poster session)
San Juan, Puerto Rico, 2018
- “Microbial processes in soil development at a former uranium mining site”
16th Symposium on remediation (Poster session)
Jena, Germany, 2017
- “Microbial processes in soil development at a former uranium mining site”
12th Symposium of the VAAM special group Molecular Biology of Fungi (Poster session)
Jena, Germany, 2017

Additional workshops and courses

Scientific Image Processing and Analysis Friedrich Schiller University	Jena, Germany 2018
Scientific Writing and Publishing for Natural Scientists Friedrich Schiller University	Jena, Germany 2018
Good scientific practice for young researches Friedrich Schiller University	Jena, Germany 2017
Terrestrial Biosphere Max Planck Institute for Biogeochemistry	Jena, Germany 2017
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Practical course “Molecular cloning” for upgrading the qualification Institute of Gene Biology	Moscow, Russia 2010

Supplementary Material

Table S2. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between variants of the experiment in content of total soil nitrogen. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate; non_inoc – non-inoculated variant of the experiment, inoc – inoculated variant of the experiment. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

ANOVA						Kruskal-Wallis test				Post Hoc comparison						
Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variants of comparison		Mean Difference	SE	t	p tukey	p bonf
variant	6.000e -4	1	6.000e -4	4.500	0.101					B_BS	B_MR	-0.020	0.009	-2.121	0.101	0.101
Residuals	5.333e -4	4	1.333e -4													
variant	0.002	1	0.002	0.543	0.502					O_BS	O_MR	0.037	0.050	0.737	0.502	0.502
Residuals	0.015	4	0.004													
variant	1.500e -4	1	1.500e -4	0.346	0.588					P_BS	P_MR	-0.010	0.017	-0.588	0.588	0.588
Residuals	0.002	4	4.333e -4													
variant	0.002	1	0.002	12.100	0.025					B_POT	B_MR	0.037	0.011	3.479	0.025*	0.025
Residuals	6.667e -4	4	1.667e -4													
variant	0.064	1	0.064	42.777	0.007					O_POT	O_MR	0.232	0.035	6.540	0.007*	0.007
Residuals	0.005	3	0.002													
variant	8.167e -4	1	8.167e -4	6.125	0.069					P_POT	P_MR	0.023	0.009	2.475	0.069	0.069
Residuals	5.333e -4	4	1.333e -4													
variant						variant	5.804	2	0.055	B_MR	O_MR	-0.200	0.024	-8.165	< .001	< .001*
Residuals											P_MR	0.010	0.024	0.408	0.914	1.000
										O_MR	P_MR	0.210	0.024	8.573	< .001	< .001*
variant						variant	6.161	2	0.046	B_BS	O_BS	-0.143	0.036	-3.970	0.017	0.022*
Residuals											P_BS	0.020	0.036	0.554	0.848	1.000
										O_BS	P_BS	0.163	0.036	4.524	0.010	0.012*

Table S3. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between variants of the experiment in content of total soil phosphorus. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate; non_inoc – non-inoculated variant of the experiment, inoc – inoculated variant of the experiment. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

ANOVA						Kruskal-Wallis test				Post Hoc comparison						
Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variants of comparison		Mean Difference	SE	t	p tukey	p bonf
variant	6.468.167	1	6.468.167	0.187	0.688					B_BS	B_MR	-65.667	151.857	-0.432	0.688	0.688
Residuals	138.362.667	4	34.590.667													
variant	384.000	1	384.000	0.014	0.910					O_BS	O_MR	16.000	133.502	0.120	0.910	0.910
Residuals	106.937.333	4	26.734.333													
variant	770.667	1	770.667	0.305	0.610					P_BS	P_MR	22.667	41.060	0.552	0.610	0.610
Residuals	10.115.333	4	2.528.833													
variant	14.900.167	1	14.900.167	0.269	0.632					B_POT	B_MR	99.667	192.284	0.518	0.632	0.632
Residuals	221.838.667	4	55.459.667													
variant	3.876.033	1	3.876.033	0.276	0.635					O_POT	O_MR	56.833	108.091	0.526	0.635	0.635
Residuals	42.061.167	3	14.020.389													
variant	206.461.500	1	206.461.500	11.666	0.027					P_POT	P_MR	371.000	108.623	3.415	0.027*	0.027
Residuals	70.793.333	4	17.698.333													
variant	1.568.167	1	1.568.167	0.033	0.865					B_POT	SUB	-32.333	178.493	-0.181	0.865	0.865
Residuals	191.158.667	4	47.789.667													
variant	2.066.700	1	2.066.700	0.159	0.717					O_POT	SUB	41.500	104.129	0.399	0.717	0.717
Residuals	39.034.500	3	13.011.500													
variant	10.416.667	1	10.416.667	0.451	0.539					P_POT	SUB	-83.333	124.139	-0.671	0.539	0.539
Residuals	92.462.667	4	23.115.667													
variant	310.230.889	2	155.115.444	9.371	0.014					B_MR	O_MR	-116.667	105.050	-1.111	0.543	0.928
Residuals	99.319.333	6	16.553.222								P_MR	322.333	105.050	3.068	0.050	0.066
										O_MR	P_MR	439.000	105.050	4.179	0.014*	0.017
variant	281.004.222	2	140.502.111	5.401	0.046					B_BS	O_BS	-198.333	131.697	-1.506	0.353	0.548
Residuals	156.096.000	6	26.016.000								P_BS	234.000	131.697	1.777	0.255	0.378
										O_BS	P_BS	432.333	131.697	3.283	0.038*	0.050
variant	18.702.167	2	9.351.083	0.199	0.826					B_POT	O_POT	-73.833	198.063	-0.373	0.927	1.000
Residuals	235.373.833	5	47.074.767								P_POT	51.000	177.153	0.288	0.956	1.000
										O_POT	P_POT	124.833	198.063	0.630	0.811	1.000
						variant	0.196	1	0.658	B_non_inoc_MR	B_non_inoc_BS	131.333	168.039	0.782	0.478	0.478
variant	7.921.000	1	7.921.000	0.562	0.532					O_non_inoc_MR	O_non_inoc_BS	-89.000	118.703	-0.750	0.532	0.532
Residuals	28.181.000	2	14.090.500													
						variant	3.857	1	0.050	P_non_inoc_MR	P_non_inoc_BS	195.000	102.792	1.897	0.131	0.131
						variant	2.400	1	0.121	B_inoc_MR	B_inoc_BS	-252.500	233.121	-1.083	0.392	0.392
						variant	0.429	1	0.513	O_inoc_MR	O_inoc_BS	183.000	214.405	0.854	0.441	0.441
						variant	3.857	1	0.050	P_inoc_MR	P_inoc_BS	-77.667	57.500	-1.351	0.248	0.248
						variant	0.333	1	0.564	B_non_inoc_MR	B_inoc_MR	-111.833	212.248	-0.527	0.635	0.635
variant	79.567.500	1	79.567.500	1.331	0.332					O_non_inoc_MR	O_inoc_MR	257.500	223.163	1.154	0.332	0.332
Residuals	179.286.500	3	59.762.167								P_inoc_MR					
						variant	3.857	1	0.050	P_non_inoc_MR	B_inoc_MR	-201.667	102.860	-1.961	0.121	0.121

Table S5. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between field tree variants in metals' content. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Metal	ANOVA						Kruskal-Wallis test				Post Hoc comparison						
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variants of comparison		Mean Difference	SE	t	p tukey	p bonf
Al							variant	3.857	1	0.050	B_MR	O_MR	313.933	13.062	24.033	< .001	< .001*
							variant	3.857	1	0.050	B_MR	P_MR	302.500	16.301	18.558	< .001	< .001*
							variant	3.857	1	0.050	O_MR	P_MR	-11.433	9.763	-1.171	0.307	0.307
							variant	3.857	1	0.050	B_BS	O_BS	294.400	19.582	15.034	< .001	< .001*
	variant	80.731.360	1	80.731.360	13.388	0.022					B_BS	P_BS	231.993	63.405	3.659	0.022*	0.022
	Residuals	24.120.796	4	6.030.199													
							variant	3.857	1	0.050	O_BS	P_BS	-62.407	60.305	-1.035	0.359	0.359
Cd	variant	0.017	1	0.017	6.228	0.067					B_MR	O_MR	-0.106	0.042	-2.496	0.067	0.067
	Residuals	0.011	4	0.003													
							variant	3.857	1	0.050	B_MR	P_MR	-0.102	0.093	-1.096	0.335	0.335
	variant	2.282e -5	1	2.282e -5	0.001	0.971					O_MR	P_MR	0.004	0.102	0.038	0.971	0.971
	Residuals	0.062	4	0.016													
	variant	0.006	1	0.006	10.835	0.030					B_BS	O_BS	-0.061	0.018	-3.292	0.030*	0.030
	Residuals	0.002	4	5.078e -4													
	variant	4.267e -4	1	4.267e -4	0.801	0.421					B_BS	P_BS	-0.017	0.019	-0.895	0.421	0.421
	Residuals	0.002	4	5.327e -4													
	variant	0.003	1	0.003	3.232	0.147					O_BS	P_BS	0.044	0.024	1.798	0.147	0.147
Residuals	0.004	4	8.862e -4														
Co	variant	0.019	1	0.019	3.003	0.158					B_MR	O_MR	-0.113	0.065	-1.733	0.158	0.158
	Residuals	0.026	4	0.006													
							variant	0.429	1	0.513	B_MR	P_MR	-0.523	0.684	-0.765	0.487	0.487
							variant	0.429	1	0.513	O_MR	P_MR	-0.410	0.683	-0.600	0.581	0.581
	variant	0.016	1	0.016	0.629	0.472					B_BS	O_BS	-0.102	0.128	-0.793	0.472	0.472
	Residuals	0.099	4	0.025													
	variant	0.072	1	0.072	0.555	0.498					B_BS	P_BS	-0.220	0.295	-0.745	0.498	0.498
	Residuals	0.522	4	0.130													
	variant	0.021	1	0.021	0.168	0.703					O_BS	P_BS	-0.118	0.287	-0.410	0.703	0.703
	Residuals	0.496	4	0.124													
Cr	variant	0.002	1	0.002	2.797	0.170					B_MR	O_MR	0.033	0.020	1.672	0.170	0.170
	Residuals	0.002	4	5.723e -4													
							variant	3.857	1	0.050	B_MR	P_MR	0.162	0.017	9.699	< .001	< .001*
							variant	3.857	1	0.050	O_MR	P_MR	0.129	0.010	12.751	< .001	< .001*
							variant	0.429	1	0.513	B_BS	O_BS	0.077	0.051	1.522	0.203	0.203
	variant	0.001	1	0.001	0.050	0.834					B_BS	P_BS	-0.027	0.119	-0.223	0.834	0.834
	Residuals	0.086	4	0.021													
						variant	0.484	1	0.487	O_BS	P_BS	-0.104	0.108	-0.956	0.393	0.393	
Cu	variant	62.449	1	62.449	47.155	0.002					B_MR	O_MR	6.452	0.940	6.867	0.002*	0.002
	Residuals	5.297	4	1.324													
	variant	37.680	1	37.680	4.523	0.101					B_MR	P_MR	5.012	2.357	2.127	0.101	0.101
	Residuals	33.321	4	8.330													
							variant	0.048	1	0.827	O_MR	P_MR	-1.440	2.296	-0.627	0.564	0.564
	variant	54.361	1	54.361	25.091	0.007					B_BS	O_BS	6.020	1.202	5.009	0.007*	0.007

	Residuals	8.666	4	2.167													
	variant	27.692	1	27.692	1.559	0.280					B_BS	P_BS	4.297	3.441	1.249	0.280	0.280
	Residuals	71.045		17.761													
							variant	0.429	1	0.513	O_BS	P_BS	-1.723	3.250	-0.530	0.624	0.624
Fe	variant	3.814.626	1	3.814.626	33.202	0.005					B_MR	O_MR	50.429	8.752	5.762	0.005*	0.005
	Residuals	459.569	4	114.892													
	variant	4.057.248	1	4.057.248	35.764	0.004					B_MR	P_MR	52.008	8.697	5.980	0.004*	0.004
	Residuals	453.785	4	113.446													
	variant	3.740	1	3.740	2.083	0.222					O_MR	P_MR	1.579	1.094	1.443	0.222	0.222
	Residuals	7.180	4	1.795													
							variant	3.857	1	0.050	B_BS	O_BS	29.103	14.426	2.017	0.114	0.114
							variant	3.857	1	0.050	B_BS	P_BS	27.500	14.608	1.882	0.133	0.133
							variant	0.429	1	0.513	O_BS	P_BS	-1.603	2.309	-0.694	0.526	0.526
Mn	variant	7.707.105	1	7.707.105	9.144	0.039					B_MR	O_MR	-71.680	23.705	-3.024	0.039*	0.039
	Residuals	3.371.609	4	842.902													
							variant	3.857	1	0.050	B_MR	P_MR	-102.014	53.243	-1.916	0.128	0.128
	variant	1.380.167	1	1.380.167	0.271	0.630					O_MR	P_MR	-30.333	58.271	-0.521	0.630	0.630
	Residuals	20.373.399	4	5.093.350													
	variant	1.659.967	1	1.659.967	10.548	0.031					B_BS	O_BS	-33.266	10.243	-3.248	0.031	0.031
	Residuals	629.476	4	157.369													
							variant	3.857	1	0.050	B_BS	P_BS	-29.786	13.427	-2.218	0.091	0.091
	variant	18.173	1	18.173	0.043	0.846					O_BS	P_BS	3.481	16.794	0.207	0.846	0.846
	Residuals	1.692.180	4	423.045													
Mo	variant	0.026	1	0.026	46.593	0.002					B_MR	O_MR	-0.132	0.019	-6.826	0.002*	0.002
	Residuals	0.002	4	5.592e -4													
							variant	2.634	1	0.105	B_MR	P_MR	-0.017	0.016	-1.061	0.349	0.349
	variant	0.020	1	0.020	20.496	0.011					O_MR	P_MR	0.114	0.025	4.527	0.011*	0.011
	Residuals	0.004	4	9.589e -4													
	variant	0.013	1	0.013	32.828	0.005					B_BS	O_BS	-0.095	0.017	-5.730	0.005*	0.005
	Residuals	0.002	4	4.095e -4													
	variant	0.000	1	0.000	0.000	1.000					B_BS	P_BS	1.133e -17	8.165e -4	1.388e -14	1.000	1.000
	Residuals	4.000e -6	4	1.000e -6													
	variant	0.013	1	0.013	32.828	0.005					O_BS	P_BS	0.095	0.017	5.730	0.005*	0.005
	Residuals	0.002	4	4.095e -4													
Ni	variant	0.806	1	0.806	11.769	0.027					B_MR	O_MR	-0.733	0.214	-3.431	0.027*	0.027
	Residuals	0.274	4	0.068													
							variant	2.333	1	0.127	B_MR	P_MR	-2.478	2.230	-1.111	0.329	0.329
							variant	0.048	1	0.827	O_MR	P_MR	-1.745	2.239	-0.779	0.479	0.479
	variant	0.614	1	0.614	19.640	0.011					B_BS	O_BS	-0.640	0.144	-4.432	0.011*	0.011
	Residuals	0.125	4	0.031													
	variant	0.653	1	0.653	2.883	0.165					B_BS	P_BS	-0.660	0.389	-1.698	0.165	0.165
	Residuals	0.907	4	0.227													
	variant	6.000e -4	1	6.000e -4	0.003	0.962					O_BS	P_BS	-0.020	0.395	-0.051	0.962	0.962
	Residuals	0.936	4	0.234													
Pb							variant	3.857	1	0.050	B_MR	O_MR	-0.068	0.026	-2.660	0.056	0.056
							variant	3.857	1	0.050	B_MR	P_MR	-0.431	0.041	-10.526	< .001	< .001*

Sr	variant	0.198	1	0.198	56.343	0.002				O_MR	P_MR	-0.363	0.048	-7.506	0.002*	0.002	
	Residuals	0.014	4	0.004													
	variant	2.640e -4	1	2.640e -4	0.199	0.679				B_BS	O_BS	-0.013	0.030	-0.446	0.679	0.679	
	Residuals	0.005	4	0.001													
							variant	3.857	1	0.050	B_BS	P_BS	-1.093	0.726	-1.506	0.207	0.207
							variant	3.857	1	0.050	O_BS	P_BS	-1.080	0.726	-1.488	0.211	0.211
										B_MR	O_MR	-2.387	0.397	-6.018	0.004*	0.004	
							variant	3.857	1	0.050	B_MR	P_MR	-22.742	10.520	-2.162	0.097	0.097
							variant	3.857	1	0.050	O_MR	P_MR	-20.355	10.528	-1.933	0.125	0.125
V	variant	5.715	1	5.715	13.903	0.020				B_BS	O_BS	-1.952	0.524	-3.729	0.020*	0.020	
	Residuals	1.644	4	0.411													
							variant	3.857	1	0.050	B_BS	P_BS	-11.055	0.266	-41.548	< .001	< .001*
							variant	3.857	1	0.050	O_BS	P_BS	-9.103	0.587	-15.501	< .001	< .001*
	variant	0.472	1	0.472	7.739	0.050				B_MR	O_MR	-0.561	0.202	-2.782	0.050	0.050	
	Residuals	0.244	4	0.061													
							variant	3.137	1	0.077	B_MR	P_MR	-0.036	0.029	-1.240	0.283	0.283
	variant	0.414	1	0.414	6.646	0.061				O_MR	P_MR	0.525	0.204	2.578	0.061	0.061	
	Residuals	0.249	4	0.062													
	variant	1.052	1	1.052	72.216	0.001				B_BS	O_BS	-0.837	0.099	-8.498	0.001*	0.001	
Zn	Residuals	0.058	4	0.015													
	variant	0.000	1	0.000	0.000	1.000				B_BS	P_BS	1.133e -17	8.165e -4	1.388e -14	1.000	1.000	
	Residuals	4.000e -6	4	1.000e -6													
	variant	1.052	1	1.052	72.216	0.001				O_BS	P_BS	0.837	0.099	8.498	0.001*	0.001	
	Residuals	0.058	4	0.015													
							variant	2.333	1	0.127	B_MR	O_MR	-2.193	1.184	-1.852	0.138	0.138
							variant	0.429	1	0.513	B_MR	P_MR	-2.053	2.371	-0.866	0.436	0.436
	variant	0.029	1	0.029	0.003	0.960				O_MR	P_MR	0.140	2.643	0.053	0.960	0.960	
	Residuals	41.897	4	10.474													
							variant	0.049	1	0.825	B_BS	O_BS	-0.583	0.727	-0.802	0.468	0.468
Cs							variant	0.049	1	0.825	B_BS	P_BS	-0.760	1.052	-0.722	0.510	0.510
							variant	0.000	1	1.000	O_BS	P_BS	-0.177	1.262	-0.140	0.895	0.895
	variant	3.702e -4	1	3.702e -4	0.050	0.835				B_MR	O_MR	0.016	0.071	0.223	0.835	0.835	
	Residuals	0.030	4	0.007													
	variant	0.007	1	0.007	6.759	0.060				B_MR	P_MR	0.067	0.026	2.600	0.060	0.060	
	Residuals	0.004	4	0.001													
							variant	0.048	1	0.827	O_MR	P_MR	0.052	0.070	0.738	0.501	0.501
							variant	0.429	1	0.513	B_BS	O_BS	0.051	0.032	1.606	0.183	0.183
	variant	0.003	1	0.003	4.874	0.092				B_BS	P_BS	0.047	0.021	2.208	0.092	0.092	
	Residuals	0.003	4	6.711e -4													
U	variant	2.360e -5	1	2.360e -5	0.012	0.918				O_BS	P_BS	-0.004	0.036	-0.109	0.918	0.918	
	Residuals	0.008	4	0.002													
							variant	3.857	1	0.050	B_MR	O_MR	2.153	0.142	15.131	< .001	< .001*
	variant	6.441	1	6.441	4.402	0.104				B_MR	P_MR	-2.072	0.988	-2.098	0.104	0.104	
	Residuals	5.853	4	1.463													
	variant	26.773	1	26.773	18.249	0.013				O_MR	P_MR	-4.225	0.989	-4.272	0.013*	0.013	

[illegible]

	variant	2.042e -4	1	2.042e -4	1.065	0.360					O_MR	O_BS	0.012	0.011	1.032	0.360	0.360
	Residuals	7.667e -4	4	1.917e -4													
							variant	2.634	1	0.105	P_MR	P_BS	-0.109	0.108	-1.009	0.370	0.370
Cu	variant	3.649	3	1.216	0.708	0.577					B_MR	B_BS	0.467	1.070	0.436	0.970	1.000
	Residuals	12.018	7	1.717								B_inoc_MR	1.153	1.196	0.964	0.773	1.000
												B_non_inoc_MR	1.433	1.070	1.340	0.569	1.000
											B_BS	B_inoc_MR	0.687	1.196	0.574	0.937	1.000
												B_non_inoc_MR	0.967	1.070	0.904	0.804	1.000
											B_inoc_MR	B_non_inoc_MR	0.280	1.196	0.234	0.995	1.000
							variant	8.197	3	0.042	O_MR	O_BS	0.034	0.469	0.073	1.000	1.000
												O_inoc_MR	-4.868	0.469	-10.387	< .001	< .001*
												O_non_inoc_MR	-4.634	0.524	-8.844	< .001	< .001*
											O_BS	O_inoc_MR	-4.902	0.469	-10.460	< .001	< .001*
												O_non_inoc_MR	-4.668	0.524	-8.910	< .001	< .001*
											O_inoc_MR	O_non_inoc_MR	0.234	0.524	0.446	0.968	1.000
							variant	0.949	3	0.814	P_MR	P_BS	-0.249	2.791	-0.089	1.000	1.000
												P_inoc_MR	-3.619	2.791	-1.297	0.590	1.000
												P_non_inoc_MR	-3.514	2.791	-1.259	0.610	1.000
											P_BS	P_inoc_MR	-3.370	2.791	-1.207	0.639	1.000
											P_non_inoc_MR	-3.266	2.791	-1.170	0.660	1.000	
										P_inoc_MR	P_non_inoc_MR	0.104	2.791	0.037	1.000	1.000	
Fe							variant	7.955	3	0.047	B_MR	B_BS	22.913	12.741	1.798	0.348	0.691
												B_inoc_MR	46.682	14.245	3.277	0.052	0.081
												B_non_inoc_MR	47.153	12.741	3.701	0.030	0.046*
											B_BS	B_inoc_MR	23.768	14.245	1.669	0.404	0.835
												B_non_inoc_MR	24.240	12.741	1.903	0.308	0.593
											B_inoc_MR	B_non_inoc_MR	0.472	14.245	0.033	1.000	1.000
							variant	8.356	3	0.039	O_MR	O_BS	1.588	0.795	1.997	0.274	0.516
												O_inoc_MR	-2.529	0.795	-3.181	0.059	0.093
												O_non_inoc_MR	-2.309	0.889	-2.597	0.127	0.213
											O_BS	O_inoc_MR	-4.117	0.795	-5.177	0.005	0.008*
												O_non_inoc_MR	-3.897	0.889	-4.383	0.013	0.019*
											O_inoc	O_non_inoc_MR	0.220	0.889	0.248	0.994	1.000
							variant	4.333	3	0.228	P_MR	P_BS	-1.595	1.680	-0.949	0.780	1.000
												P_inoc_MR	-4.651	1.680	-2.769	0.092	0.146
												P_non_inoc_MR	-4.049	1.680	-2.410	0.152	0.255
											P_BS	P_inoc_MR	-3.057	1.680	-1.819	0.332	0.638
											P_non_inoc_MR	-2.454	1.680	-1.461	0.501	1.000	
										P_inoc_MR	P_non_inoc_MR	0.603	1.680	0.359	0.983	1.000	
Mn	variant	103.427	3	34.476	13.584	0.003					B_MR	B_BS	0.424	1.301	0.326	0.987	1.000
	Residuals	17.766	7	2.538								B_inoc_MR	-6.977	1.454	-4.797	0.008*	0.012
												B_non_inoc_MR	-5.013	1.301	-3.854	0.025*	0.038
											B_BS	B_inoc_MR	-7.401	1.454	-5.089	0.006*	0.008
												B_non_inoc_MR	-5.437	1.301	-4.180	0.017*	0.025
											B_inoc_MR	B_non_inoc_MR	1.964	1.454	1.351	0.563	1.000
							variant	8.561	3	0.036	O_MR	O_BS	38.838	19.509	1.991	0.276	0.521
												O_inoc_MR	65.075	19.509	3.336	0.048	0.075
											O_non_inoc_MR	68.386	21.811	3.135	0.063	0.099	
										O_BS	O_inoc_MR	26.236	19.509	1.345	0.567	1.000	

											O_non_inoc_MR	29.548	21.811	1.355	0.561	1.000	
										O_inoc_MR	O_non_inoc_MR	3.311	21.811	0.152	0.999	1.000	
							variant	7.462	3	0.059	P_MR	P_BS	72.652	38.831	1.871	0.311	0.590
											P_inoc	91.612	38.831	2.359	0.163	0.276	
											P_non_inoc	95.450	38.831	2.458	0.142	0.237	
										P_BS	P_inoc	18.960	38.831	0.488	0.960	1.000	
											P_non_inoc	22.797	38.831	0.587	0.933	1.000	
											P_inoc_MR	P_non_inoc	3.838	38.831	0.099	1.000	1.000
Mo	variant	0.002	1	0.002	2.137	0.218					O_MR	O_BS	0.037	0.025	1.462	0.218	0.218
	Residuals	0.004	4	9.677e -4													
Ni							variant	2.634	1	0.105	P_MR	P_BS	0.017	0.016	1.061	0.349	0.349
	variant	0.025	3	0.008	0.844	0.512				B_MR	B_BS	0.110	0.081	1.362	0.557	1.000	
	Residuals	0.069	7	0.010							B_inoc_MR	0.121	0.091	1.338	0.570	1.000	
											B_non_inoc_MR	0.064	0.081	0.786	0.859	1.000	
										B_BS	B_inoc_MR	0.011	0.091	0.120	0.999	1.000	
											B_non_inoc_MR	-0.047	0.081	-0.576	0.936	1.000	
											B_inoc_MR	B_non_inoc_MR	-0.058	0.091	-0.635	0.918	1.000
											O_MR	O_BS	0.203	0.180	1.130	0.684	1.000
	variant	1.816	3	0.605	12.468	0.003					O_inoc_MR	0.880	0.180	4.892	0.007*	0.011	
	Residuals	0.340	7	0.049								O_non_inoc_MR	0.937	0.201	4.657	0.010*	0.014
											O_BS	O_inoc_MR	0.677	0.180	3.761	0.028*	0.042
												O_non_inoc_MR	0.733	0.201	3.646	0.033*	0.049
											O_inoc_MR	O_non_inoc_MR	0.057	0.201	0.282	0.992	1.000
							variant	6.385	3	0.094	P_MR	P_BS	1.928	1.599	1.206	0.640	1.000
												P_inoc_MR	2.545	1.599	1.591	0.434	0.901
												P_non_inoc_MR	2.635	1.599	1.648	0.407	0.828
Pb											P_BS	P_inoc_MR	0.617	1.599	0.386	0.979	1.000
												P_non_inoc_MR	0.707	1.599	0.442	0.969	1.000
											P_inoc_MR	P_non_inoc_MR	0.090	1.599	0.056	1.000	1.000
							variant	4.224	3	0.238	B_MR	B_BS	-0.022	0.016	-1.318	0.581	1.000
												B_inoc_MR	-0.004	0.018	-0.217	0.996	1.000
												B_non_inoc_MR	-0.003	0.016	-0.169	0.998	1.000
											B_BS	B_inoc_MR	0.018	0.018	0.962	0.774	1.000
												B_non_inoc_MR	0.019	0.016	1.150	0.674	1.000
											B_inoc_MR	B_non_inoc_MR	0.001	0.018	0.066	1.000	1.000
							variant	5.826	3	0.120	O_MR	O_BS	0.033	0.025	1.345	0.566	1.000
												O_inoc_MR	0.065	0.025	2.630	0.122	0.204
												O_non_inoc_MR	0.065	0.028	2.359	0.173	0.303
											O_BS	O_inoc_MR	0.032	0.025	1.284	0.599	1.000
												O_non_inoc_MR	0.032	0.028	1.155	0.670	1.000
											O_inoc_MR	O_non_inoc_MR	1.833e -4	0.028	0.007	1.000	1.000
							variant	8.641	3	0.034	P_MR	P_BS	-0.683	0.514	-1.330	0.571	1.000
Sr												P_inoc_MR	0.427	0.514	0.831	0.839	1.000
												P_non_inoc_MR	0.428	0.514	0.833	0.837	1.000
											P_BS	P_inoc_MR	1.110	0.514	2.161	0.214	0.376
												P_non_inoc_MR	1.111	0.514	2.163	0.213	0.375
											P_inoc_MR	P_non_inoc_MR	0.001	0.514	0.003	1.000	1.000
							variant	7.636	3	0.054	B_MR	B_BS	-0.004	0.041	-0.097	1.000	1.000
											B_inoc_MR	-1.099	0.046	-23.884	< .001	< .001*	

											B_BS	B_non_inoc_MR	-1.073	0.041	-26.064	< .001	< .001*	
											B_inoc_MR	B_inoc_MR	-1.095	0.046	-23.797	< .001	< .001*	
												B_non_inoc_MR	-1.069	0.041	-25.966	< .001	< .001*	
											B_inoc_MR	B_non_inoc_MR	0.026	0.046	0.572	0.937	1.000	
							variant	8.318	3	0.040	O_MR	O_BS	0.431	0.497	0.868	0.821	1.000	
												O_inoc_MR	1.282	0.497	2.582	0.130	0.218	
												O_non_inoc_MR	1.377	0.555	2.481	0.148	0.253	
											O_BS	O_inoc_MR	0.851	0.497	1.714	0.384	0.781	
												O_non_inoc_MR	0.947	0.555	1.705	0.388	0.792	
											O_inoc_MR	O_non_inoc_MR	0.095	0.555	0.171	0.998	1.000	
							variant	9.051	3	0.029	P_MR	P_BS	11.683	7.441	1.570	0.445	0.930	
												P_inoc_MR	21.558	7.441	2.897	0.077	0.120	
												P_non_inoc_MR	21.663	7.441	2.911	0.075	0.117	
											P_BS	P_inoc_MR	9.875	7.441	1.327	0.573	1.000	
												P_non_inoc_MR	9.980	7.441	1.341	0.565	1.000	
											P_inoc_MR	P_non_inoc_MR	0.105	7.441	0.014	1.000	1.000	
	Zn	variant	0.039	3	0.013	0.328	0.805					B_MR	B_BS	0.057	0.163	0.349	0.984	1.000
		Residuals	0.280	7	0.040								B_inoc_MR	0.154	0.183	0.843	0.833	1.000
												B_non_inoc_MR	0.131	0.163	0.800	0.853	1.000	
											B_BS	B_inoc_MR	0.097	0.183	0.531	0.949	1.000	
												B_non_inoc_MR	0.074	0.163	0.451	0.967	1.000	
											B_inoc_MR	B_non_inoc_MR	-0.023	0.183	-0.128	0.999	1.000	
							variant	5.925	3	0.115	O_MR	O_BS	1.667	1.042	1.600	0.436	0.922	
												O_inoc_MR	2.256	1.042	2.166	0.222	0.402	
												O_non_inoc_MR	2.422	1.165	2.079	0.248	0.457	
											O_BS	O_inoc_MR	0.590	1.042	0.566	0.939	1.000	
												O_non_inoc_MR	0.755	1.165	0.648	0.913	1.000	
											O_inoc_MR	O_non_inoc_MR	0.165	1.165	0.142	0.999	1.000	
							variant	1.094	3	0.779	P_MR	P_BS	1.350	1.829	0.738	0.879	1.000	
												P_inoc_MR	2.010	1.829	1.099	0.700	1.000	
												P_non_inoc_MR	2.058	1.829	1.125	0.685	1.000	
											P_BS	P_inoc_MR	0.660	1.829	0.361	0.983	1.000	
												P_non_inoc_MR	0.708	1.829	0.387	0.979	1.000	
											P_inoc_MR	P_non_inoc_MR	0.048	1.829	0.026	1.000	1.000	
Cs	variant	7.172e -4	3	2.391e -4	0.570	0.652					B_MR	B_BS	0.003	0.017	0.207	0.997	1.000	
	Residuals	0.003	7	4.194e -4								B_inoc_MR	0.015	0.019	0.783	0.860	1.000	
												B_non_inoc_MR	0.019	0.017	1.155	0.671	1.000	
											B_BS	B_inoc_MR	0.011	0.019	0.597	0.930	1.000	
												B_non_inoc_MR	0.016	0.017	0.948	0.782	1.000	
											B_inoc_MR	B_non_inoc_MR	0.005	0.019	0.250	0.994	1.000	
							variant	1.530	3	0.675	O_MR	O_BS	0.038	0.056	0.684	0.900	1.000	
												O_inoc_MR	0.008	0.056	0.150	0.999	1.000	
												O_non_inoc_MR	0.003	0.063	0.045	1.000	1.000	
											O_BS	O_inoc_MR	-0.030	0.056	-0.533	0.948	1.000	
												O_non_inoc_MR	-0.036	0.063	-0.567	0.939	1.000	
											O_inoc_MR	O_non_inoc_MR	-0.006	0.063	-0.090	1.000	1.000	
	variant	0.004	3	0.001	2.330	0.151					P_MR	P_BS	-0.017	0.019	-0.913	0.799	1.000	
	Residuals	0.004	8	5.236e -4								P_inoc_MR	-0.037	0.019	-1.984	0.270	0.495	
												P_non_inoc_MR	-0.045	0.019	-2.391	0.156	0.263	

U											P_BS	P_inoc_MR	-0.020	0.019	-1.070	0.716	1.000
												P_non_inoc_MR	-0.028	0.019	-1.477	0.492	1.000
											P_inoc_MR	P_non_inoc_MR	-0.008	0.019	-0.407	0.976	1.000
						variant	8.561	3	0.036		B_MR	B_BS	-0.371	0.227	-1.637	0.419	0.874
												B_inoc_MR	1.394	0.253	5.499	0.004	0.005*
												B_non_inoc_MR	1.366	0.227	6.028	0.002	0.003*
											B_BS	B_inoc_MR	1.765	0.253	6.963	< .001	0.001*
												B_non_inoc_MR	1.737	0.227	7.665	< .001	< .001*
											B_inoc_MR	B_non_inoc_MR	-0.027	0.253	-0.107	1.000	1.000
						variant	7.879	3	0.049		O_MR	O_BS	-0.110	0.086	-1.275	0.604	1.000
												O_inoc_MR	-0.757	0.086	-8.754	< .001	< .001*
												O_non_inoc_MR	-0.755	0.097	-7.808	< .001	< .001*
											O_BS	O_inoc_MR	-0.646	0.086	-7.479	< .001	< .001*
												O_non_inoc_MR	-0.644	0.097	-6.668	0.001	0.002*
											O_inoc_MR	O_non_inoc_MR	0.002	0.097	0.022	1.000	1.000
						variant	9.462	3	0.024		P_MR	P_BS	1.024	0.870	1.177	0.657	1.000
												P_inoc_MR	3.504	0.870	4.027	0.016	0.023*
												P_non_inoc_MR	3.477	0.870	3.996	0.017	0.024*
											P_BS	P_inoc_MR	2.480	0.870	2.851	0.082	0.129
												P_non_inoc_MR	2.453	0.870	2.819	0.086	0.135
											P_inoc_MR	P_non_inoc_MR	-0.027	0.870	-0.032	1.000	1.000

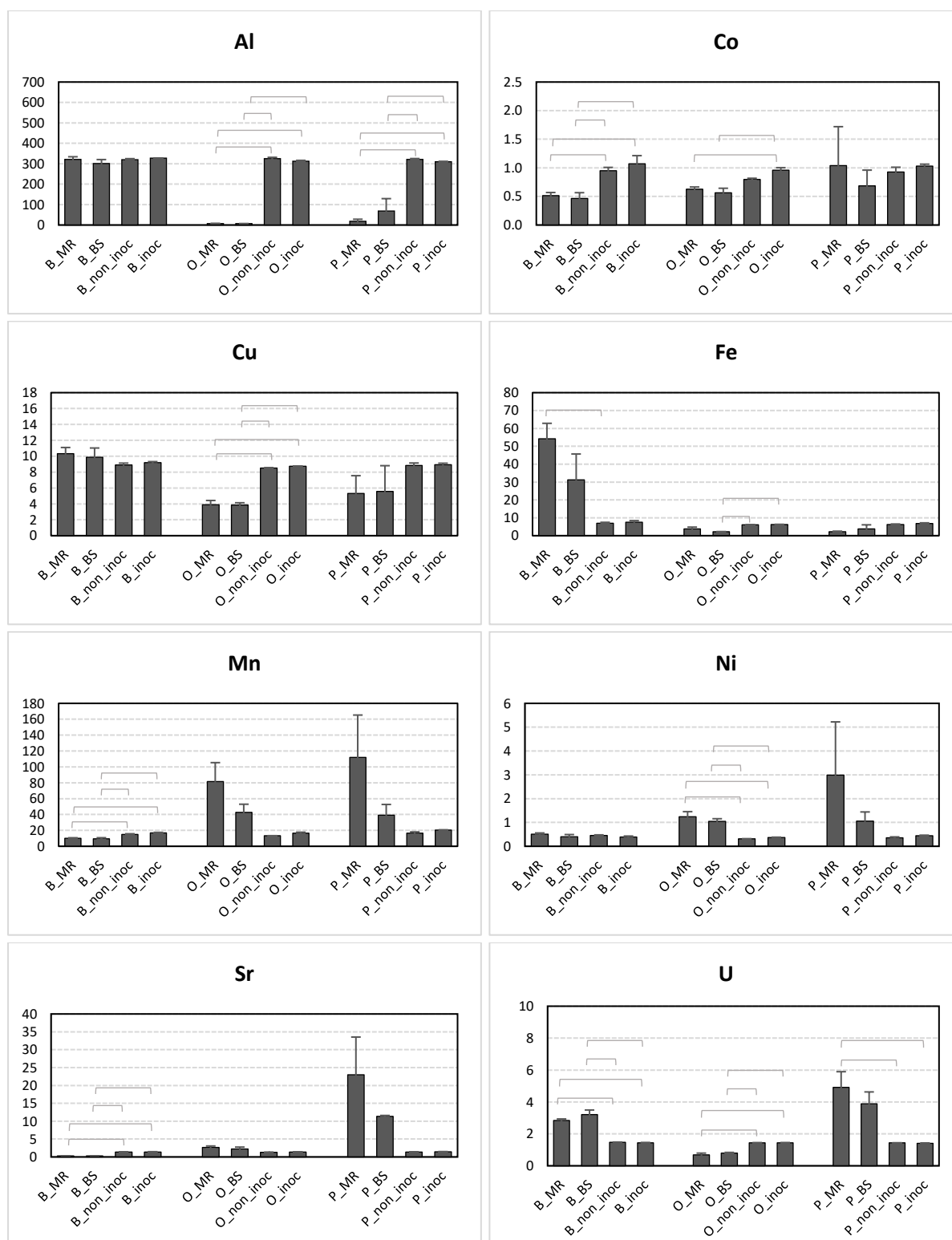


Figure S1. Sum content of toxic metals' bioavailable fractions: comparison of variants of the experiment within a tree species. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil; non_inoc – non-inoculated variant of the experiment, inoc – inoculated variant of the experiment. Brackets represent significant differences ($p < 0.05$). y-axis represents concentration in µg/g.

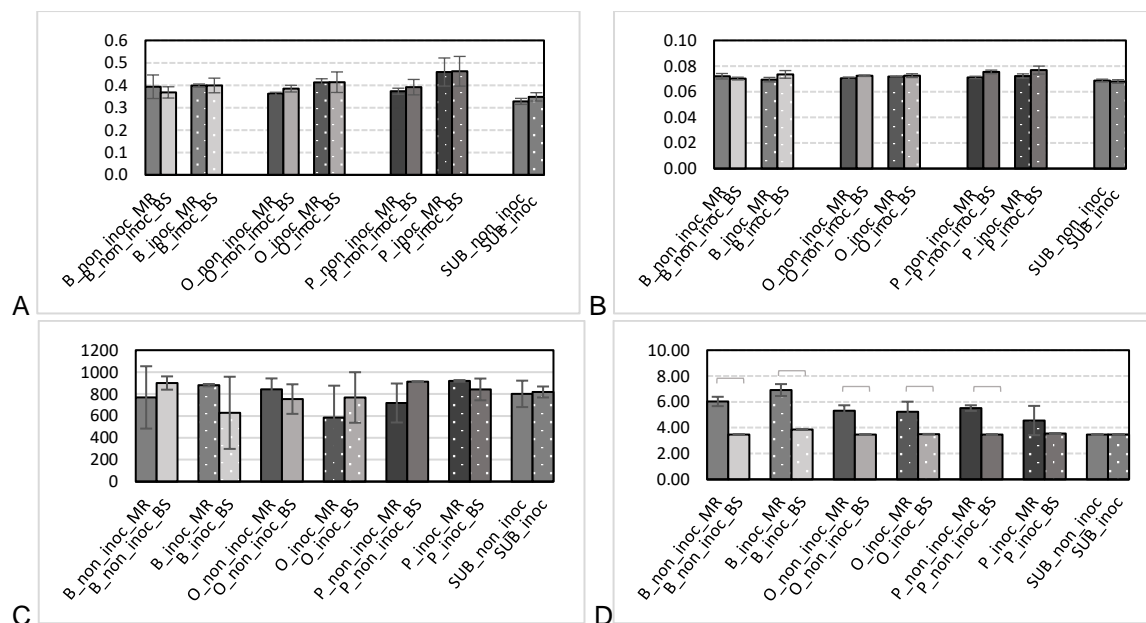


Figure S2. Chemical analysis of soil in pot experiment after additional inoculation of plants with ECM blend: **A** – content of total carbon (%), **B** – content of total nitrogen (%), **C** – content of total phosphorus (mg/kg), **D** – pH values. B – birch, O – oak, P – pine; MR – here mycorrhizosphere of plant, BS – here bulk soil of pot plant, SUB – control pot substrate; non_inoc – non-inoculated variant of the experiment, inoc – inoculated variant of the experiment. Brackets represent significant differences ($p < 0.05$).

Table S7. ANOSIM test output of pairwise comparisons between field plant mycorrhizospheres and bulk soil based on soil chemical parameters.

Mean rank within groups	42.17
Mean rank between groups	81.64
R	0.516
p	0.0001

Table S8. ANOSIM p-values of pairwise comparisons between field plant mycorrhizospheres and bulk soil based on soil chemical parameters. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

	B_MR	B_BS	O_MR	O_BS	P_MR	P_BS
B_MR						
B_BS	0.60					
O_MR	0.10	0.10				
O_BS	0.10	0.10	0.30			
P_MR	0.10	0.10	0.20	0.20		
P_BS	0.10	0.10	0.10	0.10	0.60	

Table S9. PERMANOVA test output of pairwise comparisons between field plant mycorrhizospheres and bulk soil based on soil chemical parameters.

Total sum of squares	272
Within-group sum of squares	117.6
F:	3.149
p	0.0001

Table S10. PERMANOVA p-values of pairwise comparisons between field plant mycorrhizospheres and bulk soil based on soil chemical parameters. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

	B_MR	B_BS	O_MR	O_BS	P_MR	P_BS
B_MR						
B_BS	0.61					
O_MR	0.09	0.10				
O_BS	0.10	0.10	0.39			
P_MR	0.10	0.10	0.10	0.20		
P_BS	0.11	0.10	0.10	0.10	0.71	

Table S11. Table of loadings represents correlation between important principal components and soil parameters between field plants mycorrhizospheres and pot plants mycorrhizospheres. The most contributing principal components are shown in bold.

Soil characteristics	PC 1	PC 2	PC 3
Al	-0.3850	0.1032	-0.0138
Co	0.0675	0.4035	0.3130
Cu	-0.2765	0.2428	0.1020
Fe	-0.1487	-0.065	-0.1143
Mn	0.3284	0.2067	0.1910
Ni	0.2423	0.3196	0.2252
Pb	0.3283	0.1895	-0.1791
Sr	0.2271	0.1044	-0.4694
Zn	0.1914	0.2481	0.4281
Cs	-0.1633	-0.0052	0.2799
U	0.1891	0.3135	-0.2635
TC	0.2800	-0.3058	0.1880
TN	0.2761	-0.2947	0.1992
C/N	0.2978	-0.2523	0.0224
TP	-0.2419	-0.1252	0.3470
pH	-0.1476	0.3913	-0.1087

Table S12. ANOSIM test output of pairwise comparisons between field plant mycorrhizospheres and pot plant mycorrhizospheres based on soil chemical parameters.

Mean rank within groups	38.81
Mean rank between groups	72.46
R	0.4948
p	0.0001

Table S13. ANOSIM p-values of pairwise comparisons between field plant mycorrhizospheres and pot plant mycorrhizospheres based on soil chemical parameters. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, POT – mycorrhizosphere of pot plant.

	B_MR	B_POT	O_MR	O_POT	P_MR	P_POT
B_MR						
B_POT	0.10					
O_MR	0.10	0.10				
O_POT	0.10	0.10	0.10			
P_MR	0.10	0.10	0.09	0.30		
P_POT	0.10	0.10	0.10	0.71	0.10	

Table S14. PERMANOVA test output of pairwise comparisons between field plant mycorrhizospheres and pot plant mycorrhizospheres based on soil chemical parameters.

Total sum of squares	259
Within-group sum of squares	87.3
F:	4.252
p	0.0001

Table S15. PERMANOVA p-values of pairwise comparisons between field plant mycorrhizospheres and pot plant mycorrhizospheres based on soil chemical parameters. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, POT – mycorrhizosphere of pot plant.

	B_MR	B_POT	O_MR	O_POT	P_MR	P_POT
B_MR						
B_POT	0.10					
O_MR	0.10	0.10				
O_POT	0.10	0.10	0.10			
P_MR	0.10	0.10	0.10	0.10		
P_POT	0.10	0.10	0.10	0.61	0.10	

Table S16. Table of loadings represents correlation between important principal components and soil parameters between field plants mycorrhizospheres and pot plants mycorrhizospheres. The most contributing principal components are shown in bold.

Soil characteristics	PC 1	PC 2	PC 3
Al	-0.3850	0.1032	-0.0138
Co	0.0675	0.4035	0.3130
Cu	-0.2765	0.2428	0.1020
Fe	-0.1487	-0.065	-0.1143
Mn	0.3284	0.2067	0.1910
Ni	0.2423	0.3196	0.2252
Pb	0.3283	0.1895	-0.1791
Sr	0.2271	0.1044	-0.4694
Zn	0.1914	0.2481	0.4281
Cs	-0.1633	-0.0052	0.2799
U	0.1891	0.3135	-0.2635
TC	0.2800	-0.3058	0.1880
TN	0.2761	-0.2947	0.1992
C/N	0.2978	-0.2523	0.0224
TP	-0.2419	-0.1252	0.3470
pH	-0.1476	0.3913	-0.1087

Table S17. ANOSIM test output of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on soil chemical parameters.

Mean rank within groups	12.2
Mean rank between groups	31.51
R	0.7022
p	0.0054

Table S18. ANOSIM p-values of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on soil chemical parameters. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

	B_POT	O_POT	P_POT	SUB
B_POT				
O_POT	0.30			
P_POT	0.20	0.69		
SUB	0.10	0.10	0.10	

Table S19. PERMANOVA test output of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on soil chemical parameters.

Total sum of squares	150
Within-group sum of squares	36.07
F:	7.37
p	0.0034

Table S20. PERMANOVA p-values of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on the soil chemical parameters. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

	B_POT	O_POT	P_POT	SUB
B_POT				
O_POT	0.30			
P_POT	0.20	0.59		
SUB	0.10	0.10	0.10	

Table S21. Table of loadings represents correlation between important principal components and soil parameters in pots. The most contributing principal components are shown in bold.

Soil characteristics	PC 1	PC 2
Al	0.2219	-0.3125
Co	-0.2580	0.3307
Cu	0.1805	0.5165
Fe	-0.2713	0.2470
Mn	-0.2921	0.1843
Ni	0.2805	0.2410
Pb	0.2996	-0.0820
Sr	0.2898	0.1499
Zn	0.3025	0.0009
Cs	0.3043	-0.1700
U	0.2209	-0.0299
TC	0.2460	0.3352
TN	-7.2e-30	1.14e-28
C/N	0.2286	0.3603
TP	0.3446	-0.2680
pH	0.3057	-0.0133

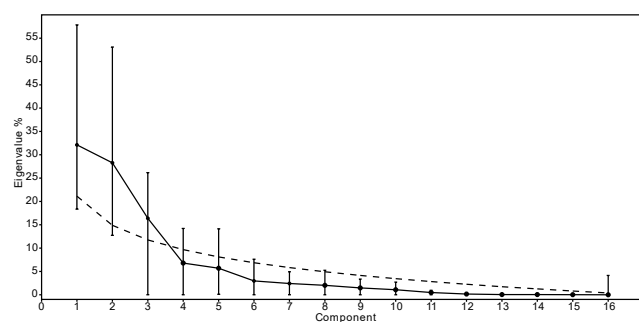


Figure S3. Scree plot of eigenvalues in principal component analysis based on chemical parameters between field sampling sites.

Table S22. Table of loadings represents correlation between important principal components and soil parameters at the field. The most contributing principal components are shown in bold.

Soil characteristics	PC 1	PC 2	PC 3
Al	0.0924	-0.4336	0.1386
Co	0.3086	0.1883	0.3357
Cu	0.2035	-0.2980	0.2866
Fe	0.0311	-0.3958	0.1510
Mn	0.2055	0.3440	0.2333
Ni	0.2706	0.2631	0.2868
Pb	0.2332	0.0971	-0.1057
Sr	0.1939	0.1789	-0.3957
Zn	0.2091	0.2442	0.3883
Cs	0.0273	-0.1711	0.2737
U	0.3971	-0.0277	-0.1068
TC	-0.3122	0.2747	0.1363
TN	-0.3118	0.2555	0.1719
C/N	-0.1925	0.2562	-0.1390
TP	-0.3106	-0.0379	0.2843
pH	0.3406	0.0682	-0.2707

Table S23. Morphological description of morphotypes.

Host tree	Variant of the experiment	Morphotype	Morphology	Emanating hyphae	Rhizomorphs	Exploration type
Birch	Field plant	B_F_MT1	unramified/monopodial pinnate, straight unramified ends, smooth mantle, light brown to dark brown colour	lacking	no	contact
Birch	Field plant	B_F_MT2	unramified, straight unramified ends, woolly mantle, black colour	frequent, black	no	short
Birch	Field plant	B_F_MT3	unramified/monopodial pinnate, straight unramified ends, smooth mantle, beige colour with silver dots	lacking	no	contact
Birch	Pot non-inoculated plant	B_non_inoc_MT1	unramified, straight unramified ends, smooth mantle, light brown colour	lacking	no	contact
Birch	Pot non-inoculated plant	B_non_inoc_MT2	unramified, straight unramified ends, woolly mantle, black colour	frequent, black	no	short
Birch	Pot non-inoculated plant	B_non_inoc_MT3	unramified, straight unramified ends, woolly mantle, light brown colour with silver dots	frequent, white	no	short
Birch	Pot non-inoculated plant	B_non_inoc_MT4	unramified, straight unramified ends, woolly mantle, black colour, brown tips	frequent, black	no	short
Birch	Pot non-inoculated plant	B_non_inoc_MT5	unramified, straight/bent unramified ends, grainy mantle, dark brown colour	infrequent, white/beige	no	short
Birch	Pot inoculated plant	B_inoc_MT1	unramified, straight unramified ends, smooth mantle, light brown to beige colour	lacking	no	contact
Birch	Pot inoculated plant	B_inoc_MT2	unramified, straight unramified ends, woolly mantle, black colour	frequent, black	no	short
Birch	Pot inoculated plant	B_inoc_MT3	unramified/monopodial pinnate, straight unramified ends, smooth mantle, dark brown colour	lacking	no	contact
Oak	Field plant	O_F_MT1	unramified, straight unramified ends, woolly mantle, black colour	frequent, black	no	short
Oak	Field plant	O_F_MT2	unramified, straight unramified ends, smooth mantle, light brown colour	lacking	no	contact
Oak	Field plant	O_F_MT3	unramified, straight unramified end, grainy mantle, dark brown to black colour	infrequent, white	no	contact
Oak	Field plant	O_F_MT4	unramified/irregular pinnate/dichotomous-like, tortuous unramified ends, stringy mantle, brown colour	abundant, white	yes, with restricted points of connection with mantle and interconnected filaments, white colour	medium, fringe subtype
Oak	Pot non-inoculated plant	O_non_inoc_MT1	unramified, straight unramified ends, woolly mantle, black colour	infrequent, black	no	short
Oak	Pot non-inoculated plant	O_non_inoc_MT2	unramified, straight unramified ends, smooth mantle, light brown colour	lacking	no	contact
Oak	Pot non-inoculated plant	O_non_inoc_MT3	unramified, straight unramified ends, woolly mantle, silver colour, light brown tips	infrequent, white	restricted points of connection with mantle, unsheathed, white colour	medium, smooth subtype
Oak	Pot inoculated plant	O_inoc_MT1	unramified, straight unramified ends, smooth/grainy mantle, beige colour	lacking	no	contact
Oak	Pot inoculated plant	O_inoc_MT2	unramified, straight unramified ends, woolly mantle, black colour	abundant, black	no	short
Oak	Pot inoculated plant	O_inoc_MT3	unramified/dichotomous, straight unramified ends, woolly mantle, white to grey colour	infrequent, white	no	short
Pine	Field plant	P_F_MT1	unramified/dichotomous, straight unramified ends, reticulate mantle, light brown colour	lacking	no	contact
Pine	Field plant	P_F_MT2	unramified, straight unramified ends, woolly mantle, black colour	abundant, black	no	short
Pine	Field plant	P_F_MT3	coralloid, straight unramified ends, reticulate mantle, white colour	lacking	yes, restricted points of connection with mantle, hairy, white-pinkish colour	medium, fringe subtype
Pine	Field plant	P_F_MT4	dichotomous, straight unramified ends, woolly mantle, brown colour	abundant, white	yes, hyphal fans, interconnected filaments, white colour	medium, mat subtype
Pine	Pot non-inoculated plant	P_non_inoc_MT1	unramified/dichotomous, straight unramified ends, smooth mantle, light brown to beige colour	lacking	no	contact
Pine	Pot non-inoculated plant	P_non_inoc_MT2	unramified/dichotomous, straight unramified ends, smooth mantle, white to grey colour	lacking	no	contact

Pine	Pot non-inoculated plant	P_non_inoc_MT3	unramified, straight unramified ends, woolly mantle, dark brown to black colour	abundant, black	no	short
Pine	Pot non-inoculated plant	P_non_inoc_MT4	unramified/dichotomous, straight unramified ends, woolly/grainy mantle, light beige colour	frequent, white	yes, growing off in flat angles, interconnected filaments, yellow colour	long
Pine	Pot inoculated plant	P_inoc_MT1	unramified/dichotomous, straight unramified ends, smooth mantle, light brown to beige colour	lacking	no	contact
Pine	Pot inoculated plant	P_inoc_MT2	unramified, straight unramified ends, woolly mantle, black colour, brown tips	abundant, black	no	short
Pine	Pot inoculated plant	P_inoc_MT3	unramified, straight unramified ends, woolly/grainy mantle, beige colour with silver dots	infrequent, white	no	short
Pine	Pot inoculated plant	P_inoc_MT4	unramified, bent unramified ends, woolly mantle, silver colour	infrequent, white	no	short

Table S24. Mycorrhiza sequence results. For morphotypes with grey cells sequence analysis was not successful.

Morphotype	Length (bp)	Closest match, UNITE (parameters)						Closest match, NCBI (parameters)					
		Accession number	UNITE Taxon Name	Score (bits)	E-value	Identity, %	EcM lineage	Accession number	Taxon Name	Query cover, %	E-value	Total score	Percent identity, %
B_F_MT1	642	UDB011464	<i>Lactarius Pers., 1797</i>	1159	0.0	100	russula-lactarius	KJ705202	<i>Lactarius mammosus</i>	97	0.0	1158	100
B_F_MT2	502	FN669230	<i>Meliniomyces bicolor Hambl. & Sigler, 2005</i>	883	0.0	99	meliniomyces	FN669230	<i>Meliniomyces sp.</i>	100	0.0	900	99
B_F_MT3	638	UDB025177	<i>Mallocybe anon.</i>	1141	0.0	99	inocybe	HQ604443	<i>Inocybe lacera</i>	100	0.0	1157	99.4
B_non_inoc_MT1													
B_non_inoc_MT2													
B_non_inoc_MT3													
B_non_inoc_MT4													
B_non_inoc_MT5													
B_inoc_MT1													
B_inoc_MT2													
B_inoc_MT3													
O_F_MT1	492	UDB002586	<i>Meliniomyces bicolor Hambl. & Sigler, 2005</i>	874	0.0	99	meliniomyces	KU176262	Uncultured <i>Meliniomyces</i>	98	0.0	865	99
O_F_MT2													
O_F_MT3	446	UDB053316	<i>Helotiales</i>	791	0.0	99	-	KY684476	Uncultured <i>Meliniomyces</i>	99	0.0	800	99.1
O_F_MT4	479	MK234571	<i>Cortinarius (Pers.) Gray, 1821</i>	643	0.0	100	cortinarius	MK234571	<i>Cortinarius bivelus</i>	100	0.0	883	100
O_non_inoc_MT1													
O_non_inoc_MT2													
O_non_inoc_MT3	530	KT334710	<i>Hyaloscyphaceae</i>	957	0.0	100	-	KT334710	Uncultured fungus	100	0.0	979	100
O_inoc_MT1													
O_inoc_MT2													
O_inoc_MT3	595	UDB009020	<i>Pisolithus Alb. & Schwein.</i>	1068	0.0	99	pisolithus-scleroderma	FR748134	<i>Pisolithus capsulifer</i>	100	0.0	1094	99.8
P_F_MT1	632	UDB016490	<i>Thelephoraceae</i>	1113	0.0	99	tomentella-thelephora	KC759473	Uncultured <i>Tomentella</i>	91	0.0	1074	100
P_F_MT2	492	FN679031	<i>Meliniomyces bicolor Hambl. & Sigler, 2005</i>	854	0.0	99	meliniomyces	KF428297	<i>Helotiaceae sp.</i>	100	0.0	876	98.8
P_F_MT3	848	UDB027685	<i>Rhizopogon mohelnensis Velen., 1931</i>	1223	0.0	99	suillus-rhizopogon	JX898967	Uncultured <i>Rhizopogon</i>	99	0.0	1549	99.7
P_F_MT4	644	UDB019593	<i>Tricholoma argyraceum (Bull.) Gillet, 1874</i>	1157	0.0	99	tricholoma	MG367247	<i>Tricholoma argyraceum</i>	100	0.0	1179	99.7
P_non_inoc_MT1	645	UDB015348	<i>Mallocybe anon.</i>	1159	0.0	99	inocybe	AB669659	Uncultured mycorrhizal fungus	100	0.0	1177	99.5
P_non_inoc_MT2													
P_non_inoc_MT3													
P_non_inoc_MT4													
P_inoc_MT1	640	AB669659	<i>Mallocybe anon.</i>	1135	0.0	99	inocybe	FM992932	Uncultured ectomycorrhiza (<i>Inocybe</i>)	98	0.0	1158	99.7
P_inoc_MT2	291	KF007259	<i>Hyaloscyphaceae</i>	495	2e-138	98	-	AB986370	<i>Hyaloscyphaceae sp.</i>	99	7e-138	501	97.9
P_inoc_MT3	500	UDB017362	<i>Meliniomyces bicolor Hambl. & Sigler, 2005</i>	897	0.0	99	meliniomyces	JX507662	<i>Helotiales sp.</i>	99	0.0	905	99.4
P_inoc_MT4	695	UDB025458	<i>Rhizopogon mohelnensis Velen., 1931</i>	1216	0.0	99	suillus-rhizopogon	HG426016	Uncultured <i>Rhizopogon</i>	97	0.0	1242	99.7

Table S25. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between variants of the experiment in diversity indices of ECM communities. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

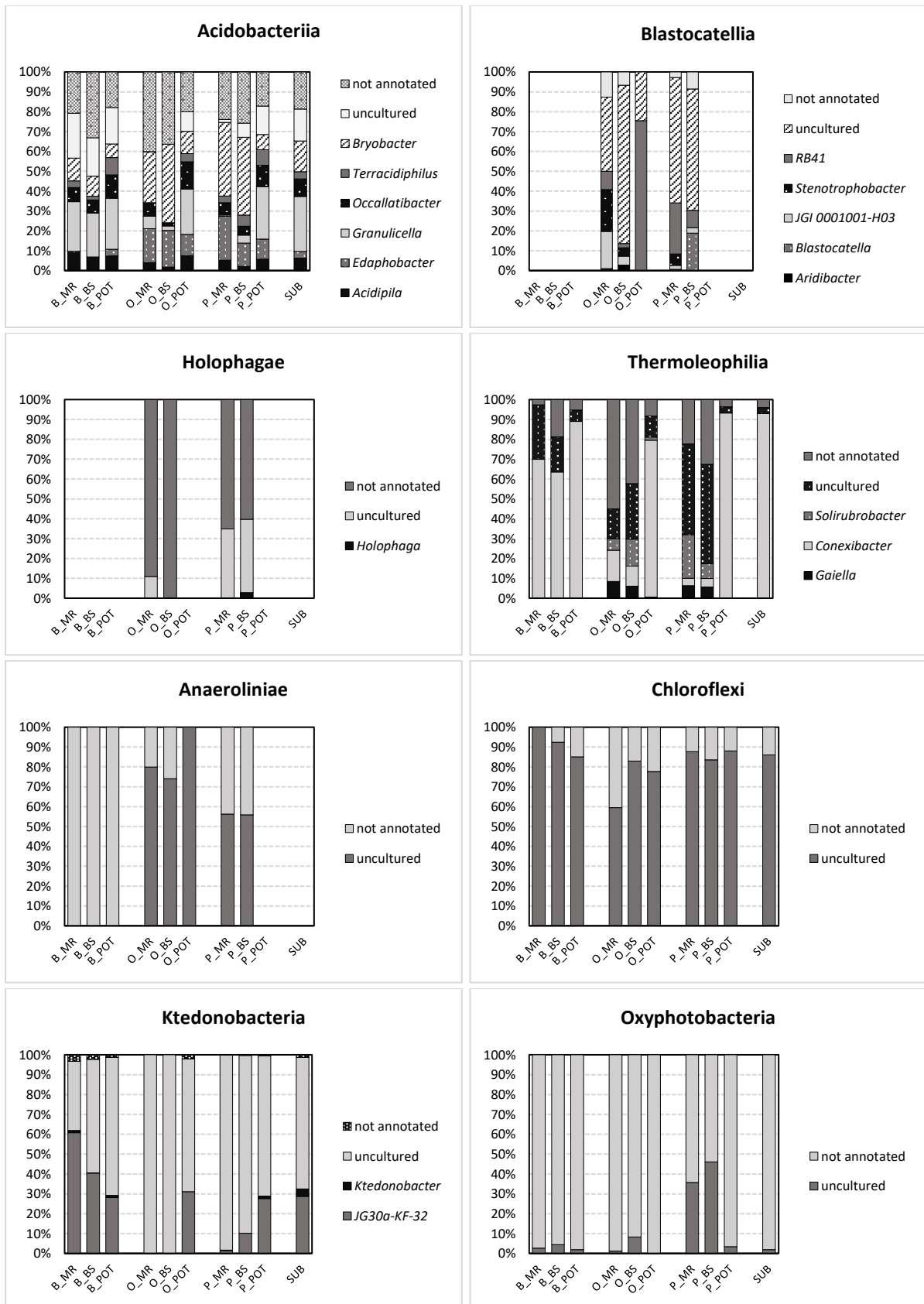
Diversity Index	ANOVA						Post Hoc comparison					
	Cases	Sum of Squares	df	Mean Square	F	p	Variants of comparisons		Mean Difference	SE	t	p tukey
<i>Simpson dominance index</i>	variant	0.053	2	0.027	0.940	0.441	field birch	pot birch inoc	-0.067	0.146	-0.464	0.890
	Residuals	0.169	6	0.028				pot birch non-inoc	0.131	0.128	1.020	0.593
							pot birch inoc	pot birch non-inoc	0.198	0.153	1.293	0.449
	variant	0.120	2	0.060	2.245	0.176	field oak	pot oak inoc	0.153	0.119	1.286	0.446
	Residuals	0.187	7	0.027				pot oak non-inoc	0.275	0.137	2.013	0.179
							pot oak inoc	pot oak non-inoc	0.122	0.149	0.816	0.706
	variant	0.013	2	0.006	0.340	0.722	field pine	pot pine inoc	0.065	0.099	0.657	0.794
	Residuals	0.148	8	0.019				pot pine non-inoc	-0.021	0.099	-0.215	0.975
<i>Gini-Simpson index</i>							pot pine inoc	pot pine non-inoc	-0.087	0.111	-0.780	0.725
	variant	0.053	2	0.027	0.940	0.441	field birch	pot birch inoc	0.068	0.146	0.464	0.890
	Residuals	0.169	6	0.028				pot birch non-inoc	-0.131	0.128	-1.020	0.593
							pot birch inoc	pot birch non-inoc	-0.198	0.153	-1.293	0.449
	variant	0.120	2	0.060	2.245	0.176	field oak	pot oak inoc	-0.153	0.119	-1.286	0.446
	Residuals	0.187	7	0.027				pot oak non-inoc	-0.275	0.137	-2.013	0.179
							pot oak inoc	pot oak non-inoc	-0.122	0.149	-0.816	0.706
	variant	0.013	2	0.006	0.340	0.722	field pine	pot pine inoc	-0.065	0.099	-0.657	0.794
<i>Shannon diversity index</i>	Residuals	0.148	8	0.019				pot pine non-inoc	0.021	0.099	0.215	0.975
							pot pine inoc	pot pine non-inoc	0.087	0.111	0.780	0.725
	variant	0.168	2	0.084	1.208	0.362	field birch	pot birch inoc	0.082	0.228	0.362	0.931
	Residuals	0.417	6	0.069				pot birch non-inoc	-0.254	0.201	-1.263	0.463
							pot birch inoc	pot birch non-inoc	-0.337	0.241	-1.400	0.399
	variant	0.340	2	0.170	2.650	0.139	field oak	pot oak inoc	-0.227	0.185	-1.226	0.476
	Residuals	0.449	7	0.064				pot oak non-inoc	-0.475	0.212	-2.242	0.131
							pot oak inoc	pot oak non-inoc	-0.248	0.231	-1.074	0.558
<i>Berger-Parker index</i>	variant	0.025	2	0.013	0.271	0.770	field pine	pot pine inoc	-0.114	0.158	-0.721	0.758
	Residuals	0.375	8	0.047				pot pine non-inoc	-0.064	0.158	-0.405	0.915
							pot pine inoc	pot pine non-inoc	0.050	0.177	0.283	0.957
	variant	0.036	2	0.018	0.683	0.540	field birch	pot birch inoc	-0.027	0.140	-0.197	0.979
	Residuals	0.157	6	0.026				pot birch non-inoc	0.122	0.123	0.993	0.608
							pot birch inoc	pot birch non-inoc	0.150	0.148	1.017	0.594
	variant	0.086	2	0.043	1.278	0.337	field oak	pot oak inoc	0.131	0.134	0.978	0.612
	Residuals	0.237	7	0.034				pot oak non-inoc	0.233	0.154	1.515	0.341
							pot oak inoc	pot oak non-inoc	0.102	0.168	0.606	0.822
	variant	0.023	2	0.012	0.476	0.638	field pine	pot pine inoc	0.086	0.115	0.750	0.742
	Residuals	0.197	8	0.025				pot pine non-inoc	-0.034	0.115	-0.297	0.953
							pot pine inoc	pot pine non-inoc	-0.120	0.128	-0.936	0.634

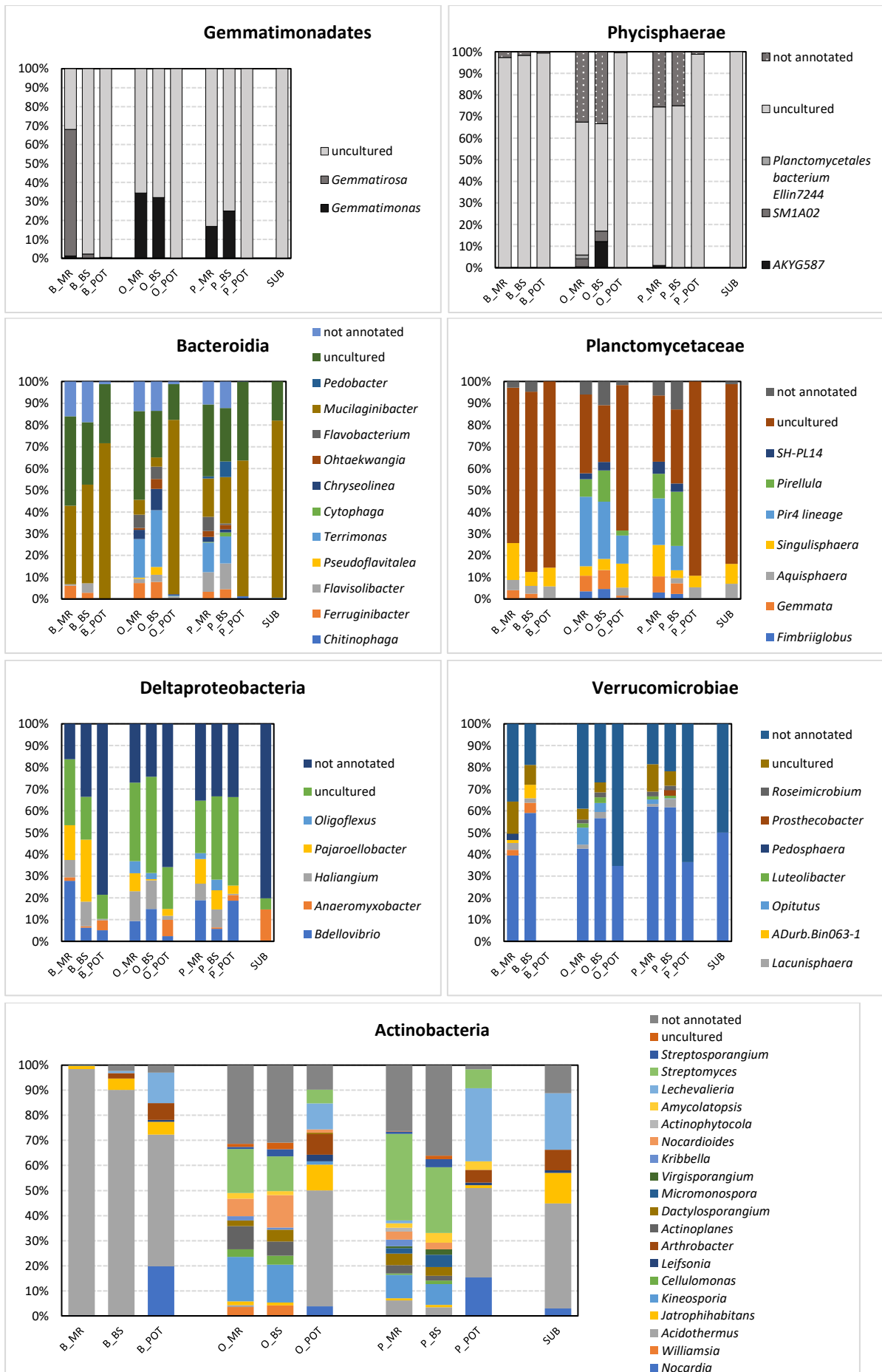
Table S26. Indices of similarity between ECM communities determined for trees within variants of the experiment.

Index	Estimate	s.e.	95%Lower	95%Upper
Field Birches				
Sorensen	0.970	0.017	0.936	1.000
Jaccard	0.889	0.052	0.786	0.991
Bray-Curtis	1.000	0.000	1.000	1.000
non-inoculated Pot Birches				
Sorensen	0.875	0.050	0.777	0.973
Jaccard	0.700	0.087	0.529	0.871
Bray-Curtis	0.930	0.057	0.819	1.000
inoculated Pot Birches				
Sorensen	0.924	0.151	0.628	1.220
Jaccard	0.858	0.214	0.440	1.277
Bray-Curtis	1.000	0.162	0.682	1.000
Field Oaks				
Sorensen	0.833	0.035	0.765	0.902
Jaccard	0.500	0.077	0.350	0.650
Bray-Curtis	0.907	0.047	0.816	0.999
non-inoculated Pot Oaks				
Sorensen	1.000	0.000	1.000	1.000
Jaccard	1.000	0.000	1.000	1.000
Bray-Curtis	1.000	0.000	1.000	1.000
inoculated Pot Oaks				
Sorensen	0.938	0.008	0.922	0.953
Jaccard	0.833	0.017	0.800	0.866
Bray-Curtis	1.000	0.005	0.990	1.000
Field Pines				
Sorensen	0.833	0.000	0.833	0.833
Jaccard	0.500	0.000	0.500	0.500
Bray-Curtis	0.954	0.011	0.932	0.975
non-inoculated Pot Pines				
Sorensen	0.955	0.017	0.922	0.987
Jaccard	0.875	0.039	0.798	0.952
Bray-Curtis	1.000	0.030	0.942	1.000
inoculated Pot Pines				
Sorensen	0.833	0.042	0.752	0.915
Jaccard	0.625	0.078	0.473	0.777
Bray-Curtis	0.852	0.074	0.706	0.997

Table S27. Kruskal-Wallis test, post hoc test output for significance of differences between variants of the experiment in relative abundance of exploration types of mycorrhiza. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Exploration type of mycorrhiza	Kruskal-Wallis				Post Hoc comparisons					
	Factor	Statistic	df	p	Variant of comparisons		Mean Difference	SE	t	p bonf
Contact	variant	31.095	2	1.770e -7	birch field	birch pot inoc	-31.899	5.391	-5.917	7.581e -8*
						birch pot non-inoc	-13.786	4.636	-2.974	0.010*
					birch pot inoc	birch pot non-inoc	18.113	4.767	3.800	6.523e -4*
	variant	37.596	2	6.856e -9	oak field	oak pot inoc	-18.687	5.117	-3.652	0.001*
						oak pot non-inoc	-20.625	5.532	-3.728	9.337e -4*
					oak pot inoc	oak pot non-inoc	-1.938	5.117	-0.379	1.000
	variant	8.054	2	0.018	pine field	pine pot inoc	-0.357	3.107	-0.115	1.000
						pine pot non-inoc	-6.935	2.892	-2.398	0.054
					pine pot inoc	pine pot non-inoc	-6.577	2.595	-2.535	0.038*
Short-distance	variant	39.906	2	2.161e -9	birch field	birch pot inoc	4.934	3.604	1.369	0.520
						birch pot non-inoc	-15.585	3.099	-5.029	4.594e -6*
					birch pot inoc	birch pot non-inoc	-20.519	3.187	-6.439	5.738e -9*
	variant	4.468	2	0.107	oak field	oak pot inoc	-7.559	3.422	-2.209	0.088
						oak pot non-inoc	-1.409	3.699	-0.381	1.000
					oak pot inoc	oak pot non-inoc	6.150	3.422	1.797	0.225
	variant	48.719	2	2.635e -11	pine field	pine pot inoc	-0.485	3.776	-0.128	1.000
						pine pot non-inoc	16.468	3.515	4.685	2.267e -5*
					pine pot inoc	pine pot non-inoc	16.952	3.153	5.376	1.167e -6*
Medium-distance	variant	55.259	2	1.001e -12	oak field	oak pot inoc	2.537	2.760	0.919	1.000
						oak pot non-inoc	-12.325	2.984	-4.131	2.171e -4*
					oak pot inoc	oak pot non-inoc	-14.863	2.760	-5.385	1.315e -6*
	variant	10.619	2	0.005	pine field	pine pot inoc	0.459	0.166	2.763	0.020*
						pine pot non-inoc	0.459	0.155	2.969	0.011*
					pine pot inoc	pine pot non-inoc	7.772e -16	0.139	5.600e -15	1.000
Long-distance	variant	43.881	2	2.961e -10	pine field	pine pot inoc	0.996	4.116	0.242	1.000
						pine pot non-inoc	-19.863	3.831	-5.184	2.727e -6*
					pine pot inoc	pine pot non-inoc	-20.859	3.437	-6.069	4.811e -8*





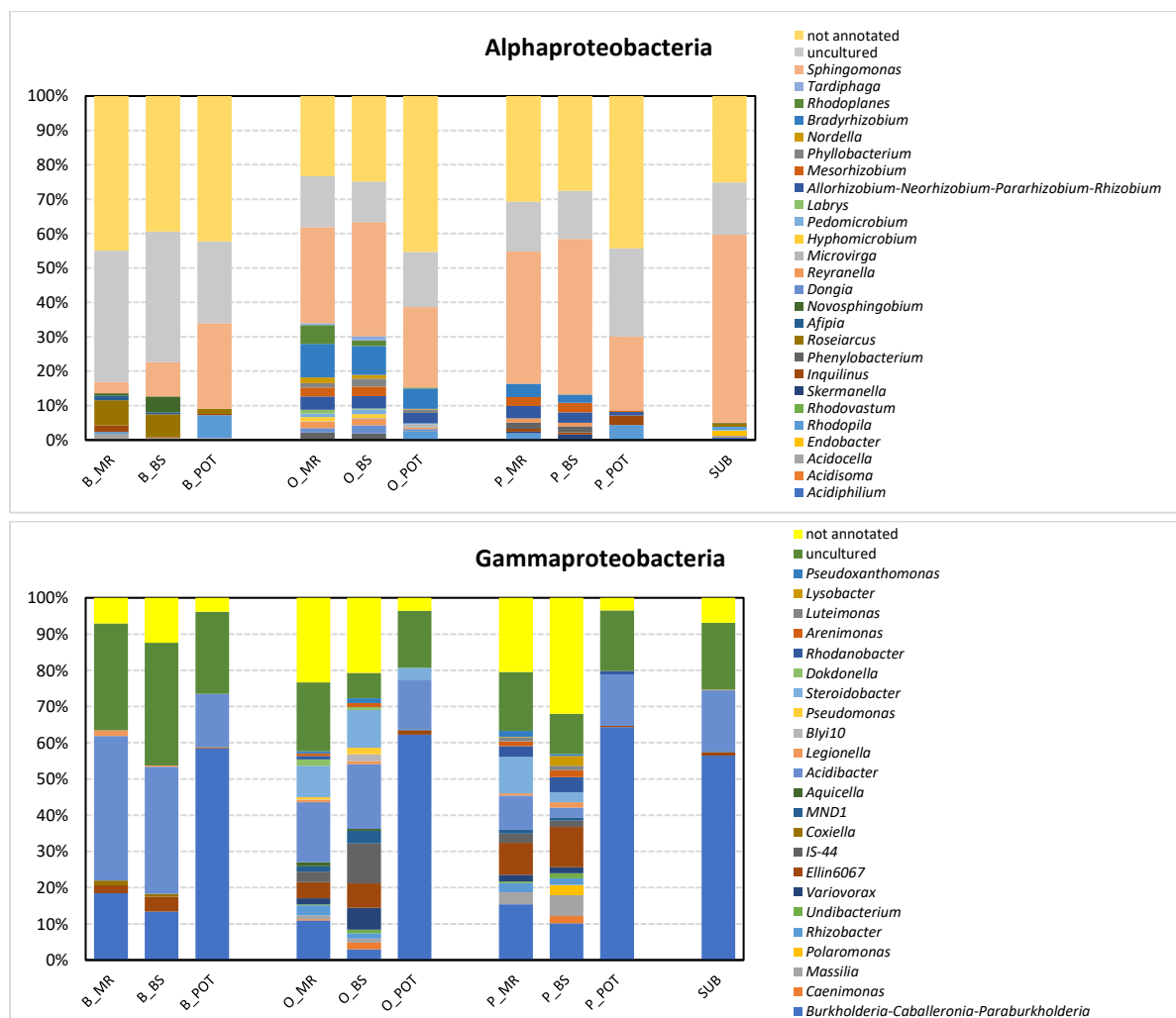


Figure S4. Characterisation of the most representative bacterial classes at genus level. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. y-axis represents relative abundance of bacterial genera within a considered bacterial class.

Table S28. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between variants of the experiment in diversity indices of bacterial communities. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Diversity Index	ANOVA						Kruskal-Wallis				Post Hoc comparisons						
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variant of comparisons		Mean Difference	SE	t	p tukey	p bonf
Richness	variant	2.521.500	1	2.521.500	0.746	0.436					B_MR	B_BS	-41.000	47.468	-0.864	0.436	0.436
	Residuals	13.519.333	4	3.379.833													
	variant	48.061.500	1	48.061.500	5.343	0.082					O_MR	O_BS	179.000	77.442	2.311	0.082	0.082
	Residuals	35.983.333	4	8.995.833													
	variant	337.500	1	337.500	0.010	0.927					P_MR	P_BS	-15.000	153.513	-0.098	0.927	0.927
	Residuals	141.397.333	4	35.349.333													
	variant	22.326.000	1	22.326.000	8.851	0.041					B_MR	B_POT	122.000	41.007	2.975	0.041*	0.041
	Residuals	10.089.333	4	2.522.333													
	variant	44.083.333	1	44.083.333	6.275	0.087					O_MR	O_POT	191.667	76.516	2.505	0.087	0.087
	Residuals	21.076.667	3	7.025.556													
	variant	150.416.667	1	150.416.667	4.270	0.108					P_MR	P_POT	316.667	153.239	2.066	0.108	0.108
	Residuals	140.893.333	4	35.223.333													
	variant	1.232.667	1	1.232.667	1.880	0.242					B_POT	SUB	28.667	20.907	1.371	0.242	0.242
	Residuals	2.622.667	4	655.667													
	variant	12.241.200	1	12.241.200	4.164	0.134					O_POT	SUB	101.000	49.497	2.041	0.134	0.134
	Residuals	8.820.000	3	2.940.000													
	variant	16.667	1	16.667	0.035	0.860					P_POT	SUB	-3.333	17.714	-0.188	0.860	0.860
	Residuals	1.882.667	4	470.667													
	variant	47.051.556	2	23.525.778	0.875	0.464					B_MR	O_MR	-142.000	133.907	-1.060	0.570	0.989
	Residuals	161.380.000	6	26.896.667								P_MR	-162.667	133.907	-1.215	0.488	0.810
Simpson dominance index											O_MR	P_MR	-20.667	133.907	-0.154	0.987	1.000
	variant	196.683.556	2	98.341.778	19.988	0.002					B_BS	O_BS	-362.000	57.271	-6.321	0.002*	0.002
	Residuals	29.520.000	6	4.920.000								P_BS	-188.667	57.271	-3.294	0.038*	0.050
											O_BS	P_BS	173.333	57.271	3.027	0.053	0.070
	variant	9.923e -7	1	9.923e -7	0.384	0.569					B_MR	B_BS	8.133e -4	0.001	0.620	0.569	0.569
	Residuals	1.034e -5	4	2.585e -6													
							variant	1.190	1	0.275	O_MR	O_BS	0.005	0.004	1.154	0.313	0.313
							variant	0.429	1	0.513	P_MR	P_BS	-0.006	0.007	-0.899	0.419	0.419
	variant	6.787e -5	1	6.787e -5	17.007	0.015					B_MR	B_POT	-0.007	0.002	-4.124	0.015*	0.015
	Residuals	1.596e -5	4	3.991e -6													
	variant	6.694e -5	1	6.694e -5	8.910	0.058					O_MR	O_POT	-0.007	0.003	-2.985	0.058	0.058
	Residuals	2.254e -5	3	7.514e -6													
	variant	3.099e -6	1	3.099e -6	0.041	0.850					P_MR	P_POT	-0.001	0.007	-0.202	0.850	0.850
	Residuals	3.042e -4	4	7.605e -5													
	variant	1.012e -4	1	1.012e -4	32.639	0.005					B_POT	SUB	-0.008	0.001	-5.713	0.005*	0.005
	Residuals	1.240e -5	4	3.100e -6													
	variant	2.543e -4	1	2.543e -4	34.126	0.010					O_POT	SUB	-0.015	0.002	-5.842	0.010*	0.010
	Residuals	2.235e -5	3	7.451e -6													
	variant	2.445e -4	1	2.445e -4	47.712	0.002					P_POT	SUB	-0.013	0.002	-6.907	0.002	0.002
	Residuals	2.050e -5	4	5.124e -6													
variant	1.119e -4	2	5.596e -5	1.113	0.388					B_MR	O_MR	0.007	0.006	1.224	0.483	0.800	
Residuals	3.016e -4	6	5.026e -5								P_MR	-7.360e -4	0.006	-0.127	0.991	1.000	
										O_MR	P_MR	-0.008	0.006	-1.351	0.421	0.676	

	variant	6.078e-5	2	3.039e-5	1.860	0.235					B_BS	O_BS	0.003	0.003	0.994	0.607	1.000
	Residuals	9.804e-5	6	1.634e-5								P_BS	0.006	0.003	1.928	0.211	0.306
Gini-Simpson index											O_BS	P_BS	0.003	0.003	0.934	0.641	1.000
	variant	1.042e-6	1	1.042e-6	0.392	0.565					B_MR	B_BS	-8.333e-4	0.001	-0.626	0.565	0.565
	Residuals	1.063e-5	4	2.657e-6													
							variant	1.190	1	0.275	O_MR	O_BS	-0.005	0.004	-1.151	0.314	0.314
							variant	0.429	1	0.513	P_MR	P_BS	0.006	0.007	0.900	0.419	0.419
	variant	6.801e-5	1	6.801e-5	16.785	0.015					B_MR	B_POT	0.007	0.002	4.097	0.015*	0.015
	Residuals	1.621e-5	4	4.052e-6													
	variant	6.660e-5	1	6.660e-5	9.023	0.057					O_MR	O_POT	0.007	0.002	3.004	0.057	0.057
	Residuals	2.214e-5	3	7.382e-6													
	variant	3.082e-6	1	3.082e-6	0.040	0.850					P_MR	P_POT	0.001	0.007	0.201	0.850	0.850
	Residuals	3.044e-4	4	7.610e-5													
	variant	1.009e-4	1	1.009e-4	32.694	0.005					B_POT	SUB	0.008	0.001	5.718	0.005*	0.005
	Residuals	1.234e-5	4	3.085e-6													
	variant	2.540e-4	1	2.540e-4	34.666	0.010					O_POT	SUB	0.015	0.002	5.888	0.010*	0.010
	Residuals	2.199e-5	3	7.328e-6													
	variant	2.432e-4	1	2.432e-4	48.869	0.002					P_POT	SUB	0.013	0.002	6.991	0.002*	0.002
	Residuals	1.991e-5	4	4.977e-6													
Shannon diversity index	variant	1.119e-4	2	5.594e-5	1.110	0.389					B_MR	O_MR	-0.007	0.006	-1.219	0.485	0.805
	Residuals	3.023e-4	6	5.038e-5								P_MR	7.667e-4	0.006	0.132	0.990	1.000
											O_MR	P_MR	0.008	0.006	1.352	0.421	0.676
	variant	6.083e-5	2	3.041e-5	1.860	0.235					B_BS	O_BS	-0.003	0.003	-0.999	0.604	1.000
	Residuals	9.813e-5	6	1.635e-5								P_BS	-0.006	0.003	-1.928	0.211	0.306
											O_BS	P_BS	-0.003	0.003	-0.929	0.644	1.000
	variant	0.013	1	0.013	0.587	0.486					B_MR	B_BS	-0.092	0.120	-0.766	0.486	0.486
	Residuals	0.086	4	0.021													
	variant	0.005	1	0.005	0.172	0.700					O_MR	O_BS	0.056	0.134	0.415	0.700	0.700
	Residuals	0.108	4	0.027													
	variant	0.044	1	0.044	0.206	0.673					P_MR	P_BS	0.170	0.375	0.454	0.673	0.673
	Residuals	0.844	4	0.211													
	variant	0.156	1	0.156	8.061	0.047					B_MR	B_POT	0.322	0.113	2.839	0.047*	0.047
	Residuals	0.077	4	0.019													
	variant	0.464	1	0.464	11.031	0.045					O_MR	O_POT	0.622	0.187	3.321	0.045*	0.045
	Residuals	0.126	3	0.042													
							variant	1.190	1	0.275	P_MR	P_POT	0.613	0.376	1.629	0.179	0.179
	variant	0.040	1	0.040	5.853	0.073					B_POT	SUB	0.164	0.068	2.419	0.073	0.073
	Residuals	0.027	4	0.007													
	variant	0.235	1	0.235	6.840	0.079					O_POT	SUB	0.442	0.169	2.615	0.079	0.079
	Residuals	0.103	3	0.034													
	variant	0.054	1	0.054	6.629	0.062					P_POT	SUB	0.189	0.073	2.575	0.062	0.062
	Residuals	0.032	4	0.008													
	variant	0.503	2	0.252	1.644	0.270					B_MR	O_MR	-0.578	0.319	-1.810	0.245	0.361
	Residuals	0.918	6	0.153								P_MR	-0.316	0.319	-0.989	0.610	1.000
											O_MR	P_MR	0.262	0.319	0.821	0.705	1.000
	variant	0.882	2	0.441	22.065	0.002					B_BS	O_BS	-0.726	0.115	-6.285	0.002*	0.002
	Residuals	0.120	6	0.020								P_BS	-0.578	0.115	-5.006	0.006*	0.007
											O_BS	P_BS	0.148	0.115	1.279	0.456	0.744

Berger-Parker index	variant	1.261e -4	1	1.261e -4	2.254	0.208					B_MR	B_BS	-0.009	0.006	-1.501	0.208	0.208
	Residuals	2.239e -4	4	5.597e -5													
	variant	0.003	1	0.003	2.475	0.191					O_MR	O_BS	0.041	0.026	1.573	0.191	0.191
	Residuals	0.004	4	0.001													
							variant	1.190	1	0.275	P_MR	P_BS	-0.035	0.032	-1.089	0.337	0.337
	variant	5.022e -4	1	5.022e -4	7.596	0.051					B_MR	B_POT	-0.018	0.007	-2.756	0.051	0.051
	Residuals	2.644e -4	4	6.611e -5													
	variant	6.446e -4	1	6.446e -4	7.516	0.071					O_MR	O_POT	-0.023	0.008	-2.742	0.071	0.071
	Residuals	2.573e -4	3	8.576e -5													
	variant	7.457e -4	1	7.457e -4	0.483	0.525					P_MR	P_POT	0.022	0.032	0.695	0.525	0.525
	Residuals	0.006	4	0.002													
	variant	0.002	1	0.002	43.058	0.003					B_POT	SUB	-0.041	0.006	-6.562	0.003*	0.003
	Residuals	2.310e -4	4	5.775e -5													
	variant	0.005	1	0.005	68.884	0.004					O_POT	SUB	-0.061	0.007	-8.300	0.004*	0.004
	Residuals	1.969e -4	3	6.563e -5													
	variant	0.006	1	0.006	79.542	< .001					P_POT	SUB	-0.066	0.007	-8.919	< .001*	< .001
	Residuals	3.246e -4	4	8.115e -5													
							variant	3.289	2	0.193	B_MR	O_MR	0.026	0.027	0.956	0.628	1.000
												P_MR	-0.016	0.027	-0.588	0.831	1.000
											O_MR	P_MR	-0.041	0.027	-1.544	0.338	0.521
							variant	2.489	2	0.288	B_BS	O_BS	-0.025	0.021	-1.151	0.521	0.881
												P_BS	0.010	0.021	0.479	0.883	1.000
											O_BS	P_BS	0.035	0.021	1.630	0.305	0.463

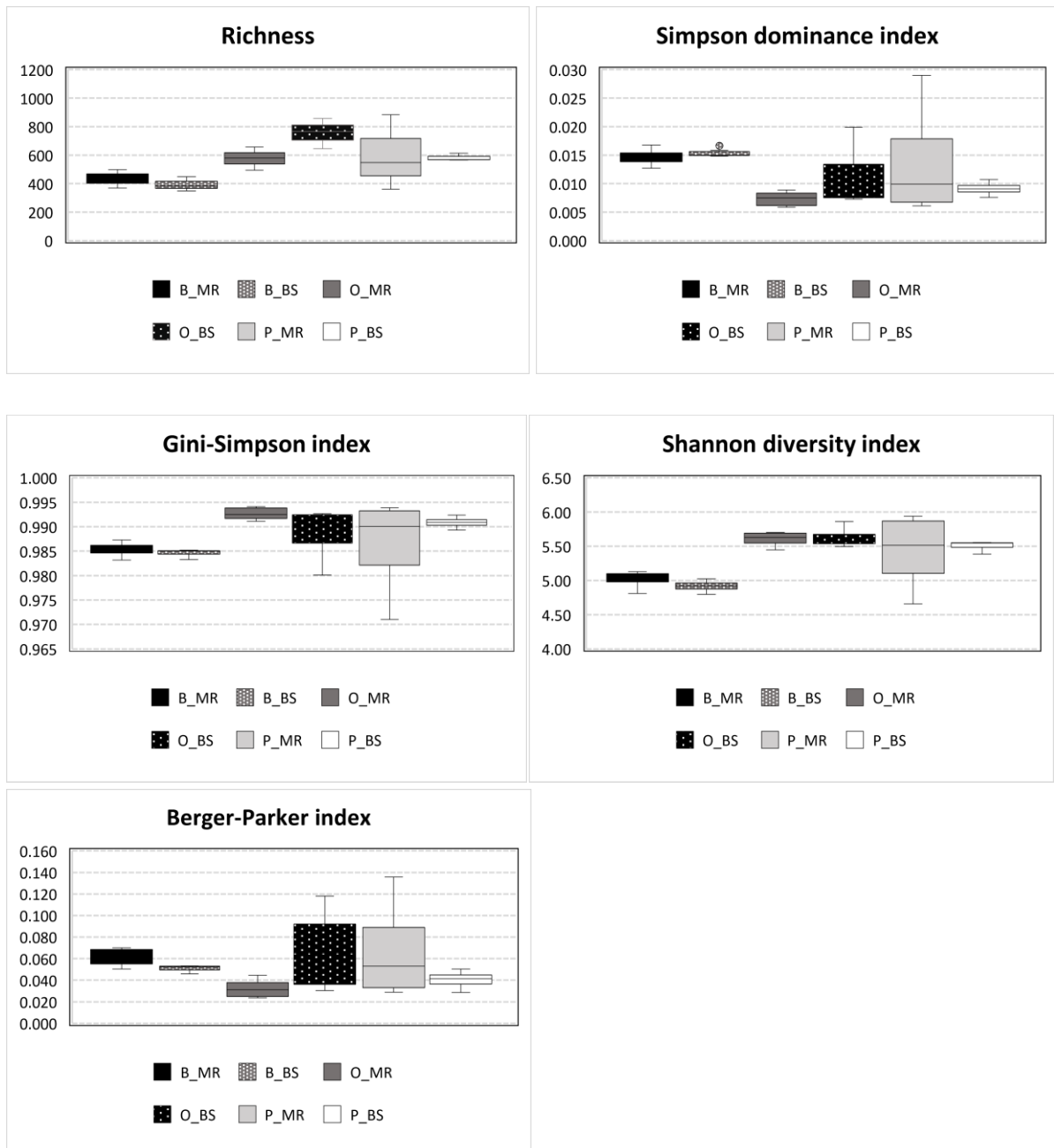


Figure S5. Pairwise comparisons of bacterial community diversity indices based on ASVs between field plant mycorrhizosphere and corresponding bulk soil. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil. Brackets indicate significant differences ($p < 0.05$).

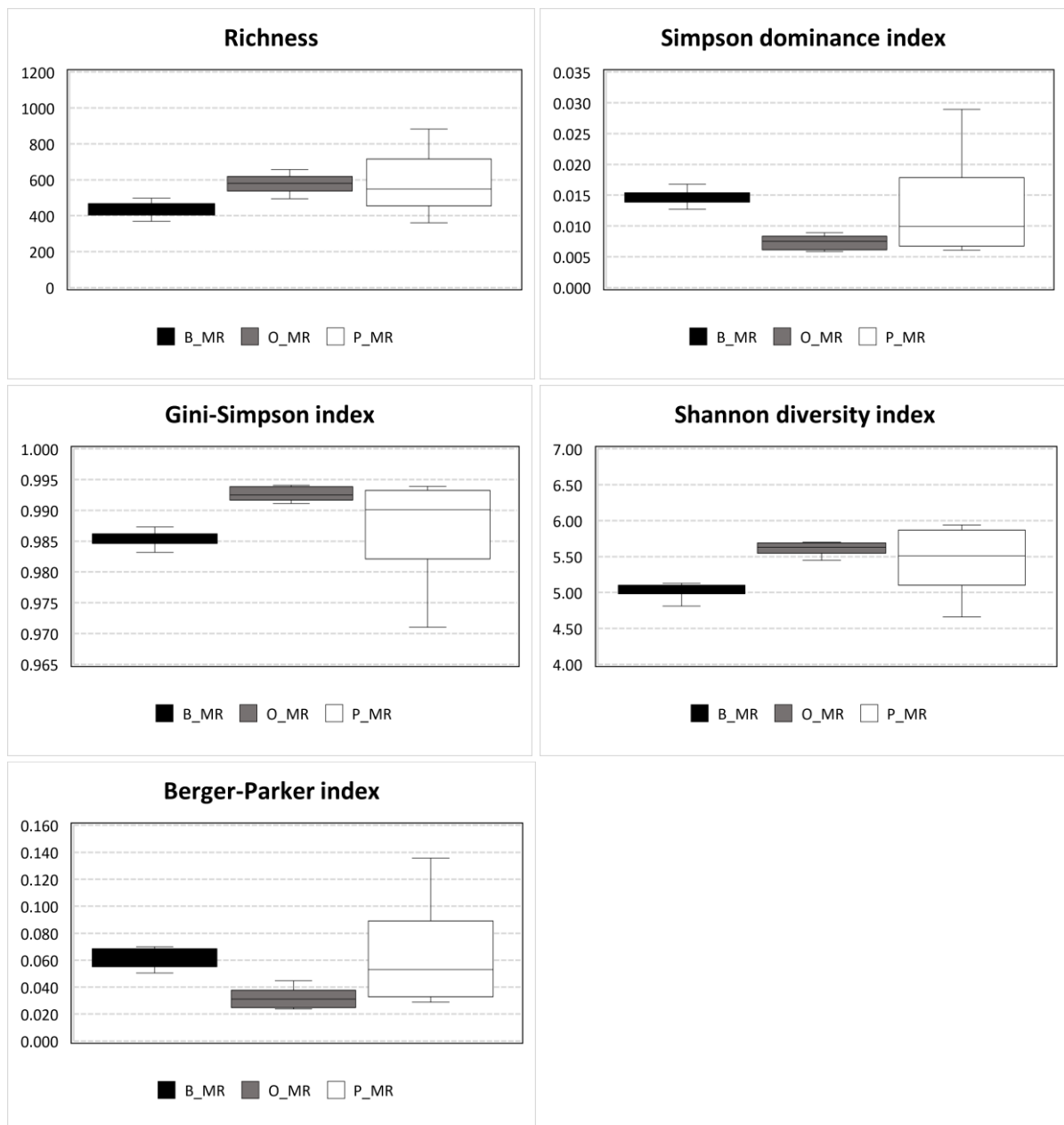


Figure S6. Pairwise comparisons of bacterial community diversity indices based on ASVs between field plant mycorrhizospheres. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant. Brackets indicate significant differences ($p < 0.05$).

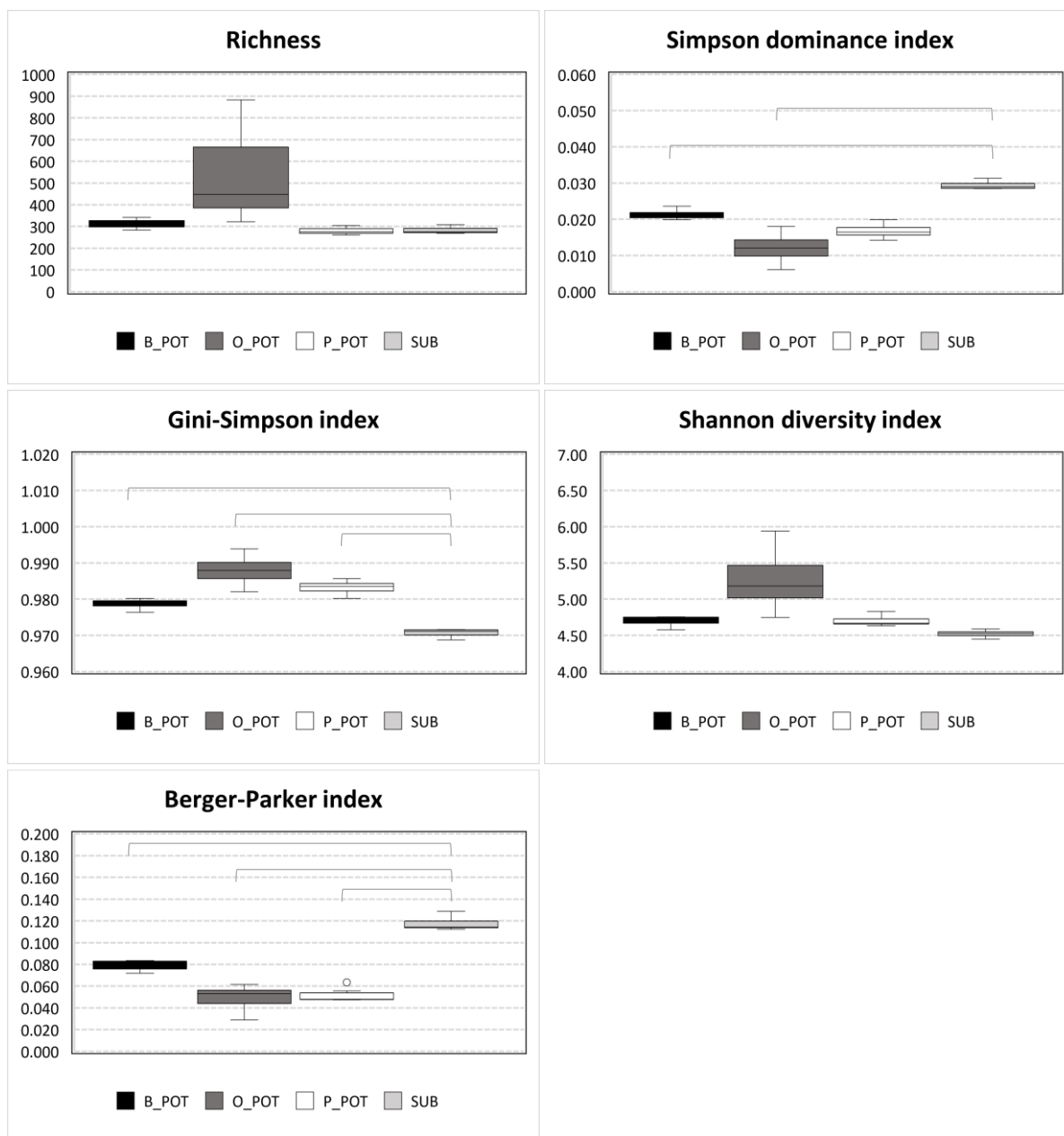


Figure S7. Pairwise comparisons of bacterial community diversity indices based on ASVs between pot plant mycorrhizosphere and control pot substrate. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Brackets indicate significant differences ($p < 0.05$).

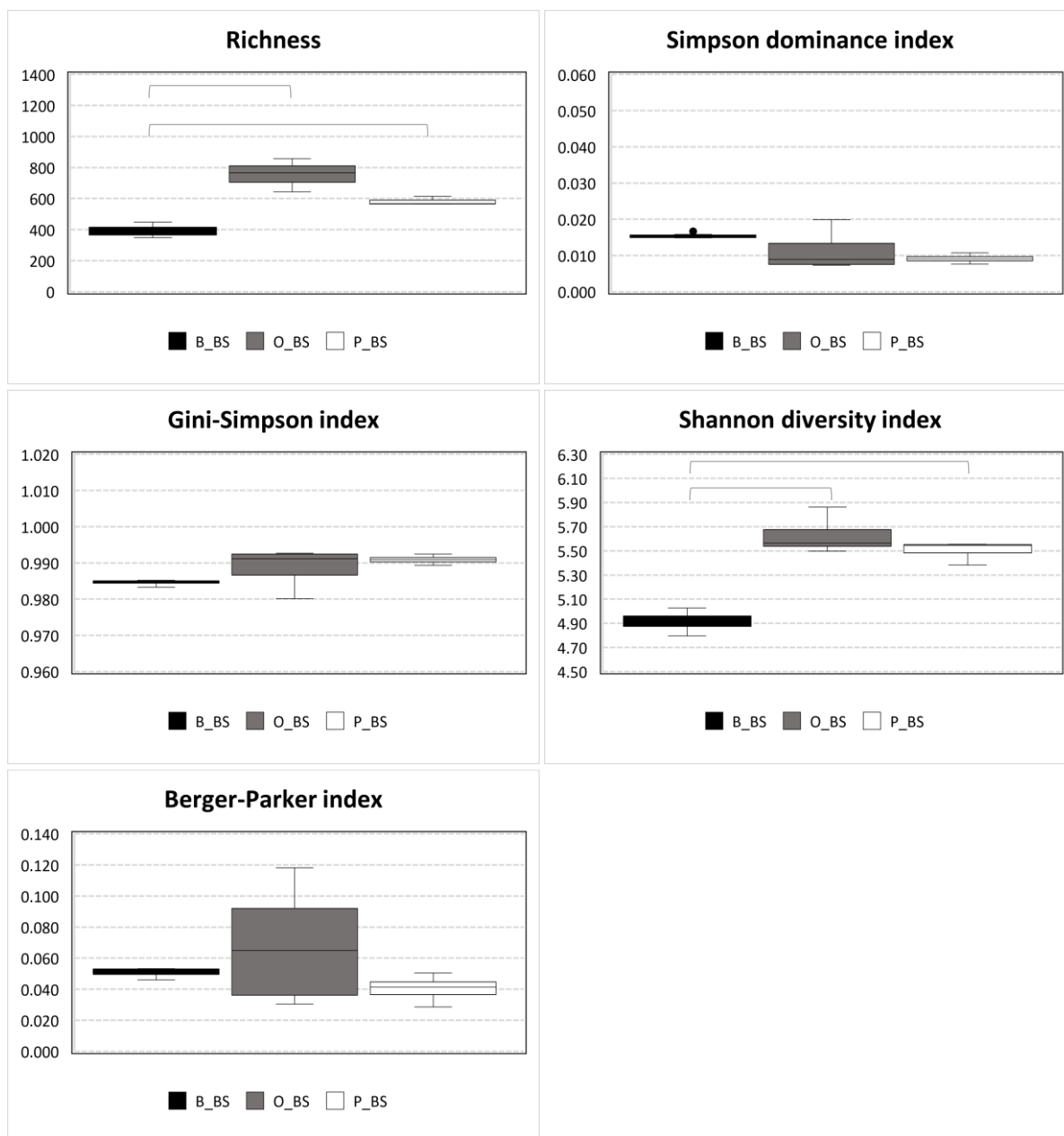


Figure S8. Pairwise comparisons of bacterial community diversity indices based on ASVs between bulk soil at different sampling sites. B – birch, O – oak, P – pine; BS – bulk soil. Brackets indicate significant differences ($p < 0.05$).

Table S29. Indices of similarity between bacterial communities determined for trees within variants of the experiment. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

Index	Estimate	s.e.	95%Lower	95%Upper
B_MR				
Sorensen	0.572	0.002	0.569	0.575
Jaccard	0.308	0.001	0.305	0.311
Bray-Curtis	0.508	0.001	0.505	0.511
O_MR				
Sorensen	0.317	0.002	0.314	0.321
Jaccard	0.134	0.001	0.132	0.136
Bray-Curtis	0.265	0.001	0.262	0.268
P_MR				
Sorensen	0.222	0.002	0.218	0.226
Jaccard	0.087	0.001	0.085	0.089
Bray-Curtis	0.144	0.001	0.142	0.146
B_BS				
Sorensen	0.541	0.002	0.537	0.546
Jaccard	0.282	0.002	0.2785	0.286
Bray-Curtis	0.520	0.002	0.518	0.523
O_BS				
Sorensen	0.413	0.001	0.411	0.415
Jaccard	0.190	0.001	0.189	0.191
Bray-Curtis	0.351	0.001	0.348	0.353
P_BS				
Sorensen	0.242	0.001	0.240	0.244
Jaccard	0.096	0.001	0.095	0.097
Bray-Curtis	0.199	0.001	0.197	0.202
B_POT				
Sorensen	0.756	0.002	0.753	0.760
Jaccard	0.509	0.003	0.504	0.514
Bray-Curtis	0.784	0.001	0.781	0.786
O_POT				
Sorensen	0.535	0.001	0.533	0.538
Jaccard	0.365	0.001	0.363	0.368
Bray-Curtis	0.614	0.002	0.609	0.619
P_POT				
Sorensen	0.673	0.002	0.669	0.678
Jaccard	0.407	0.002	0.403	0.412
Bray-Curtis	0.708	0.002	0.704	0.712
SUB				
Sorensen	0.729	0.003	0.723	0.735
Jaccard	0.473	0.004	0.465	0.480
Bray-Curtis	0.814	0.002	0.811	0.817

Table S30. ANOSIM test output of pairwise comparisons between field plant mycorrhizospheres based on the relative abundance of the most representative bacterial taxa.

Mean rank within groups	11
Mean rank between groups	21
R	0.5556
p	0.0135

Table S31. ANOSIM p-values of pairwise comparisons between field plant mycorrhizospheres based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant.

	B_MR	O_MR	P_MR
B_MR			
O_MR	0.10		
P_MR	0.10	0.19	

Table S32. PERMANOVA test output of pairwise comparisons between field plant mycorrhizospheres based on the relative abundance of the most representative bacterial taxa.

Total sum of squares	0.4383
Within-group sum of squares	0.1884
F	3.979
p	0.0196

Table S33. PERMANOVA p-values of pairwise comparisons between field plant mycorrhizospheres based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant.

	B_MR	O_MR	P_MR
B_MR			
O_MR	0.11		
P_MR	0.10	0.60	

Table S34. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between field plant mycorrhizospheres in relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Taxon	ANOVA						Kruskal-Wallis				Post Hoc comparisons						
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variant of comparisons		Mean Difference	SE	t	p tukey	p bonf
Acidobacteriia	variant	498.611	2	249.306	7.065	0.026					B_MR	O_MR	16.156	4.850	3.331	0.036*	0.047
	Residuals	211.739	6	35.290								P_MR	15.395	4.850	3.174	0.044*	0.058
											O_MR	P_MR	-0.761	4.850	-0.157	0.987	1.000
Blastocatellia (Subgroup 4)	variant	7.947	1	7.947	1.608	0.274					O_MR	P_MR	2.302	1.815	1.268	0.274	0.274
	Residuals	19.772	4	4.943													
Holophagae							variant	0.429	1	0.513	O_MR	P_MR	-1.493	1.456	-1.026	0.363	0.363
Acidimicrobiia	variant	0.067	2	0.034	0.580	0.589					B_MR	O_MR	-0.036	0.196	-0.181	0.982	1.000
	Residuals	0.347	6	0.058								P_MR	-0.198	0.196	-1.010	0.598	1.000
											O_MR	P_MR	-0.163	0.196	-0.829	0.700	1.000
Actinobacteria	variant	22.414	2	11.207	3.090	0.120					B_MR	O_MR	0.077	1.555	0.049	0.999	1.000
	Residuals	21.764	6	3.627								P_MR	-3.309	1.555	-2.128	0.164	0.232
											O_MR	P_MR	-3.385	1.555	-2.177	0.154	0.217
Thermoleophilia							variant	5.067	2	0.079	B_MR	O_MR	-1.950	1.035	-1.885	0.223	0.325
												P_MR	-0.742	1.035	-0.718	0.763	1.000
											O_MR	P_MR	1.207	1.035	1.167	0.512	0.862
Bacteroidia							variant	0.800	2	0.670	B_MR	O_MR	-4.817	2.568	-1.876	0.225	0.329
												P_MR	-4.158	2.568	-1.619	0.309	0.470
											O_MR	P_MR	0.659	2.568	0.257	0.965	1.000
Anaerolineae							variant	5.956	2	0.051	B_MR	O_MR	-0.561	0.838	-0.670	0.789	1.000
												P_MR	-1.954	0.838	-2.332	0.126	0.175
											O_MR	P_MR	-1.393	0.838	-1.662	0.293	0.443
Chloroflexia							variant	5.067	2	0.079	B_MR	O_MR	-0.070	0.900	-0.078	0.997	1.000
												P_MR	-1.271	0.900	-1.412	0.393	0.623
											O_MR	P_MR	-1.202	0.900	-1.334	0.429	0.691
Ktedonobacteria							variant	5.067	2	0.079	B_MR	O_MR	10.881	4.731	2.300	0.132	0.183
												P_MR	5.879	4.731	1.243	0.474	0.781
											O_MR	P_MR	-5.003	4.731	-1.058	0.572	0.993
Oxyphotobacteria							variant	1.156	2	0.561	B_MR	O_MR	1.678	1.258	1.334	0.429	0.692
												P_MR	2.017	1.258	1.603	0.315	0.480
											O_MR	P_MR	0.338	1.258	0.269	0.961	1.000
Gemmatimonadetes							variant	5.422	2	0.066	B_MR	O_MR	-1.909	0.973	-1.962	0.203	0.292
												P_MR	-1.099	0.973	-1.129	0.533	0.906
											O_MR	P_MR	0.810	0.973	0.832	0.698	1.000
Phycisphaerae	variant	62.964	2	31.482	6.381	0.033					B_MR	O_MR	5.835	1.814	3.218	0.042*	0.055
	Residuals	29.601	6	4.934								P_MR	5.355	1.814	2.953	0.058	0.077
											O_MR	P_MR	-0.480	1.814	-0.265	0.962	1.000
Planctomycetacea	variant	6.264	2	3.132	1.108	0.389					B_MR	O_MR	0.198	1.373	0.144	0.989	1.000
	Residuals	16.956	6	2.826								P_MR	1.860	1.373	1.355	0.419	0.672
											O_MR	P_MR	1.662	1.373	1.211	0.490	0.814
Alphaproteobacteria	variant	112.277	2	56.139	3.483	0.099					B_MR	O_MR	-8.617	3.278	-2.629	0.087	0.117
	Residuals	96.712	6	16.119								P_MR	-4.976	3.278	-1.518	0.348	0.540
											O_MR	P_MR	3.642	3.278	1.111	0.542	0.927
Deltaproteobacteria	variant	1.663	2	0.831	1.122	0.386					B_MR	O_MR	-1.051	0.703	-1.495	0.357	0.556

	Residuals	4.447	6	0.741							P_MR	-0.474	0.703	-0.674	0.786	1.000
										O_MR	P_MR	0.577	0.703	0.821	0.705	1.000
<i>Gammaproteobacteria</i>	variant	25.562	2	12.781	5.544	0.043				B_MR	O_MR	-3.413	1.240	-2.753	0.074	0.099
	Residuals	13.832	6	2.305							P_MR	-3.718	1.240	-2.999	0.054	0.072
										O_MR	P_MR	-0.305	1.240	-0.246	0.967	1.000
<i>Verrucomicrobiae</i>	variant	72.681	2	36.340	4.856	0.056				B_MR	O_MR	-6.717	2.234	-3.007	0.054	0.071
	Residuals	44.898	6	7.483							P_MR	-1.776	2.234	-0.795	0.719	1.000
										O_MR	P_MR	4.941	2.234	2.212	0.147	0.207

Table S35. SIMPER analysis demonstrating contribution (%) of the most abundant bacterial classes to Bray-Curtis dissimilarity between field plant mycorrhizospheres. Only bacterial classes with contribution higher than 10% are included in table. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant.

	B_MR	O_MR	P_MR
B_MR		Acidobacteriia (22.3%) Ktedonobacteria (14.94%) Alphaproteobacteria (11.87%)	Acidobacteriia (22.16%) Ktedonobacteria (13.42%)
O_MR			Acidobacteriia (12.63%) Verrucomicrobiae (11.64%) Ktedonobacteria (11.57%) Alphaproteobacteria (11.2%)
P_MR			

Table S36. ANOSIM test output of pairwise comparisons between bulk soil at different sampling sites based on the relative abundance of the most representative bacterial taxa.

Mean rank within groups	8.333
Mean rank between groups	21.89
R	0.7531
P	0.0165

Table S37. ANOSIM p-values of pairwise comparisons between bulk soil at different sampling sites based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; BS – bulk soil.

	B_BS	O_BS	P_BS
B_BS			
O_BS	0.10		
P_BS	0.09	0.50	

Table S38. PERMANOVA test output of pairwise comparisons between bulk soil at different sampling sites based on the relative abundance of the most representative bacterial taxa.

Total sum of squares	0.5634
Within-group sum of squares	0.1544
F	7.945
P	0.0112

Table S39. PERMANOVA p-values of pairwise comparisons between bulk soil at different sampling sites based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; BS – bulk soil.

	B_BS	O_BS	P_BS
B_BS			
O_BS	0.10		
P_BS	0.10	0.30	

Table S40. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between bulk soil at different sampling sites in relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; BS – bulk soil. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Taxon	ANOVA						Kruskal-Wallis				Post Hoc comparisons						
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variant of comparisons		Mean Difference	SE	t	p tukey	p bonf
Acidobacteriia							variant	5.422	2	0.066	B_BS	O_BS	18.475	3.264	5.661	0.003	0.004*
												P_BS	16.345	3.264	5.008	0.006	0.007*
												O_BS	P_BS	-2.130	3.264	-0.652	0.798
Blastocatellia (Subgroup 4)	variant	74.588	1	74.588	4.314	0.106					O_BS	P_BS	7.052	3.395	2.077	0.106	0.106
	Residuals	69.158	4	17.290													
Holophagae							variant	0.048	1	0.827	O_BS	P_BS	-1.693	1.878	-0.901	0.418	0.418
Acidimicrobiia	variant	0.051	2	0.025	0.745	0.514					B_BS	O_BS	-0.043	0.150	-0.284	0.957	1.000
	Residuals	0.204	6	0.034								P_BS	0.133	0.150	0.886	0.668	1.000
												O_BS	P_BS	0.176	0.150	1.170	0.511
Actinobacteria	variant	7.479	2	3.739	8.086	0.020					B_BS	O_BS	-0.079	0.555	-0.142	0.989	1.000
	Residuals	2.775	6	0.462								P_BS	-1.972	0.555	-3.551	0.028*	0.036
												O_BS	P_BS	-1.893	0.555	-3.409	0.033*
Thermoleophilia	variant	1.714	2	0.857	7.274	0.025					B_BS	O_BS	-1.068	0.280	-3.811	0.021*	0.027
	Residuals	0.707	6	0.118								P_BS	-0.495	0.280	-1.766	0.258	0.384
												O_BS	P_BS	0.573	0.280	2.045	0.182
Bacteroidia	variant	49.770	2	24.885	2.430	0.169					B_BS	O_BS	-2.563	2.613	-0.981	0.614	1.000
	Residuals	61.442	6	10.240								P_BS	-5.749	2.613	-2.200	0.150	0.210
												O_BS	P_BS	-3.186	2.613	-1.219	0.485
Anaerolineae							variant	5.600	2	0.061	B_BS	O_BS	-0.742	0.799	-0.929	0.644	1.000
												P_BS	-1.528	0.799	-1.913	0.215	0.313
												O_BS	P_BS	-0.786	0.799	-0.984	0.612
Chloroflexia							variant	3.822	2	0.148	B_BS	O_BS	0.396	0.352	1.126	0.534	0.910
												P_BS	-0.333	0.352	-0.947	0.633	1.000
												O_BS	P_BS	-0.730	0.352	-2.073	0.176
Ktedonobacteria	variant	438.503	2	219.252	17.032	0.003					B_BS	O_BS	16.251	2.929	5.548	0.003*	0.004
	Residuals	77.237	6	12.873								P_BS	12.727	2.929	4.344	0.011*	0.015
												O_BS	P_BS	-3.525	2.929	-1.203	0.494
Oxyphotobacteria							variant	0.356	2	0.837	B_BS	O_BS	0.164	1.149	0.143	0.989	1.000
												P_BS	-0.926	1.149	-0.805	0.714	1.000
												O_BS	P_BS	-1.090	1.149	-0.948	0.633
Gemmatimonadetes	variant	1.699	2	0.850	0.298	0.752					B_BS	O_BS	0.280	1.378	0.203	0.978	1.000
	Residuals	17.086	6	2.848								P_BS	-0.749	1.378	-0.544	0.853	1.000
												O_BS	P_BS	-1.029	1.378	-0.747	0.746
Phycisphaerae	variant	44.737	2	22.369	21.133	0.002					B_BS	O_BS	4.815	0.840	5.732	0.003*	0.004
	Residuals	6.351	6	1.058								P_BS	4.639	0.840	5.522	0.004*	0.004
												O_BS	P_BS	-0.176	0.840	-0.210	0.976
Planctomycetacea	variant	12.995	2	6.497	5.237	0.048					B_BS	O_BS	1.340	0.909	1.473	0.367	0.574
	Residuals	7.444	6	1.241								P_BS	2.939	0.909	3.232	0.041*	0.054
												O_BS	P_BS	1.600	0.909	1.759	0.261
Alphaproteobacteria	variant	395.937	2	197.969	5.695	0.041					B_BS	O_BS	-12.735	4.814	-2.646	0.085	0.115
	Residuals	208.565	6	34.761								P_BS	-15.104	4.814	-3.138	0.046*	0.060
												O_BS	P_BS	-2.369	4.814	-0.492	0.878
Deltaproteobacteria	variant	3.622	2	1.811	7.716	0.022					B_BS	O_BS	-1.182	0.396	-2.987	0.055	0.073

	Residuals	1.408	6	0.235								P_BS	0.283	0.396	0.716	0.764	1.000
											O_BS	P_BS	1.465	0.396	3.703	0.023*	0.030
<i>Gammaproteobacteria</i>	variant	58.007	2	29.003	17.653	0.003					B_BS	O_BS	-5.396	1.047	-5.156	0.005*	0.006
	Residuals	9.858	6	1.643								P_BS	-5.374	1.047	-5.135	0.005*	0.006
											O_BS	P_BS	0.022	1.047	0.021	1.000	1.000
<i>Verrucomicrobiae</i>	variant	62.821	2	31.410	5.054	0.052					B_BS	O_BS	-6.371	2.035	-3.130	0.046*	0.061
	Residuals	37.286	6	6.214								P_BS	-2.203	2.035	-1.083	0.558	0.962
											O_BS	P_BS	4.168	2.035	2.048	0.182	0.260

Table S41. SIMPER analysis demonstrating contribution (%) of the most abundant bacterial classes to Bray-Curtis dissimilarity between bulk soil at different sampling sites. Only bacterial classes with contribution higher than 10% are included in table. B – birch, O – oak, P – pine; BS – bulk soil.

	B_BS	O_BS	P_BS
B_BS		Acidobacteriia (21.66%) Ktedonobacteria (19.14%) Alphaproteobacteria (15.11%) Blastocatellia (Subgr.4) (10.39%)	Acidobacteriia (20.77%) Alphaproteobacteria (19.21%) Ktedonobacteria (16.28%)
O_BS			Blastocatellia (Subgr.4) (16.18%) Alphaproteobacteria (14.39%) Bacteroidia (10.14%)
P_BS			

Table S42. ANOSIM test output of pairwise comparisons between field plant mycorrhizospheres and bulk soil based on the relative abundance of the most representative bacterial taxa.

Mean rank within groups	43.39
Mean rank between groups	81.48
R	0.4979
p	0.0007

Table S43. ANOSIM p-values of pairwise comparisons between field plant mycorrhizospheres and bulk soil based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

	B_MR	B_BS	O_MR	O_BS	P_MR	P_BS
B_MR						
B_BS	0.49					
O_MR	0.10	0.10				
O_BS	0.10	0.10	0.51			
P_MR	0.10	0.10	0.20	0.41		
P_BS	0.10	0.10	0.10	0.50	0.80	

Table S44. PERMANOVA test output of pairwise comparisons between field plant mycorrhizospheres and bulk soil based on the relative abundance of the most representative bacterial taxa.

Total sum of squares	1.02
Within-group sum of squares	0.34
F	4.74
P	0.001

Table S45. PERMANOVA p-values of pairwise comparisons between field plant mycorrhizospheres and bulk soil based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

	B_MR	B_BS	O_MR	O_BS	P_MR	P_BS
B_MR						
B_BS	0.31					
O_MR	0.10	0.10				
O_BS	0.10	0.09	0.70			
P_MR	0.10	0.10	0.61	0.19		
P_BS	0.11	0.11	0.51	0.30	0.80	

Table S46. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between field birch mycorrhizosphere and bulk soil in relative abundance of the most representative bacterial taxa. B – birch; MR – mycorrhizosphere of field plant, BS – bulk soil. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

[illegible]

Table S47. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between field oak mycorrhizosphere and bulk soil in relative abundance of the most representative bacterial taxa. O – birch; MR – mycorrhizosphere of field plant, BS – bulk soil. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

[illegible]

Table S48. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between field pine mycorrhizosphere and bulk soil in relative abundance of the most representative bacterial taxa. P – birch; MR – mycorrhizosphere of field plant, BS – bulk soil. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

[illegible]

Table S49. SIMPER analysis demonstrating contribution (%) of the most abundant bacterial classes to Bray-Curtis dissimilarity between field plant mycorrhizospheres and bulk soil. Only bacterial classes with contribution higher than 10% are included in table. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

	B_BS	O_BS	P_BS
B_MR	Alphaproteobacteria (18.58%) Ktedonobacteria (16.64%) Acidobacteriia (14.62%) Bacteroidia (12.17%)		
O_MR		Blastocatellia (subgr.4) (20.07%) Alphaproteobacteria (14.11%) Verrucomicrobiae (11.33%)	
P_MR			Alphaproteobacteria (14.32%) Ktedonobacteria (13.14%) Acidobacteriia (11.77%)

Table S50. ANOSIM test output of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on the relative abundance of the most representative bacterial taxa.

Mean rank within groups	12.1
Mean rank between groups	31.53
R	0.7067
p	0.001

Table S51. ANOSIM p-values of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

	B_POT	O_POT	P_POT	SUB
B_POT				
O_POT	0.20			
P_POT	0.40	0.20		
SUB	0.10	0.10	0.11	

Table S52. PERMANOVA test output of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on the relative abundance of the most representative bacterial taxa.

Total sum of squares	0.1508
Within-group sum of squares	0.03812
F	6.895
p	0.0008

Table S53. PERMANOVA p-values of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

	B_POT	O_POT	P_POT	SUB
B_POT				
O_POT	0.10			
P_POT	0.30	0.20		
SUB	0.10	0.10	0.10	

Table S54. SIMPER analysis demonstrating contribution (%) of the most abundant bacterial classes to Bray-Curtis dissimilarity between pot plant mycorrhizospheres and control pot substrate. Only bacterial classes with contribution higher than 10% are included in table. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

	B_POT	O_POT	P_POT	SUB
B_POT				
O_POT	Alphaproteobacteria (24.07%) Acidobacteriia (17.24%) Oxyphotobacteria (12.47%)			
P_POT	Bacteroidia (17.48%) Oxyphotobacteria (15.01%) Ktedonobacteria (13.53%) Gammaproteobacteria (11.99%)	Alphaproteobacteria (15.64%) Phycisphaerae (14.46%) Acidobacteriia (13.14%) Bacteroidia (11.42%)		
SUB	Acidobacteriia (19.04%) Ktedonobacteria (17.77%) Phycisphaerae (13.21%) Thermoleophilia (12.3%)	Ktedonobacteria (17.77%) Alphaproteobacteria (14.22%) Thermoleophilia (10.47%) Acidimicrobiia (10.45%)	Ktedonobacteria (17.77%) Phycisphaerae (13.21%) Bacteroidia (13.75%) Acidimicrobiia (11.7%) Thermoleophilia (11.63%) Gammaproteobacteria (11.24%)	

Table S55. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between pot birch mycorrhizosphere and control pot substrate in relative abundance of the most representative bacterial taxa. B – birch; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Taxon	ANOVA						Kruskal-Wallis				Post Hoc comparisons					
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variant of comparisons	Mean Difference	SE	t	p tukey	p bonf
<i>Acidobacteriia</i>							variant	3.857	1	0.050	B_POT SUB	6.303	0.222	28.366	< .001	< .001*
<i>Acidimicrobiia</i>	variant	2.802	1	2.802	29.927	0.005					B_POT SUB	-1.367	0.250	-5.471	0.005*	0.005
	Residuals	0.374	4	0.094												
<i>Actinobacteria</i>	variant	0.104	1	0.104	0.212	0.669					B_POT SUB	-0.263	0.572	-0.460	0.669	0.669
	Residuals	1.966	4	0.492												
<i>Thermoleophilia</i>							variant	3.857	1	0.050	B_POT SUB	-4.070	0.517	-7.868	0.001	0.001*
<i>Bacteroidia</i>	variant	9.551	1	9.551	8.166	0.046					B_POT SUB	2.523	0.883	2.858	0.046*	0.046
	Residuals	4.679	4	1.170												
<i>Chloroflexia</i>							variant	3.857	1	0.050	B_POT SUB	-1.057	0.082	-12.835	< .001	< .001*
<i>Ktedonobacteria</i>	variant	52.156	1	52.156	10.307	0.033					B_POT SUB	-5.897	1.837	-3.210	0.033*	0.033
	Residuals	20.242	4	5.060												
<i>Oxyphotobacteria</i>	variant	2.614	1	2.614	0.337	0.592					B_POT SUB	1.320	2.272	0.581	0.592	0.592
	Residuals	30.978	4	7.744												
<i>Gemmatimonadetes</i>	variant	0.042	1	0.042	17.986	0.013					B_POT SUB	0.167	0.039	4.241	0.013*	0.013
	Residuals	0.009	4	0.002												
<i>Phycisphaerae</i>	variant	28.733	1	28.733	80.289	< .001					B_POT SUB	4.377	0.488	8.960	< .001*	< .001
	Residuals	1.431	4	0.358												
<i>Planctomycetacea</i>	variant	8.167e -4	1	8.167e -4	0.037	0.856					B_POT SUB	0.023	0.121	0.193	0.856	0.856
	Residuals	0.088	4	0.022												
<i>Alphaproteobacteria</i>	variant	2.535	1	2.535	4.315	0.106					B_POT SUB	1.300	0.626	2.077	0.106	0.106
	Residuals	2.350	4	0.587												
<i>Deltaproteobacteria</i>	variant	0.027	1	0.027	5.063	0.088					B_POT SUB	0.133	0.059	2.250	0.088	0.088
	Residuals	0.021	4	0.005												
<i>Gammaproteobacteria</i>	variant	8.857	1	8.857	24.607	0.008					B_POT SUB	2.430	0.490	4.961	0.008*	0.008

Table S56. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between pot oak mycorrhizosphere and control pot substrate in relative abundance of the most representative bacterial taxa. O – oak; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Taxon	ANOVA						Kruskal-Wallis				Post Hoc comparisons						
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variant of comparisons		Mean Difference	SE	t	p tukey	p bonf
Acidobacteriia							variant	0.000	1	1.000	O_POT	SUB	2.785	2.699	1.032	0.378	0.378
Acidimicrobiia	variant	1.387	1	1.387	8.192	0.064					O_POT	SUB	-1.075	0.376	-2.862	0.064	0.064
	Residuals	0.508	3	0.169													
Actinobacteria	variant	2.391	1	2.391	10.881	0.046					O_POT	SUB	1.412	0.428	3.299	0.046*	0.046
	Residuals	0.659	3	0.220													
Thermoleophilia	variant	15.566	1	15.566	29.036	0.013					O_POT	SUB	-3.602	0.668	-5.389	0.013*	0.013
	Residuals	1.608	3	0.536													
Bacteroidia	variant	10.668	1	10.668	10.623	0.047					O_POT	SUB	2.982	0.915	3.259	0.047*	0.047
	Residuals	3.013	3	1.004													
Chloroflexia	variant	0.954	1	0.954	72.800	0.003					O_POT	SUB	-0.892	0.105	-8.532	0.003*	0.003
	Residuals	0.039	3	0.013													
Ktedonobacteria	variant	62.410	1	62.410	10.151	0.050					O_POT	SUB	-7.212	2.263	-3.186	0.050	0.050
	Residuals	18.444	3	6.148													
Oxyphotobacteria	variant	0.555	1	0.555	0.330	0.606					O_POT	SUB	0.680	1.184	0.574	0.606	0.606
	Residuals	5.045	3	1.682													
Gemmatimonadetes							variant	2.193	1	0.139	O_POT	SUB	0.125	0.080	1.569	0.215	0.215
Phycisphaerae	variant	6.183	1	6.183	28.841	0.013					O_POT	SUB	2.270	0.423	5.370	0.013*	0.013
	Residuals	0.643	3	0.214													
Planctomycetacea	variant	0.254	1	0.254	2.536	0.210					O_POT	SUB	0.460	0.289	1.592	0.210	0.210
	Residuals	0.300	3	0.100													
Alphaproteobacteria	variant	48.973	1	48.973	8.970	0.058					O_POT	SUB	6.388	2.133	2.995	0.058	0.058
	Residuals	16.380	3	5.460													
Deltaproteobacteria	variant	0.084	1	0.084	136.654	0.001					O_POT	SUB	0.265	0.023	11.690	0.001*	0.001
	Residuals	0.002	3	6.167e -4													
Gammaproteobacteria	variant	8.175	1	8.175	8.203	0.064					O_POT	SUB	2.610	0.911	2.864	0.064	0.064
	Residuals	2.990	3	0.997													
Verrucomicrobiae							variant	0.093	1	0.761	O_POT	SUB	0.288	0.220	1.311	0.281	0.281

Table S57. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between pot pine mycorrhizosphere and control pot substrate in relative abundance of the most representative bacterial taxa. P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

[illegible]

Table S58. ANOSIM test output of pairwise comparisons between field plant mycorrhizosphere, pot plant mycorrhizosphere and control pot substrate based on the relative abundance of the most representative bacterial taxa.

Mean rank within groups	34.68
Mean rank between groups	102.3
R	0.7113
p	0.0001

Table S59. Matrix representing R-statistics of pairwise ANOSIM between field plant mycorrhizosphere, corresponding pot plant mycorrhizosphere and control pot substrate based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Arrows represent significant values ($p < 0.05$).

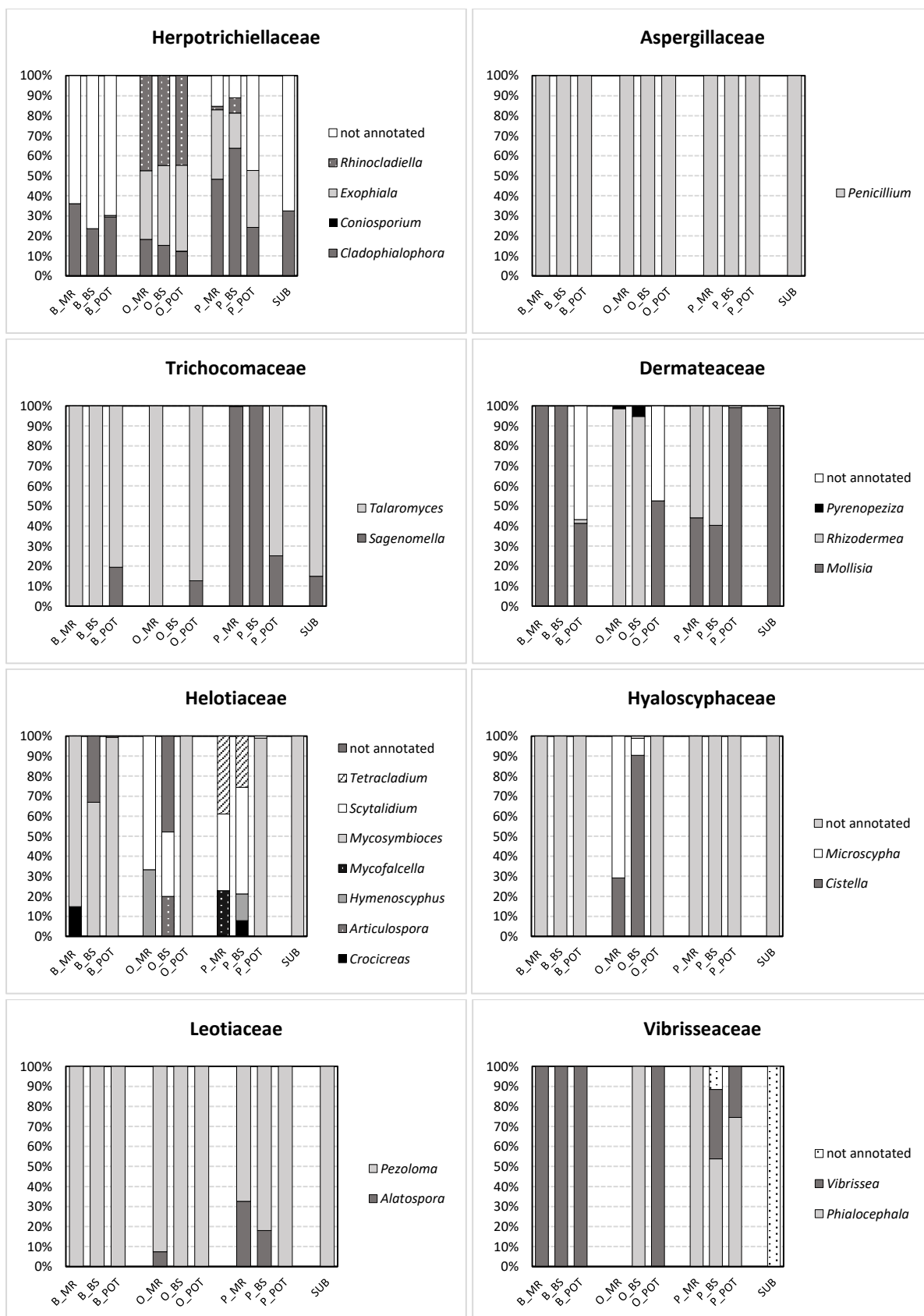
	B_MR	B_POT	O_MR	O_POT	P_MR	P_POT	SUB
B_POT	0.56						
O_POT			1				
P_POT					0.70		
SUB	1	1	1	1	0.81	1	

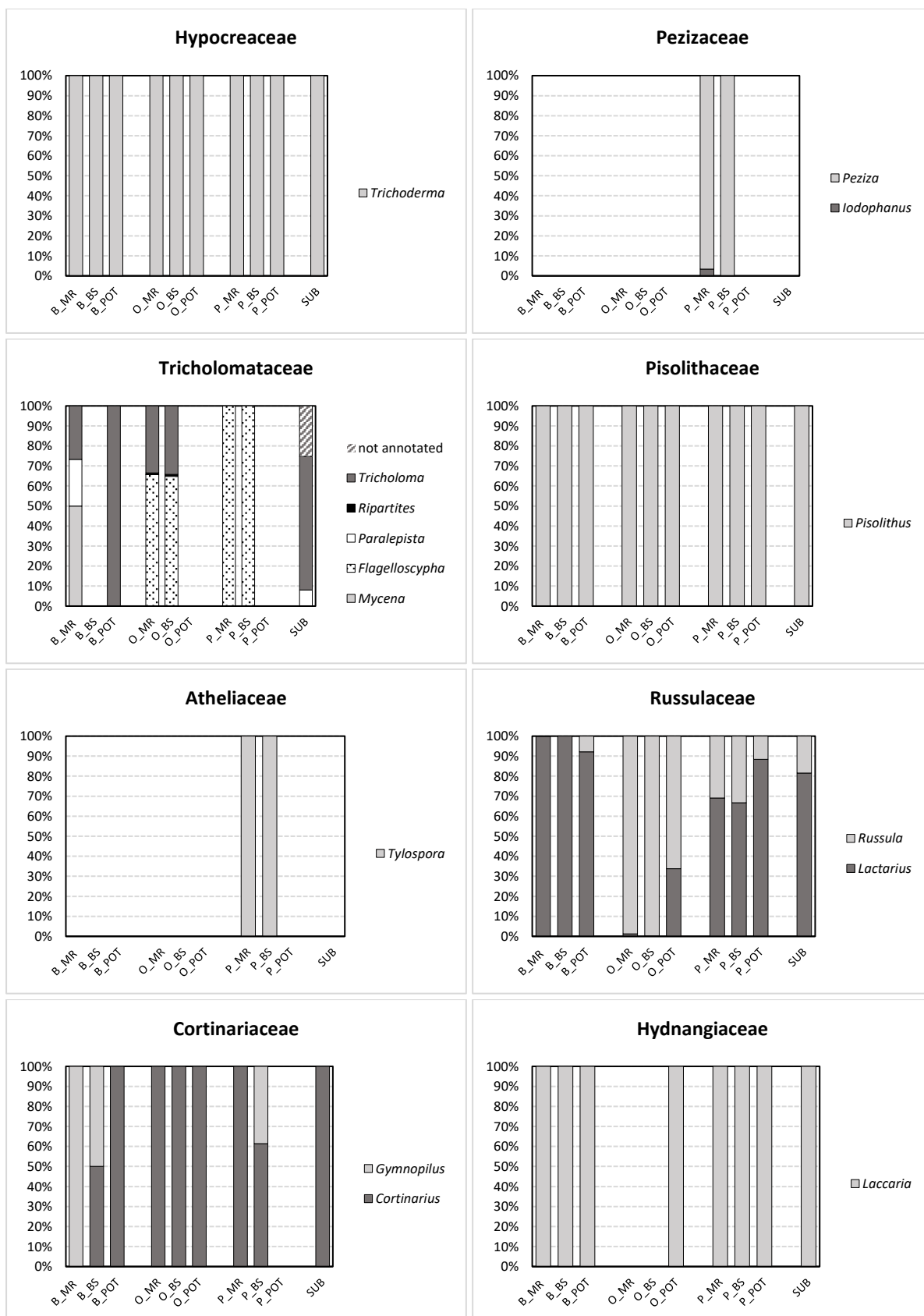
Table S60. PERMANOVA test output of pairwise comparisons between field plant mycorrhizosphere, pot plant mycorrhizosphere and control pot substrate based on the relative abundance of the most representative bacterial taxa.

Total sum of squares	1.474
Within-group sum of squares	0.2391
F	11.19
P	0.0001

Table S61. PERMANOVA p-values of pairwise comparisons field plant mycorrhizosphere, pot plant mycorrhizosphere and control pot substrate based on the relative abundance of the most representative bacterial taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Arrows represent significant values ($p < 0.05$).

	B_MR	B_POT	O_MR	O_POT	P_MR	P_POT	SUB
B_POT	0.10						
O_POT			0.10				
P_POT					0.10		
SUB	0.10	0.10	0.10	0.10	0.10	0.10	





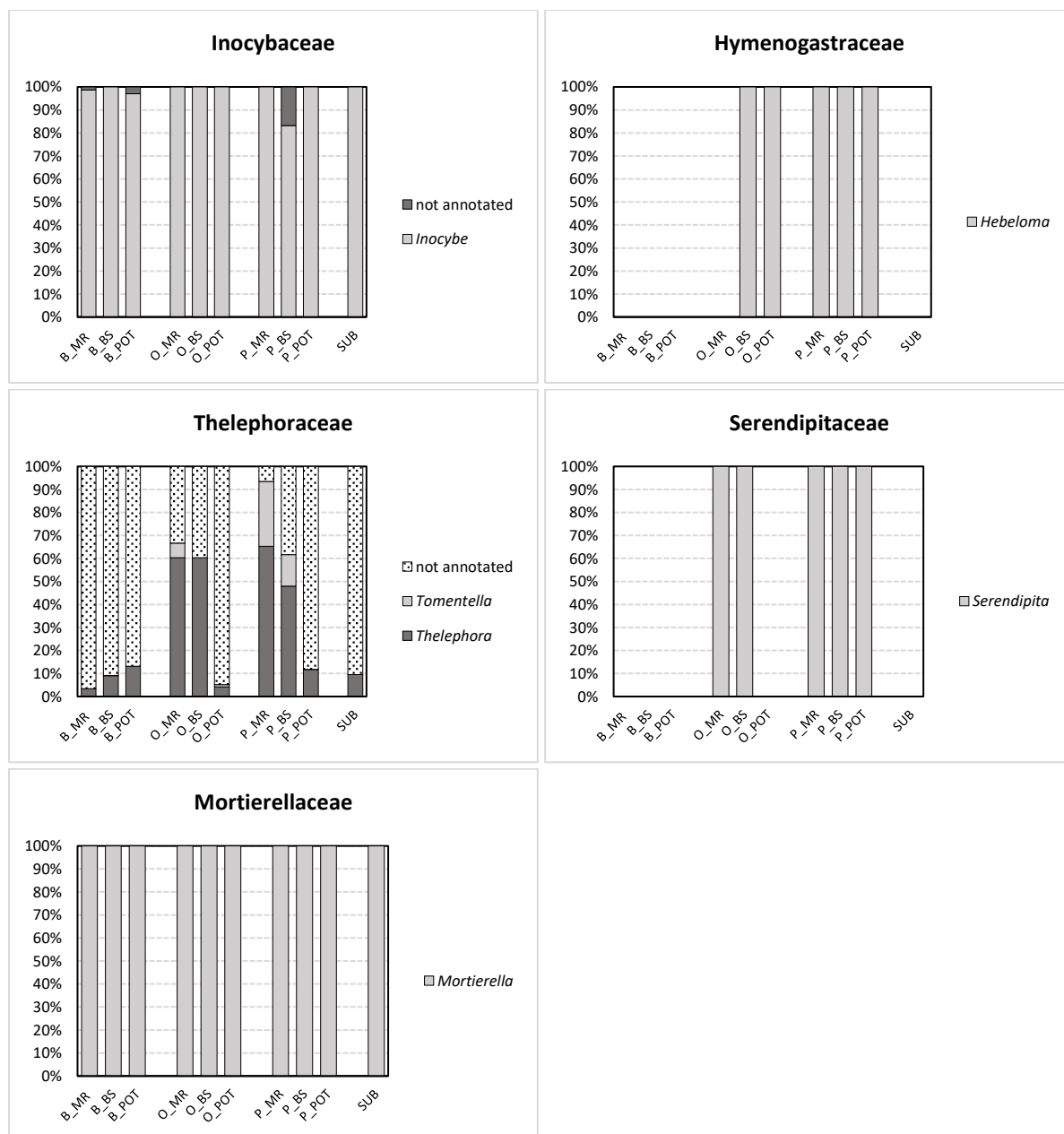


Figure S9. Characterisation of the most representative fungal families at genus level. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. y-axis represents relative abundance of fungal genera within a considered fungal family.

Table S62. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between variants of the experiment in diversity indices of fungal communities. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

are not informative.

Diversity Index	ANOVA						Kruskal-Wallis				Post Hoc comparisons						
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variant of comparisons		Mean Difference	SE	t	p tukey	p bonf
Richness	variant	2.090.667	1	2.090.667	1.052	0.363					B_MR	B_BS	37.333	36.397	1.026	0.363	0.363
	Residuals	7.948.667	4	1.987.167													
	variant	160.167	1	160.167	0.074	0.799					O_MR	O_BS	10.333	38.058	0.272	0.799	0.799
	Residuals	8.690.667	4	2.172.667													
	variant	9.204.167	1	9.204.167	3.155	0.150					P_MR	P_BS	78.333	44.100	1.776	0.150	0.150
	Residuals	11.668.667	4	2.917.167													
	variant	48.167	1	48.167	0.038	0.856					B_MR	B_POT	5.667	29.242	0.194	0.856	0.856
	Residuals	5.130.667	4	1.282.667													
	variant	326.700	1	326.700	0.085	0.789					O_MR	O_POT	16.500	56.516	0.292	0.789	0.789
	Residuals	11.498.500	3	3.832.833													
	variant	2.480.667	1	2.480.667	6.831	0.059					P_MR	P_POT	40.667	15.560	2.614	0.059	0.059
	Residuals	1.452.667	4	363.167													
	variant	8.893.500	1	8.893.500	40.425	0.003					B_POT	SUB	-77.000	12.111	-6.358	0.003*	0.003
	Residuals	880.000	4	220.000													
							variant	0.333	1	0.564	O_POT	SUB	-64.500	57.088	-1.130	0.341	0.341
	variant	6.936.000	1	6.936.000	31.527	0.005					P_POT	SUB	-68.000	12.111	-5.615	0.005*	0.005
	Residuals	880.000	4	220.000													
	variant	2.907.556	2	1.453.778	1.345	0.329					B_MR	O_MR	-23.333	26.844	-0.869	0.677	1.000
	Residuals	6.485.333	6	1.080.889								P_MR	-44.000	26.844	-1.639	0.301	0.457
											O_MR	P_MR	-20.667	26.844	-0.770	0.734	1.000
variant	15.100.222	2	7.550.111	2.076	0.206					B_BS	O_BS	3.667	49.242	0.074	0.997	1.000	
Residuals	21.822.667	6	3.637.111								P_BS	-85.000	49.242	-1.726	0.271	0.405	
										O_BS	P_BS	-88.667	49.242	-1.801	0.248	0.366	
Simpson dominance index	variant	3.481e -4	1	3.481e -4	0.080	0.791					B_MR	B_BS	-0.015	0.054	-0.283	0.791	0.791
	Residuals	0.017	4	0.004													
	variant	0.005	1	0.005	0.663	0.461					O_MR	O_BS	0.060	0.073	0.814	0.461	0.461
	Residuals	0.032	4	0.008													
	variant	0.009	1	0.009	1.970	0.233					P_MR	P_BS	-0.078	0.056	-1.403	0.233	0.233
	Residuals	0.019	4	0.005													
	variant	1.236e -5	1	1.236e -5	3.606e -4	0.986					B_MR	B_POT	0.003	0.151	0.019	0.986	0.986
	Residuals	0.137	4	0.034													
							variant	0.333	1	0.564	O_MR	O_POT	-0.164	0.159	-1.032	0.378	0.378
	variant	0.002	1	0.002	0.447	0.540					P_MR	P_POT	0.033	0.050	0.668	0.540	0.540
	Residuals	0.015	4	0.004													
							variant	3.857	1	0.050	B_POT	SUB	0.203	0.147	1.382	0.239	0.239
							variant	3.000	1	0.083	O_POT	SUB	0.270	0.155	1.739	0.180	0.180
	variant	0.014	1	0.014	30.670	0.005					P_POT	SUB	0.096	0.017	5.538	0.005*	0.005
	Residuals	0.002	4	4.474e -4													
	variant	0.016	2	0.008	1.913	0.228					B_MR	O_MR	0.100	0.053	1.869	0.227	0.333
	Residuals	0.026	6	0.004								P_MR	0.077	0.053	1.434	0.383	0.605
											O_MR	P_MR	-0.023	0.053	-0.435	0.903	1.000
	variant	0.033	2	0.017	2.338	0.177					B_BS	O_BS	0.025	0.069	0.359	0.932	1.000

	variant	0.012	1	0.012	0.795	0.423					O_MR	O_BS	0.090	0.101	0.891	0.423	0.423
	Residuals	0.061	4	0.015													
	variant	0.021	1	0.021	1.122	0.349					P_MR	P_BS	-0.118	0.111	-1.059	0.349	0.349
	Residuals	0.074	4	0.018													
	variant	7.639e -4	1	7.639e -4	0.013	0.914					B_MR	B_POT	0.023	0.195	0.116	0.914	0.914
	Residuals	0.228	4	0.057													
							variant	0.333	1	0.564	O_MR	O_POT	-0.185	0.180	-1.028	0.380	0.380
	variant	0.004	1	0.004	0.283	0.623					P_MR	P_POT	0.054	0.101	0.532	0.623	0.623
	Residuals	0.061	4	0.015													
							variant	0.429	1	0.513	B_POT	SUB	0.245	0.188	1.300	0.263	0.263
							variant	3.000	1	0.083	O_POT	SUB	0.365	0.174	2.100	0.127	0.127
	variant	0.037	1	0.037	11.694	0.027					P_POT	SUB	0.158	0.046	3.420	0.027*	0.027
	Residuals	0.013	4	0.003													
	variant	0.012	2	0.006	0.496	0.632					B_MR	O_MR	0.088	0.089	0.983	0.613	1.000
	Residuals	0.072	6	0.012								P_MR	0.056	0.089	0.630	0.810	1.000
											O_MR	P_MR	-0.032	0.089	-0.353	0.934	1.000
	variant	0.075	2	0.037	2.401	0.171					B_BS	O_BS	0.030	0.102	0.297	0.953	1.000
	Residuals	0.093	6	0.016								P_BS	0.206	0.102	2.029	0.186	0.266
											O_BS	P_BS	0.176	0.102	1.732	0.269	0.402

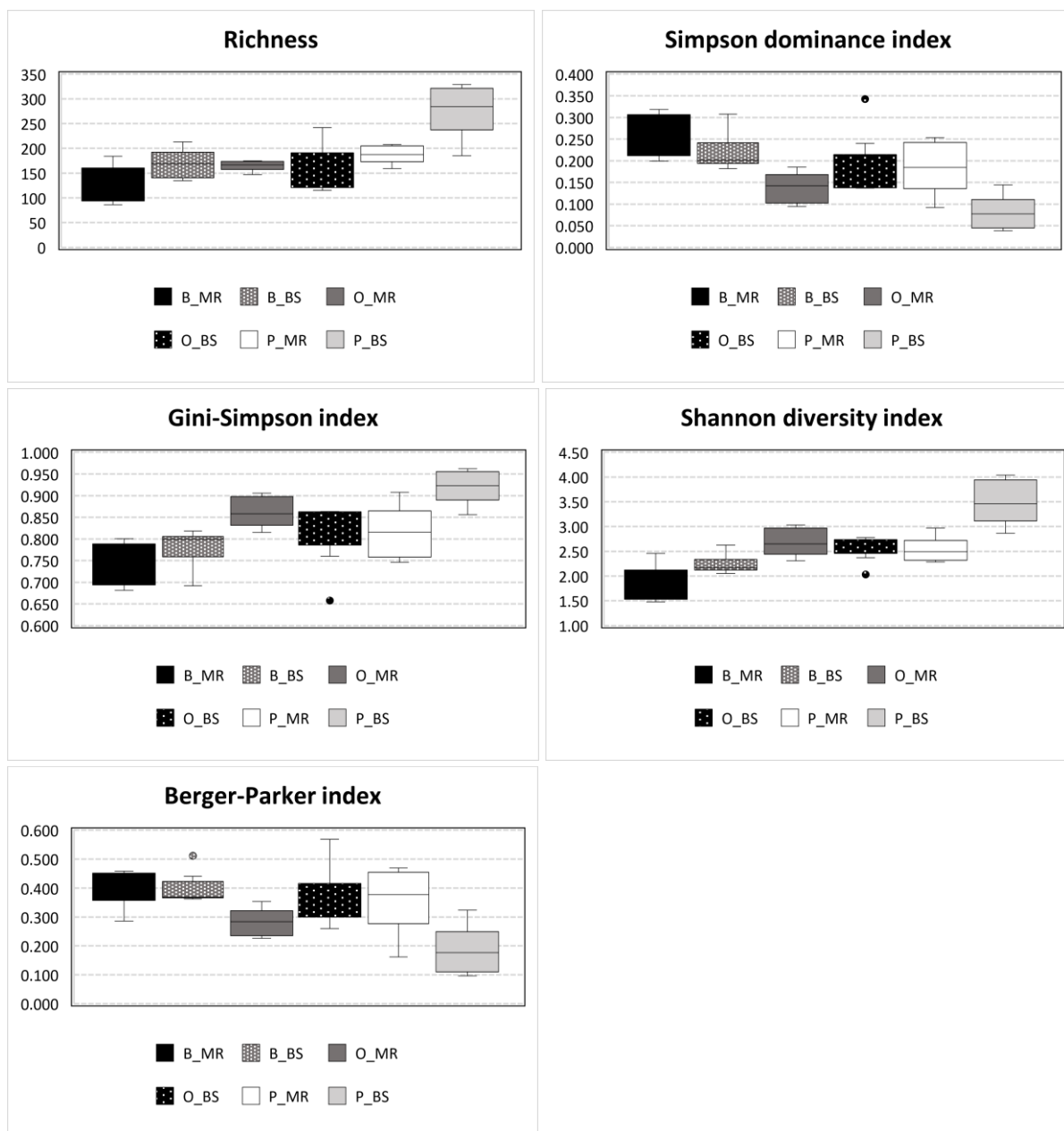


Figure S10. Pairwise comparisons of fungal community diversity indices based on ASVs between field plant mycorrhizosphere and corresponding bulk soil. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil. Brackets indicate significant differences ($p < 0.05$).

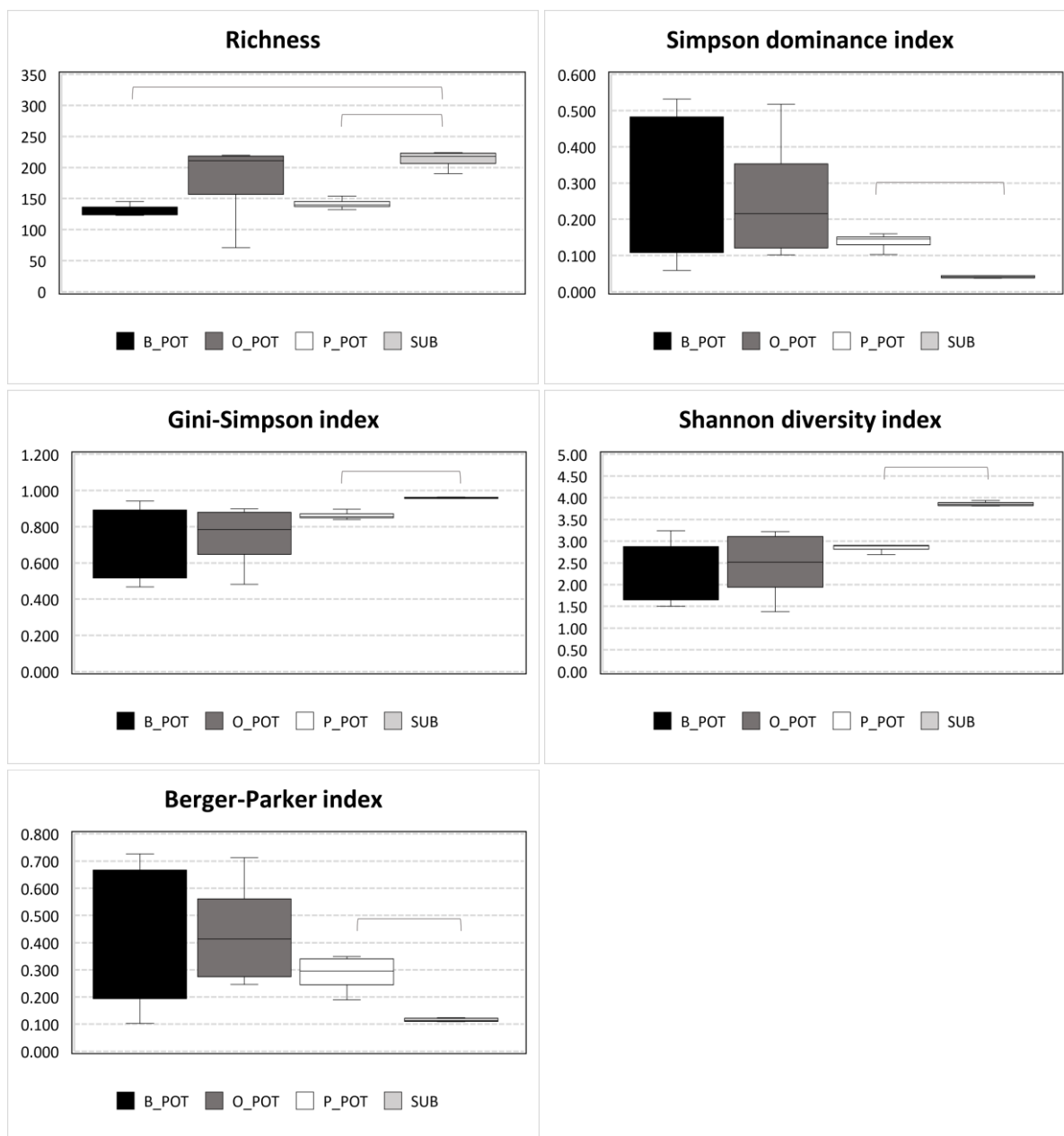


Figure S11. Pairwise comparisons of fungal community diversity indices based on ASVs between pot plant mycorrhizosphere and control pot substrate. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Brackets indicate significant differences ($p < 0.05$).

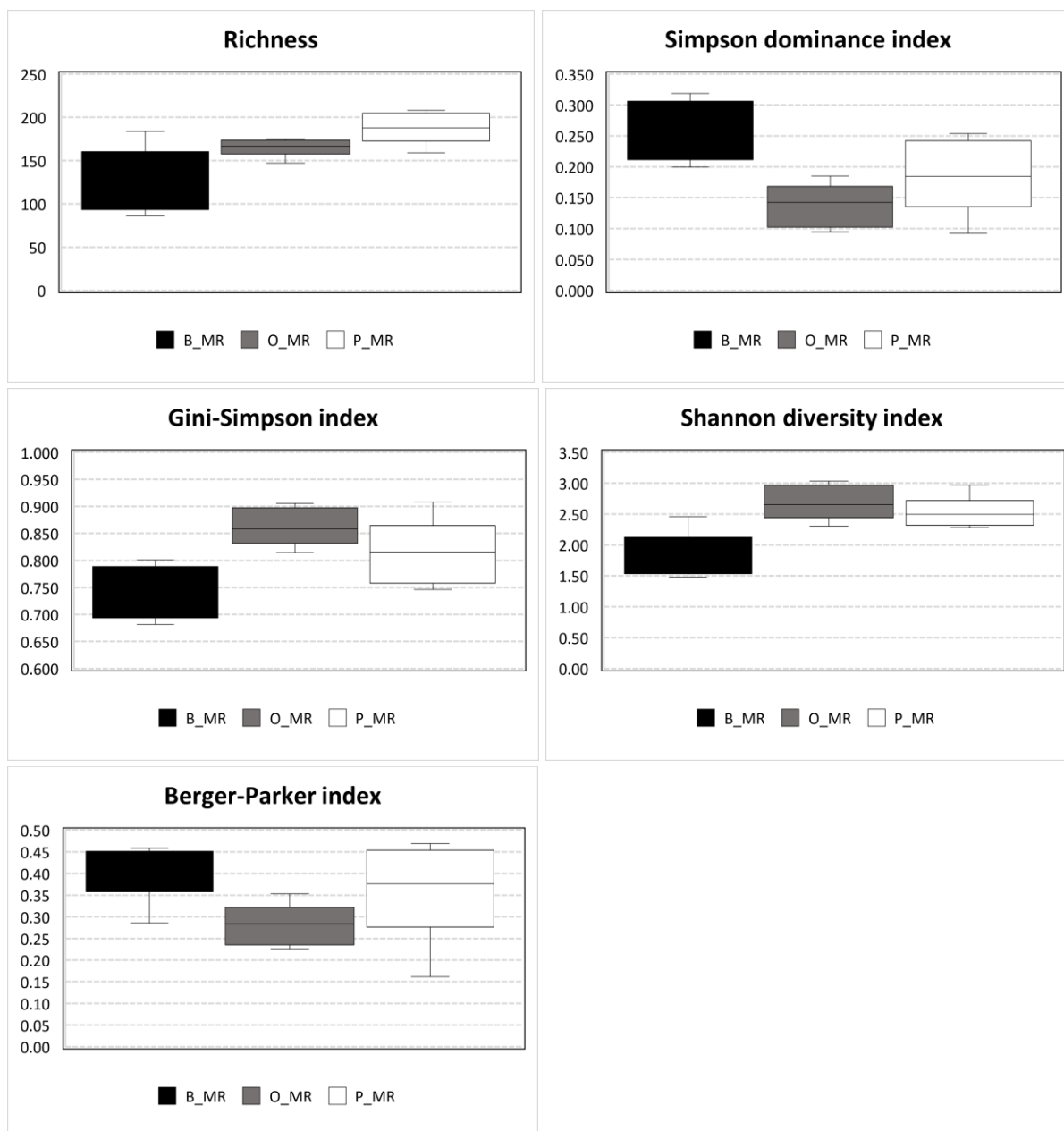


Figure S12. Pairwise comparisons of fungal community diversity indices based on ASVs between field plant mycorrhizospheres. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant. Brackets indicate significant differences ($p < 0.05$).

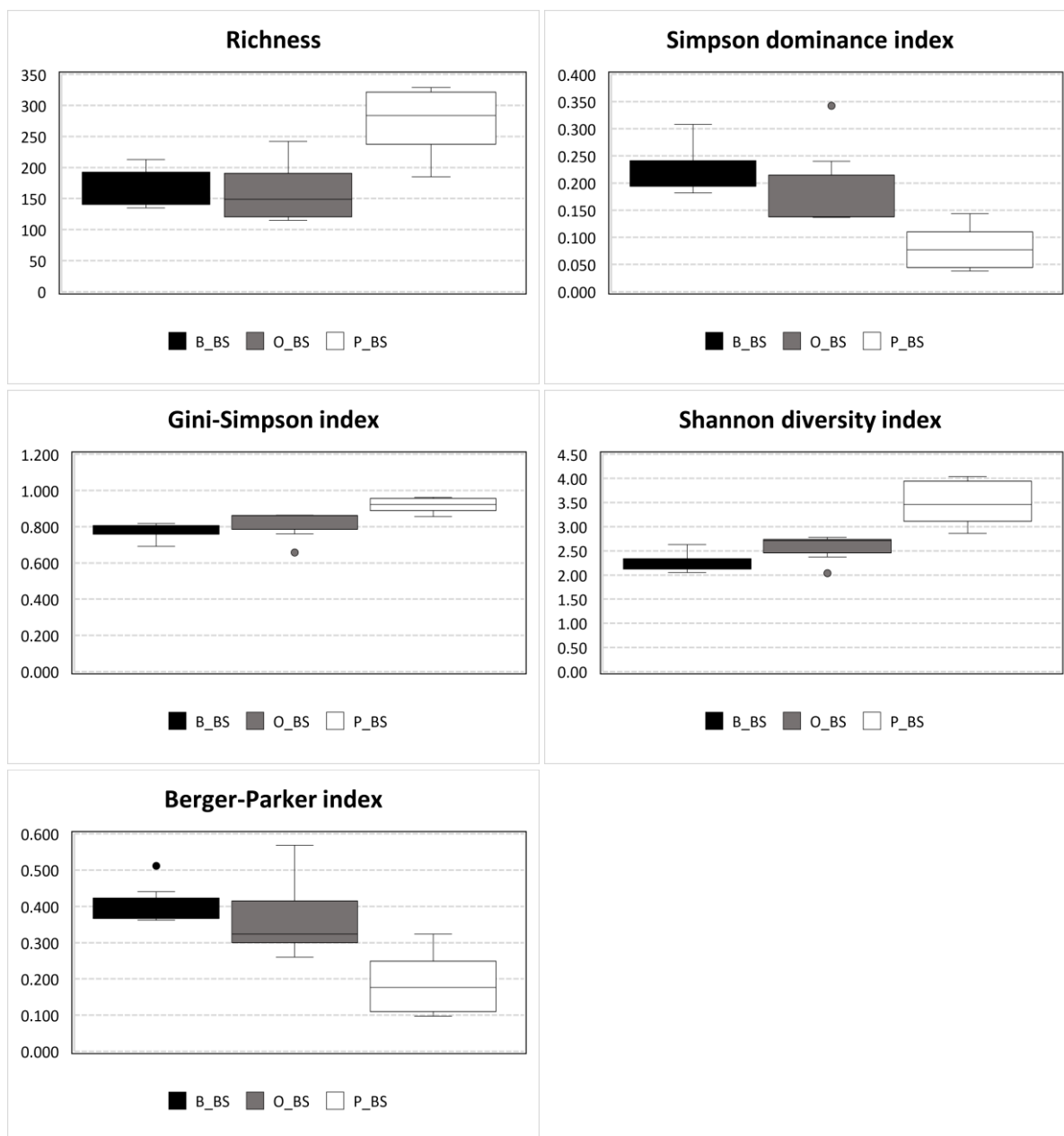


Figure S13. Pairwise comparisons of fungal community diversity indices based on ASVs between bulk soil at different sampling sites. B – birch, O – oak, P – pine; BS – bulk soil. Brackets indicate significant differences ($p < 0.05$).

Table S63. Indices of similarity between fungal communities determined for trees within variants of the experiment. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil, POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

Index	Estimate	s.e.	95%Lower	95%Upper
B_MR				
Sorensen	0.202	0.013	0.177	0.227
Jaccard	0.078	0.006	0.066	0.090
Bray-Curtis	0.437	0.001	0.435	0.439
O_MR				
Sorensen	0.199	0.007	0.187	0.212
Jaccard	0.077	0.003	0.071	0.082
Bray-Curtis	0.062	0.000	0.061	0.062
P_MR				
Sorensen	0.145	0.009	0.128	0.162
Jaccard	0.054	0.004	0.047	0.061
Bray-Curtis	0.083	0.002	0.078	0.087
B_BS				
Sorensen	0.267	0.007	0.253	0.281
Jaccard	0.108	0.004	0.101	0.115
Bray-Curtis	0.509	0.001	0.507	0.512
O_BS				
Sorensen	0.180	0.011	0.159	0.201
Jaccard	0.068	0.005	0.059	0.077
Bray-Curtis	0.104	0.001	0.103	0.106
P_BS				
Sorensen	0.149	0.005	0.140	0.158
Jaccard	0.055	0.002	0.052	0.059
Bray-Curtis	0.173	0.001	0.171	0.145
B_POT				
Sorensen	0.199	0.007	0.186	0.213
Jaccard	0.077	0.003	0.071	0.083
Bray-Curtis	0.062	0.000	0.061	0.062
O_POT				
Sorensen	0.124	0.002	0.120	0.128
Jaccard	0.066	0.001	0.064	0.068
Bray-Curtis	0.012	0.008	0.000	0.027
P_POT				
Sorensen	0.225	0.005	0.216	0.235
Jaccard	0.088	0.002	0.084	0.093
Bray-Curtis	0.306	0.001	0.304	0.308
SUB				
Sorensen	0.214	0.006	0.204	0.225
Jaccard	0.083	0.003	0.079	0.088
Bray-Curtis	0.118	0.001	0.116	0.119

Table S64. ANOSIM test output of pairwise comparisons between field plant mycorrhizospheres based on the relative abundance of the most representative fungal taxa.

Mean rank within groups	9.556
Mean rank between groups	21.48
R	0.6626
p	0.0059

Table S65. ANOSIM p-values of pairwise comparisons between field plant mycorrhizospheres based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant.

	B_MR	O_MR	P_MR
B_MR			
O_MR	0.10		
P_MR	0.20	0.10	

Table S66. PERMANOVA test output of pairwise comparisons between field plant mycorrhizospheres based on the relative abundance of the most representative fungal taxa.

Total sum of squares	1.852
Within-group sum of squares	0.7379
F	4.528
P	0.014

Table S67. PERMANOVA p-values of pairwise comparisons between field plant mycorrhizospheres based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant.

	B_MR	O_MR	P_MR
B_MR			
O_MR	0.11		
P_MR	0.39	0.10	

Table S68. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between field plant mycorrhizospheres in relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Taxon	ANOVA						Kruskal-Wallis				Post Hoc comparisons						
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variants of comparisons		Mean Difference	SE	t	p tukey	p bonf
<i>Herpotrichiellaceae</i>							variant	5.956	2	0.051	B_MR	O_MR	-20.947	3.680	-5.693	0.003	0.004*
												P_MR	-4.457	3.680	-1.211	0.490	0.814
											O_MR	P_MR	16.490	3.680	4.481	0.010	0.013*
<i>Aspergillaceae</i>							variant	2.222	2	0.329	B_MR	O_MR	-0.805	0.806	-1.000	0.604	1.000
												P_MR	-0.205	0.806	-0.255	0.965	1.000
											O_MR	P_MR	0.600	0.806	0.745	0.748	1.000
<i>Trichocomaceae</i>							variant	4.356	2	0.113	B_MR	O_MR	0.350	2.163	0.162	0.986	1.000
												P_MR	-2.696	2.163	-1.246	0.472	0.777
											O_MR	P_MR	-3.045	2.163	-1.408	0.395	0.626
<i>Dermateaceae</i>							variant	5.067	2	0.079	B_MR	O_MR	0.206	0.442	0.467	0.889	1.000
												P_MR	-0.553	0.442	-1.250	0.470	0.773
											O_MR	P_MR	-0.759	0.442	-1.717	0.274	0.410
<i>Helotiaceae</i>							variant	2.756	2	0.252	B_MR	O_MR	-0.112	0.361	-0.309	0.949	1.000
												P_MR	-0.434	0.361	-1.200	0.495	0.826
											O_MR	P_MR	-0.322	0.361	-0.892	0.665	1.000
<i>Hyaloscyphaceae</i>							variant	7.261	2	0.027	B_MR	O_MR	7.471	1.060	7.051	< .001	0.001*
												P_MR	7.627	1.060	7.198	< .001	0.001*
											O_MR	P_MR	0.156	1.060	0.147	0.988	1.000
<i>Leotiaceae</i>	variant	288.630	2	144.315	1.728	0.255					B_MR	O_MR	11.925	7.461	1.598	0.317	0.483
	Residuals	501.020	6	83.503								P_MR	12.099	7.461	1.622	0.308	0.468
											O_MR	P_MR	0.174	7.461	0.023	1.000	1.000
<i>Vibrisseaceae</i>							variant	3.971	1	0.046	B_MR	P_MR	-0.723	0.655	-1.105	0.331	0.331
<i>Hypocreaceae</i>							variant	3.289	2	0.193	B_MR	O_MR	-0.560	3.551	-0.158	0.986	1.000
												P_MR	-5.024	3.551	-1.415	0.392	0.621
											O_MR	P_MR	-4.464	3.551	-1.257	0.466	0.766
<i>Cortinariaceae</i>							variant	4.506	2	0.105	B_MR	O_MR	-0.257	3.294	-0.078	0.997	1.000
												P_MR	-5.136	3.294	-1.559	0.332	0.510
											O_MR	P_MR	-4.879	3.294	-1.481	0.363	0.567
<i>Hydnangiaceae</i>	variant	0.001	1	0.001	5.178	0.085					B_MR	P_MR	0.028	0.012	2.276	0.085	0.085
	Residuals	9.128e -4	4	2.282e -4													
<i>Inocybaceae</i>							variant	3.289	2	0.193	B_MR	O_MR	33.644	12.843	2.620	0.088	0.119
												P_MR	4.899	12.843	0.381	0.924	1.000
											O_MR	P_MR	-28.745	12.843	-2.238	0.143	0.200
<i>Tricholomataceae</i>							variant	3.294	2	0.193	B_MR	O_MR	-2.711	2.132	-1.272	0.459	0.752
												P_MR	0.077	2.132	0.036	0.999	1.000
											O_MR	P_MR	2.788	2.132	1.308	0.442	0.717
<i>Pisolithaceae</i>	variant	0.033	2	0.016	1.010	0.419					B_MR	O_MR	0.006	0.104	0.057	0.998	1.000
	Residuals	0.097	6	0.016								P_MR	0.131	0.104	1.259	0.466	0.765
											O_MR	P_MR	0.125	0.104	1.202	0.495	0.824
<i>Russulaceae</i>	variant	3.273.909	2	1.636.955	21.057	0.002					B_MR	O_MR	-40.553	7.199	-5.633	0.003*	0.004
	Residuals	466.439	6	77.740								P_MR	-0.189	7.199	-0.026	1.000	1.000
											O_MR	P_MR	40.365	7.199	5.607	0.003*	0.004
<i>Serendipitaceae</i>							variant	0.048	1	0.827	O_MR	P_MR	1.104	2.028	0.544	0.615	0.615

<i>Thelephoraceae</i>							variant	2.489	2	0.288	B_MR	O_MR	19.777	10.801	1.831	0.238	0.350
												P_MR	12.836	10.801	1.188	0.501	0.839
											O_MR	P_MR	-6.941	10.801	-0.643	0.803	1.000
<i>Mortierellaceae</i>	variant	5.304	2	2.652	2.545	0.158					B_MR	O_MR	-1.785	0.833	-2.141	0.161	0.228
	Residuals	6.252	6	1.042								P_MR	-0.379	0.833	-0.455	0.894	1.000
											O_MR	P_MR	1.405	0.833	1.686	0.285	0.428

Table S69. SIMPER analysis demonstrating contribution (%) of the most abundant fungal families to Bray-Curtis dissimilarity between field plant mycorrhizospheres. Only fungal families with contribution higher than 10% are included in table. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant.

	B_MR	O_MR	P_MR
B_MR			
O_MR	Russulaceae (27.4%) Inocybaceae (22.93%) Herpotrichiellaceae (14.21%) Thelephoraceae (14.08%)		
P_MR	Inocybaceae (20.03%) Thelephoraceae (19.28%) Leotiaceae (13.7%)	Russulaceae (30.91%) Inocybaceae (22.41%) Herpotrichiellaceae (12.69%)	

Table S70. ANOSIM test output of pairwise comparisons between bulk soil at different sampling sites based on the relative abundance of the most representative fungal taxa.

Mean rank within groups	5.222
Mean rank between groups	22.93
R	0.9835
P	0.0035

Table S71. ANOSIM p-values of pairwise comparisons between bulk soil at different sampling sites based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; BS – bulk soil.

	B_BS	O_BS	P_BS
B_BS			
O_BS	0.10		
P_BS	0.10	0.10	

Table S72. PERMANOVA test output of pairwise comparisons between bulk soil at different sampling sites based on the relative abundance of the most representative fungal taxa.

Total sum of squares	1.536
Within-group sum of squares	0.363
F	9.694
p	0.035

Table S73. PERMANOVA p-values of pairwise comparisons between bulk soil at different sampling sites based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; BS – bulk soil.

	B_BS	O_BS	P_BS
B_BS			
O_BS	0.11		
P_BS	0.10	0.10	

Table S74. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between bulk soil at different sampling sites in relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; BS – bulk soil. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Taxon	ANOVA						Kruskal-Wallis				Post Hoc comparisons							
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variants of comparisons		Mean Difference	SE	t	p tukey	p bonf	
<i>Herpotrichiellaceae</i>	variant	169.645	2	84.823	7.965	0.020						B_BS	O_BS	-9.593	2.664	-3.600	0.026*	0.034
	Residuals	63.893	6	10.649									P_BS	-0.821	2.664	-0.308	0.949	1.000
												O_BS	P_BS	8.772	2.664	3.292	0.038*	0.050
<i>Aspergillaceae</i>							variant	5.422	2	0.066		B_BS	O_BS	-0.473	0.372	-1.271	0.460	0.752
													P_BS	-0.493	0.372	-1.324	0.434	0.701
												O_BS	P_BS	-0.020	0.372	-0.053	0.998	1.000
<i>Trichocomaceae</i>	variant	7.061	1	7.061	3.977	0.117						B_BS	P_BS	-2.170	1.088	-1.994	0.117	0.117
	Residuals	7.102	4	1.776														
<i>Dermateaceae</i>							variant	5.067	2	0.079		B_BS	O_BS	0.059	0.743	0.079	0.997	1.000
													P_BS	-1.055	0.743	-1.420	0.390	0.616
												O_BS	P_BS	-1.114	0.743	-1.499	0.356	0.553
<i>Helotiaceae</i>							variant	2.400	2	0.301		B_BS	O_BS	0.047	2.644	0.018	1.000	1.000
													P_BS	2.344	2.644	0.886	0.668	1.000
												O_BS	P_BS	2.296	2.644	0.868	0.678	1.000
<i>Hyaloscyphaceae</i>							variant	5.241	2	0.073		B_BS	O_BS	10.796	4.456	2.423	0.113	0.155
													P_BS	11.948	4.456	2.681	0.081	0.109
												O_BS	P_BS	1.152	4.456	0.258	0.964	1.000
<i>Leotiaceae</i>	variant	55.560	2	27.780	4.402	0.067						B_BS	O_BS	5.926	2.051	2.889	0.062	0.083
	Residuals	37.864	6	6.311									P_BS	4.163	2.051	2.030	0.186	0.266
												O_BS	P_BS	-1.763	2.051	-0.859	0.683	1.000
<i>Vibrisseaceae</i>							variant	5.915	2	0.052		B_BS	O_BS	-0.004	0.584	-0.007	1.000	1.000
													P_BS	-1.005	0.584	-1.719	0.274	0.409
												O_BS	P_BS	-1.001	0.584	-1.713	0.276	0.413
<i>Hypocreaceae</i>							variant	2.222	2	0.329		B_BS	O_BS	-2.152	1.517	-1.419	0.390	0.617
													P_BS	-0.326	1.517	-0.215	0.975	1.000
												O_BS	P_BS	1.826	1.517	1.204	0.493	0.822
<i>Cortinariaceae</i>							variant	1.195	2	0.550		B_BS	O_BS	-0.095	0.074	-1.279	0.455	0.744
													P_BS	-0.047	0.074	-0.630	0.810	1.000
												O_BS	P_BS	0.048	0.074	0.649	0.800	1.000
<i>Hydnangiaceae</i>	variant	0.003	1	0.003	9.728	0.036						B_BS	P_BS	0.044	0.014	3.119	0.036*	0.036
	Residuals	0.001	4	2.999e -4														
<i>Hymenogastraceae</i>							variant	2.333	1	0.127		O_BS	P_BS	-0.328	0.258	-1.270	0.273	0.273
<i>Inocybaceae</i>	variant	2.638.105	2	1.319.053	51.725	< .001						B_BS	O_BS	41.801	4.123	10.138	< .001*	< .001
	Residuals	153.006	6	25.501									P_BS	23.830	4.123	5.779	0.003*	0.004
												O_BS	P_BS	-17.971	4.123	-4.359	0.011*	0.014
<i>Tricholomataceae</i>							variant	3.857	1	0.050		O_BS	P_BS	2.973	2.834	1.049	0.353	0.353
<i>Pisolithaceae</i>	variant	0.054	2	0.027	1.560	0.285						B_BS	O_BS	0.186	0.107	1.739	0.267	0.398
	Residuals	0.103	6	0.017									P_BS	0.064	0.107	0.601	0.825	1.000
												O_BS	P_BS	-0.122	0.107	-1.137	0.528	0.896
<i>Russulaceae</i>	variant	3.275.139	2	1.637.569	11.125	0.010						B_BS	O_BS	-38.317	9.906	-3.868	0.019*	0.025
	Residuals	883.208	6	147.201									P_BS	4.002	9.906	0.404	0.915	1.000
												O_BS	P_BS	42.319	9.906	4.272	0.012*	0.016
<i>Serendipitaceae</i>	variant	0.309	1	0.309	0.019	0.896						O_BS	P_BS	0.454	3.255	0.140	0.896	0.896

	Residuals	63.557	4	15.889													
<i>Thelephoraceae</i>							variant	4.622	2	0.099	B_BS	O_BS	3.538	3.103	1.140	0.527	0.893
												P_BS	-8.373	3.103	-2.698	0.079	0.107
											O_BS	P_BS	-11.911	3.103	-3.839	0.020	0.026*
<i>Mortierellaceae</i>							variant	5.689	2	0.058	B_BS	O_BS	-1.236	1.470	-0.841	0.694	1.000
												P_BS	-4.076	1.470	-2.774	0.072	0.097
											O_BS	P_BS	-2.841	1.470	-1.933	0.210	0.304

Table S75. SIMPER analysis demonstrating contribution (%) of the most abundant fungal families to Bray-Curtis dissimilarity between bulk soil at different sampling sites. Only fungal families with contribution higher than 10% are included in table. B – birch, O – oak, P – pine; BS – bulk soil.

	B_BS	O_BS	P_BS
B_BS			
O_BS	Inocybaceae (33.44%) Russulaceae (30.62%)		
P_BS	Inocybaceae (29.1%) Russulaceae (19.15%) Hyaloscyphaceae (14.53%) Thelephoraceae (10.61%)	Russulaceae (40.73%) Inocybaceae (16.78%) Thelephoraceae (11.19%)	

Table S76. ANOSIM test output of pairwise comparisons between field plant mycorrhizosphere and bulk soil based on the relative abundance of the most representative fungal taxa.

Mean rank within groups	32.61
Mean rank between groups	82.92
R	0.6576
p	0.0001

Table S77. ANOSIM p-values of pairwise comparisons between field plants MRs vs BS based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

	B_MR	B_BS	O_MR	O_BS	P_MR	P_BS
B_MR						
B_BS	0.20					
O_MR	0.10	0.10				
O_BS	0.10	0.10	0.30			
P_MR	0.19	0.20	0.10	0.10		
P_BS	0.10	0.10	0.10	0.10	0.50	

Table S78. PERMANOVA test output of pairwise comparisons between field plant mycorrhizosphere and bulk soil based on the relative abundance of the most representative fungal taxa.

Total sum of squares	3.482
Within-group sum of squares	1.085
F	5.304
P	0.0001

Table S79. PERMANOVA p-values of pairwise comparisons between field plant mycorrhizosphere and bulk soil based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil.

	B_MR	B_BS	O_MR	O_BS	P_MR	P_BS
B_MR						
B_BS	0.20					
O_MR	0.10	0.10				
O_BS	0.10	0.10	0.40			
P_MR	0.40	0.60	0.10	0.10		
P_BS	0.10	0.10	0.10	0.10	0.70	

Table S80. SIMPER analysis demonstrating contribution (%) of the most abundant fungal families to Bray-Curtis dissimilarity between field plant mycorrhizosphere and bulk soil. Only fungal families with contribution higher than 10% are included in table. B – birch, O – oak, P – pine; EMR – mycorrhizosphere of field plant, BS – bulk soil.

	B_BS	O_BS	P_BS
B_MR	Thelephoraceae (30.78%) Leotiaceae (16.51%) Inocybaceae (15.75%) Russulaceae (13.72%) Hyaloscyphaceae (12.98%)		
O_MR		Herpotrichiellaceae (21.39%) Russulaceae (20.23%) Leotiaceae (15.57%)	
P_MR			Inocybaceae (26.7%) Russulaceae (14.71%)

Table S81. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between field birch mycorrhizosphere and bulk soil in relative abundance of the most representative fungal taxa. B – birch; MR – mycorrhizosphere of field plant, BS – bulk soil. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

[illegible]

Table S82. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between field oak mycorrhizosphere and bulk soil in relative abundance of the most representative fungal taxa. O – oak; MR – mycorrhizosphere of field plant, BS – bulk soil. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

[illegible]

Table S83. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between field pine mycorrhizosphere and bulk soil in relative abundance of the most representative fungal taxa. P – pine; MR – mycorrhizosphere of field plant, BS – bulk soil. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

[illegible]

Table S84. ANOSIM test output of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on the relative abundance of the most representative fungal taxa.

Mean rank within groups	15.6
Mean rank between groups	30.76
R	0.5511
p	0.0108

Table S85. ANOSIM p-values of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

	B_POT	O_POT	P_POT	SUB
B_POT				
O_POT	0.60			
P_POT	0.50	0.30		
SUB	0.10	0.10	0.11	

Table S86. PERMANOVA test output of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on the relative abundance of the most representative fungal taxa.

Total sum of squares	1.234
Within-group sum of squares	0.4541
F	4.009
p	0.0134

Table S87. PERMANOVA p-values of pairwise comparisons between pot plant mycorrhizospheres and control pot substrate based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

	B_POT	O_POT	P_POT	SUB
B_POT				
O_POT	0.60			
P_POT	0.20	0.70		
SUB	0.10	0.10	0.10	

Table S88. SIMPER analysis demonstrating contribution (%) of the most abundant fungal families to Bray-Curtis dissimilarity between pot plant mycorrhizospheres and control pot substrate. Only fungal families with contribution higher than 10% are included in table. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate.

	B_POT	O_POT	P_POT	SUB
B_POT				
O_POT	Thelephoraceae (38.34%) Herpotrichiellaceae (15.55%) Pisolithaceae (12.36%)			
P_POT	Thelephoraceae (25.21%) Inocybaceae (20.68%) Pisolithaceae (17.29%)	Thelephoraceae (41.32%) Herpotrichiellaceae (17.1%) Inocybaceae (15.92%)		
SUB	Thelephoraceae (42.37%) Pisolithaceae (11.33%)	Thelephoraceae (42.37%) Herpotrichiellaceae (17.06%) Leotiaceae (11.86%)	Thelephoraceae (42.37%) Inocybaceae (21.91%) Leotiaceae (11.2%)	

Table S89. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between pot birch mycorrhizosphere and control pot substrate in relative abundance of the most representative fungal taxa. B – birch; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Taxon	ANOVA						Kruskal-Wallis				Post Hoc comparisons					
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variants of comparisons	Mean Difference	SE	t	p tukey	p bonf
<i>Herpotrichiellaceae</i>	variant	105.840	1	105.840	54.887	0.002					B_POT SUB	-8.400	1.134	-7.409	0.002*	0.002
	Residuals	7.713	4	1.928												
<i>Aspergillaceae</i>	variant	5.415	1	5.415	20.434	0.011					B_POT SUB	-1.900	0.420	-4.520	0.011*	0.011
	Residuals	1.060	4	0.265												
<i>Trichocomaceae</i>							variant	3.971	1	0.046	B_POT SUB	-0.840	0.588	-1.429	0.226	0.226
<i>Dermateaceae</i>	variant	25.544	1	25.544	16.699	0.015					B_POT SUB	-4.127	1.010	-4.086	0.015*	0.015
	Residuals	6.119	4	1.530												
<i>Helotiaceae</i>							variant	3.971	1	0.046	B_POT SUB	-1.030	0.862	-1.194	0.298	0.298
<i>Hyaloscyphaceae</i>							variant	3.857	1	0.050	B_POT SUB	-4.957	0.401	-12.373	< .001	< .001*
<i>Leotiaceae</i>	variant	151.504	1	151.504	12.809	0.023					B_POT SUB	-10.050	2.808	-3.579	0.023*	0.023
	Residuals	47.313	4	11.828												
<i>Vibrissaceae</i>	variant	0.395	1	0.395	0.719	0.444					B_POT SUB	-0.513	0.605	-0.848	0.444	0.444
	Residuals	2.198	4	0.549												
<i>Hypocreaceae</i>	variant	0.157	1	0.157	0.029	0.873					B_POT SUB	-0.323	1.891	-0.171	0.873	0.873
	Residuals	21.452	4	5.363												
<i>Hydnangiaceae</i>							variant	0.441	1	0.507	B_POT SUB	5.473	5.667	0.966	0.389	0.389
<i>Inocybaceae</i>							variant	0.429	1	0.513	B_POT SUB	3.937	3.990	0.987	0.380	0.380
<i>Pisolithaceae</i>							variant	0.429	1	0.513	B_POT SUB	8.450	10.688	0.791	0.473	0.473
<i>Russulaceae</i>							variant	3.971	1	0.046	B_POT SUB	-0.433	0.038	-11.358	< .001	< .001*
<i>Thelephoraceae</i>	variant	2.740.061	1	2.740.061	7.728	0.050					B_POT SUB	42.740	15.375	2.780	0.050	0.050
	Residuals	1.418.330	4	354.582												
<i>Mortierellaceae</i>							variant	3.971	1	0.046	B_POT SUB	-1.327	0.895	-1.482	0.213	0.213

Table S90. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between pot oak mycorrhizosphere and control pot substrate in relative abundance of the most representative fungal taxa. O – oak; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

[illegible]

Table S91. One-Way ANOVA, Kruskal-Wallis test, post hoc test output for significance of differences between pot pine mycorrhizosphere and control pot substrate in relative abundance of the most representative fungal taxa. P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant p values (< 0.05). Empty grey cells are not informative.

Taxon	ANOVA						Kruskal-Wallis				Post Hoc comparisons					
	Cases	Sum of Squares	df	Mean Square	F	p	Factor	Statistic	df	p	Variants of comparisons	Mean Difference	SE	t	p tukey	p bonf
<i>Herpotrichiaceae</i>	variant	51.042	1	51.042	7.783	0.049					P_POT SUB	5.833	2.091	-2.790	0.049*	0.049
	Residuals	26.233	4	6.558												
<i>Aspergillaceae</i>	variant	4.002	1	4.002	15.006	0.018					P_POT SUB	-1.633	0.422	-3.874	0.018*	0.018
	Residuals	1.067	4	0.267												
<i>Trichocomaceae</i>							variant	3.971	1	0.046	P_POT SUB	-0.773	0.588	-1.316	0.259	0.259
<i>Dermateaceae</i>	variant	24.321	1	24.321	8.718	0.042					P_POT SUB	-4.027	1.364	-2.953	0.042	0.042
	Residuals	11.159	4	2.790												
<i>Helotiaceae</i>							variant	3.971	1	0.046	P_POT SUB	-1.063	0.862	-1.233	0.285	0.285
<i>Hyaloscyphaceae</i>	variant	1.675	1	1.675	3.716	0.126					P_POT SUB	-1.057	0.548	-1.928	0.126	0.126
	Residuals	1.803	4	0.451												
<i>Leotiaceae</i>	variant	107.950	1	107.950	3.377	0.140					P_POT SUB	-8.483	4.617	-1.838	0.140	0.140
	Residuals	127.873	4	31.968												
<i>Vibrisseaceae</i>							variant	0.048	1	0.827	P_POT SUB	1.553	2.483	0.626	0.565	0.565
<i>Hypocreaceae</i>	variant	0.213	1	0.213	0.036	0.858					P_POT SUB	0.377	1.980	0.190	0.858	0.858
	Residuals	23.512	4	5.878												
<i>Inocybaceae</i>	variant	466.578	1	466.578	13.911	0.020					P_POT SUB	17.637	4.729	3.730	0.020*	0.020
	Residuals	134.158	4	33.540												
<i>Pisolithaceae</i>	variant	13.650	1	13.650	216.960	< .001					P_POT SUB	-3.017	0.205	-14.730	< .001*	< .001
	Residuals	0.252	4	0.063												
<i>Russulaceae</i>							variant	3.971	1	0.046	P_POT SUB	-0.433	0.038	-11.358	< .001	< .001*
<i>Thelephoraceae</i>							variant	3.857	1	0.050	P_POT SUB	29.840	2.739	10.895	< .001	< .001*
<i>Mortierellaceae</i>							variant	0.048	1	0.827	P_POT SUB	-0.760	0.919	-0.827	0.455	0.455

Table S92. ANOVA test output of pairwise comparisons between field plant mycorrhizosphere, corresponding pot plant mycorrhizosphere and control pot substrate based on the relative abundance of the most representative fungal taxa.

Mean rank within groups	41.47
Mean rank between groups	101.5
R	0.6319
p	0.0001

Table S93. Matrix representing R-statistics of pairwise ANOSIM between field plant mycorrhizosphere, corresponding pot plant mycorrhizosphere and control pot substrate based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant values ($p < 0.05$).

	B_MR	B_POT	O_MR	O_POT	P_MR	P_POT	SUB
B_POT	0.63						
O_POT			0.75				
P_POT					0.30		
SUB	1	1	1	0.5	0.59		

Table S94. PERMANOVA test output of pairwise comparisons between field plant mycorrhizosphere, corresponding pot plant mycorrhizosphere and control pot substrate based on the relative abundance of the most representative fungal taxa.

Total sum of squares	4.236
Within-group sum of squares	1.385
F	4.46
P	0.0001

Table S96. PERMANOVA p-values of pairwise comparisons field plant mycorrhizosphere, pot plant mycorrhizosphere and control pot substrate based on the relative abundance of the most representative fungal taxa. B – birch, O – oak, P – pine; POT – mycorrhizosphere of pot plant, SUB – control pot substrate. Asterisks represent significant values ($p < 0.05$).

	B_MR	B_POT	O_MR	O_POT	P_MR	P_POT	SUB
B_POT	0.10						
O_POT			0.11				
P_POT					0.20		
SUB	0.10	0.10	0.10	0.10	0.10		

Table S96. Field plants mycorrhizosphere network parameters.

Degree		Betweenness Centrality		Closeness Centrality		Clustering Coefficient	
node	value	node	value	node	value	node	value
Mesorhizobium	35	Pirellula	0.40	n/d Acidimicrobiia	1.00	Inocybe	1.00
n/d Holophagae	34	n/d Ktedonobacteria	0.40	Alatospora	1.00	Penicillium	1.00
Vicinamibacter	33	n/d Acidobacteriia	0.09	Pirellula	0.47	Metapochonia	1.00
Candidatus Udaeobacter	33	n/d Saccharimonadia	0.08	n/d Ktedonobacteria	0.43	Lactarius	1.00
Dongia	32	Acidicapsa	0.07	Mesorhizobium	0.40	Lacunisphaera	0.91
Pirellula	31	BCP**	0.05	Vicinamibacter	0.40	n/d Deltaproteobacteria	0.84
Rhizobacter	31	n/d Ascomycota	0.05	Candidatus Udaeobacter	0.40	Kineosporia	0.83
Flavobacterium	31	n/d Phycisphaerae	0.04	Dongia	0.40	n/d Verrucomicrobiae	0.83
Variovorax	31	n/d Bacteroidia	0.04	n/d Holophagae	0.40	Devosia	0.83
Pir4 lineage	31	Acidipila	0.04	Rhizobacter	0.40	Phialocephala	0.81
Chryseolinea	31	n/d Anaeroliniae	0.04	Flavobacterium	0.40	Tomentella	0.80
Terrimonas	31	Singulisphaera	0.03	Variovorax	0.40	Candidatus Solibacter	0.80
ANPR*	28	Gemmatimonas	0.03	Pir4 lineage	0.39	Stenotrophobacter	0.78
Sphingomonas	27	Russula	0.03	Chryseolinea	0.39	Pajaroellobacter	0.78
Bradyrhizobium	27	Mortierella	0.03	Sphingomonas	0.39	Terracidiphilus	0.76
Chthoniobacter	27	Edaphobacter	0.03	Bradyrhizobium	0.39	Bryobacter	0.76
n/d Anaeroliniae	27	Exophiala	0.03	n/d Subgroup 6	0.39	Opitutus	0.75
Luteolibacter	27	n/d Chloroflexia	0.03	ANPR*	0.39	Luteolibacter	0.74
n/d Subgroup 6	26	Granulicella	0.03	MND1	0.39	Flavobacterium	0.73
MND1	26	Bryobacter	0.02	Chthoniobacter	0.39	Variovorax	0.73
Opitutus	26	Pezoloma	0.02	Opitutus	0.39	n/d Herpotrichiellaceae	0.73
Reyranella	26	Aquicella	0.02	n/d Anaeroliniae	0.38	Ellin6067	0.73
Ellin6067	26	Mesorhizobium	0.02	n/d Blastocatellia	0.38	Cladophialophora	0.72
n/d Blastocatellia	25	Haliangium	0.02	Reyranella	0.38	Bradyrhizobium	0.72
Exophiala	24	Phenylobacterium	0.02	Luteolibacter	0.38	Occallatibacter	0.72
Devosia	23	n/d Thelephoraceae	0.02	Ellin6067	0.38	ANPR*	0.72
Kineosporia	23	Cladophialophora	0.02	Gemmatimonas	0.38	n/d Blastocatellia	0.72
Cladophialophora	21	n/d Gemmatimonadetes	0.02	Devosia	0.37	n/d AD3	0.71
Granulicella	21	Terracidiphilus	0.02	n/d Acidobacteriia	0.37	Rhizobacter	0.71
Flavisolibacter	20	Reyranella	0.02	n/d Gemmatimonadetes	0.37	MND1	0.71
Acidothermus	20	n/d AD3	0.02	n/d Deltaproteobacteria	0.36	Dongia	0.71
n/d Acidobacteriia	19	n/d Holophagae	0.02	n/d Saccharimonadia	0.36	Pir4 lineage	0.71
n/d Deltaproteobacteria	19	n/d Gammaproteobacteria	0.02	Acidicapsa	0.36	Chthoniobacter	0.71
Thelephora	18	n/d Herpotrichiellaceae	0.02	n/d Chloroflexia	0.36	n/d Bacteria	0.70
Roseiarcus	18	Acidibacter	0.02	Acidipila	0.36	n/d Basidiomycota	0.70
Gemmatimonas	17	Chryseolinea	0.02	Mortierella	0.36	n/d Alphaproteobacteria	0.70
n/d Saccharimonadia	17	Vicinamibacter	0.02	Stenotrophobacter	0.36	Reyranella	0.70
Acidicapsa	16	Acidothermus	0.02	Terracidiphilus	0.35	Mucilaginibacter	0.69
Acidipila	16	Candidatus Udaeobacter	0.01	n/d AD3	0.35	Sphingomonas	0.69
n/d Herpotrichiellaceae	16	Mycobacterium	0.01	Haliangium	0.35	n/d Subgroup 6	0.69
n/d Gemmatimonadetes	15	n/d Melainabacteria	0.01	Terrimonas	0.35	Chryseolinea	0.68
n/d Chloroflexia	15	n/d Pleosporaceae	0.01	n/d Phycisphaerae	0.34	Thelephora	0.68
Russula	15	Conexibacter	0.01	Exophiala	0.34	Actinospica	0.68
Singulisphaera	15	Dongia	0.01	Cladophialophora	0.34	Flavisolibacter	0.67
Aquisphaera	15	n/d Hyaloscyphaceae	0.01	Kineosporia	0.33	Talaromyces	0.67
Terracidiphilus	14	Rhizobacter	0.01	Flavisolibacter	0.33	n/d Gemmatimonadetes	0.67
n/d AD3	14	Pir4 lineage	0.01	n/d Herpotrichiellaceae	0.32	Russula	0.67
Lacunisphaera	14	Streptomyces	0.01	Thelephora	0.32	Vicinamibacter	0.66
Streptomyces	14	Terrimonas	0.01	n/d Bacteria	0.32	Exophiala	0.66
Haliangium	13	Sphingomonas	0.01	Russula	0.32	Candidatus Udaeobacter	0.65
Occallatibacter	13	Chthoniobacter	0.01	n/d Thermoleophilia	0.32	Acidipila	0.65
n/d Melainabacteria	13	Flavobacterium	0.01	Lacunisphaera	0.31	Mesorhizobium	0.63
n/d Phycisphaerae	12	Variovorax	0.01	n/d Bacteroidia	0.31	n/d Holophagae	0.63

n/d Fungi	12	ANPR*	0.01	Streptomyces	0.31	Terrimonas	0.62
n/d Basidiomycota	12	Aquisphaera	0.01	n/d Fungi	0.31	Pezoloma	0.62
n/d Alphaproteobacteria	12	Roseiarcus	0.01	Serendipita	0.31	n/d Thelephoraceae	0.62
Pezoloma	12	Opitutus	0.01	Granulicella	0.31	Mortierella	0.62
Mortierella	11	MND1	0.01	n/d Basidiomycota	0.31	Haliangium	0.62
Talaromyces	11	Bradyrhizobium	0.01	Phenylobacterium	0.30	Aquisphaera	0.61
n/d Ktedonobacteria	10	Mucilaginibacter	0.01	Acidothermus	0.30	Acidicapsa	0.61
Stenotrophobacter	10	n/d Subgroup 6	0.01	Roseiarcus	0.30	n/d Phycisphaerae	0.61
Bryobacter	10	n/d Blastocatellia	0.01	Inocybe	0.30	n/d Actinobacteria	0.60
Acidibacter	10	Luteolibacter	0.01	n/d Actinobacteria	0.30	Pirellula	0.60
Mucilaginibacter	9	n/d Fungi	0.01	n/d Pleosporaceae	0.30	Roseiarcus	0.59
Pajaroellobacter	9	Talaromyces	0.01	n/d Alphaproteobacteria	0.29	n/d Fungi	0.59
n/d Hyaloscyphaceae	9	Ellin6067	0.01	Cortinarius	0.29	n/d Hyaloscyphaceae	0.58
n/d Planctomycetacia	9	n/d Thermoleophilia	0.00	BCP**	0.29	Acidibacter	0.58
Actinospica	8	Thelephora	0.00	Mucilaginibacter	0.29	n/d Anaeroliniae	0.57
Serendipita	7	Actinospica	0.00	Occallatibacter	0.29	n/d Saccharimonadia	0.57
Phialocephala	7	Devosia	0.00	Edaphobacter	0.29	n/d Chloroflexia	0.56
n/d Thelephoraceae	7	n/d Alphaproteobacteria	0.00	Bryobacter	0.29	n/d Acidobacteria	0.56
n/d Thermoleophilia	6	Flavisolibacter	0.00	Pezoloma	0.29	n/d Planctomycetacia	0.56
Phenylobacterium	6	Serendipita	0.00	Singulisphaera	0.29	n/d Melainabacteria	0.55
n/d Actinobacteria	6	n/d Actinobacteria	0.00	Aquisphaera	0.29	Acidothermus	0.55
n/d Pleosporaceae	6	Occallatibacter	0.00	n/d Melainabacteria	0.29	Serendipita	0.52
BCP**	6	n/d Planctomycetacia	0.00	Phialocephala	0.29	Streptomyces	0.52
n/d Ascomycota	6	n/d Basidiomycota	0.00	Actinospica	0.29	n/d Ktedonobacteria	0.51
n/d Bacteria	5	n/d Bacteria	0.00	Pajaroellobacter	0.29	Granulicella	0.50
n/d Bacteroidia	5	Cortinarius	0.00	Talaromyces	0.29	Singulisphaera	0.50
Cortinarius	5	n/d Deltaproteobacteria	0.00	n/d Ascomycota	0.28	Gemmatimonas	0.50
Tomentella	5	Pisolithus	0.00	Acidibacter	0.28	Cortinarius	0.50
Candidatus Solibacter	5	Kineosporia	0.00	Aquicella	0.28	Pisolithus	0.50
n/d Verrucomicrobiae	4	Stenotrophobacter	0.00	n/d Hyaloscyphaceae	0.28	n/d Thermoleophilia	0.47
Pisolithus	4	Pajaroellobacter	0.00	n/d Thelephoraceae	0.28	n/d Bacteroidia	0.40
Edaphobacter	3	Candidatus Solibacter	0.00	Tomentella	0.28	n/d Pleosporaceae	0.40
Aquicella	3	Phialocephala	0.00	n/d Gammaproteobacteria	0.27	Edaphobacter	0.33
Lactarius	3	Lacunisphaera	0.00	Gemmata	0.26	Aquicella	0.33
Inocybe	2	Tomentella	0.00	Mycobacterium	0.25	Phenylobacterium	0.27
n/d Gammaproteobacteria	2	Bdellovibrio	0.00	Penicillium	0.25	BCP**	0.27
Mycobacterium	2	n/d Verrucomicrobiae	0.00	Metapochonia	0.25	n/d Ascomycota	0.27
Penicillium	2	Lactarius	0.00	Conexibacter	0.25	n/d Acidimicrobiia	0.00
Metapochonia	2	Inocybe	0.00	Candidatus Solibacter	0.24	Alatospora	0.00
Conexibacter	2	Penicillium	0.00	n/d Planctomycetacia	0.23	n/d Gammaproteobacteria	0.00
Bdellovibrio	2	Metapochonia	0.00	n/d Verrucomicrobiae	0.23	Gemmata	0.00
n/d Acidimicrobiia	1	n/d Acidimicrobiia	0.00	Pisolithus	0.23	Mycobacterium	0.00
Alatospora	1	Alatospora	0.00	Lactarius	0.23	Conexibacter	0.00
Gemmata	1	Gemmata	0.00	n/d Oxyphotobacteria	0.23	n/d Oxyphotobacteria	0.00
n/d Oxyphotobacteria	1	n/d Oxyphotobacteria	0.00	Bdellovibrio	0.22	Bdellovibrio	0.00
Ferruginibacter	1	Ferruginibacter	0.00	Ferruginibacter	0.22	Ferruginibacter	0.00

* *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*

** *Burkholderia-Caballeronia-Paraburkholderia*

Table S97. Pot plants mycorrhizosphere network parameters.

Degree		Betweenness Centrality		Closeness Centrality		Clustering Coefficient	
node	value	node	value	node	value	node	value
n/d Ascomycota	10	BCP**	1.00	BCP**	1.00	Conexibacter	1.00
Exophiala	9	Jatrophihabitans	0.53	n/d Dermateaceae	1.00	n/d Chloroflexia	1.00
Mortierella	8	n/d Acidobacteriia	0.53	Pisolithus	1.00	Mucilaginibacter	1.00
n/d Herpotrichiellaceae	8	Exophiala	0.41	Jatrophihabitans	0.75	Streptomyces	1.00
ANPR*	7	n/d Herpotrichiellaceae	0.39	n/d Thermoleophilina	0.67	Oidiodendron	1.00
n/d Bacteroidia	6	n/d Thermoleophilina	0.33	Inocybe	0.67	Lechevalieria	0.83
Trichoderma	5	Terracidiphilus	0.33	n/d Gammaproteobacteria	0.67	Chthoniobacter	0.83
n/d Fungi	5	n/d Bacteria	0.33	n/d Acidobacteriia	0.60	Candidatus Xiphinematobacter	0.83
Cladophialophora	5	Nocardia	0.33	Occallatibacter	0.60	Trichoderma	0.70
Catenulispora	5	n/d Ascomycota	0.28	Rhodopila	0.60	Occallatibacter	0.67
Vibrissea	5	Occallatibacter	0.20	Conexibacter	0.60	Rhodopila	0.67
Mucilaginibacter	4	Rhodopila	0.20	n/d Chloroflexia	0.60	Edaphobacter	0.67
Lechevalieria	4	n/d Bacteroidia	0.15	n/d Bacteria	0.55	n/d Deltaproteobacteria	0.67
Chthoniobacter	4	Catenulispora	0.15	Terracidiphilus	0.50	Sphingomonas	0.67
Candidatus Xiphinematobacter	4	ANPR*	0.13	Exophiala	0.48	Acidothermus	0.67
Jatrophihabitans	4	Vibrissea	0.12	n/d Herpotrichiellaceae	0.48	n/d Fungi	0.60
n/d Thermoleophilina	4	Mycobacterium	0.12	n/d Ascomycota	0.46	Jatrophihabitans	0.50
Mycobacterium	4	Mortierella	0.10	Nocardia	0.46	n/d Thermoleophilina	0.50
Pezoloma	4	Pezoloma	0.09	Arthrobacter	0.43	Mycobacterium	0.50
Conexibacter	3	Umbelopsis	0.06	Mortierella	0.42	Cladophialophora	0.50
n/d Chloroflexia	3	Candidatus Solibacter	0.06	n/d Fungi	0.39	n/d Bacteroidia	0.47
Occallatibacter	3	Singulisphaera	0.06	Pezoloma	0.38	Mortierella	0.46
Rhodopila	3	Coccomyxa subellipsoidea C-169	0.05	Lechevalieria	0.38	Catenulispora	0.40
Edaphobacter	3	Trichoderma	0.03	ANPR*	0.38	Vibrissea	0.40
n/d Deltaproteobacteria	3	n/d Fungi	0.03	n/d AD3	0.38	n/d Acidobacteriia	0.33
Sphingomonas	3	Cladophialophora	0.01	n/d Bacteroidia	0.37	Terracidiphilus	0.33
Acidothermus	3	Lechevalieria	0.01	Catenulispora	0.37	Pezoloma	0.33
n/d Acidobacteriia	3	Chthoniobacter	0.01	Mycobacterium	0.36	Umbelopsis	0.33
Terracidiphilus	3	Candidatus Xiphinematobacter	0.01	Trichoderma	0.36	n/d Alphaproteobacteria	0.33
Umbelopsis	3	Edaphobacter	0.01	Chthoniobacter	0.36	n/d Ascomycota	0.29
n/d Alphaproteobacteria	3	n/d Deltaproteobacteria	0.01	Candidatus Xiphinematobacter	0.36	ANPR*	0.29
Streptomyces	2	n/d Alphaproteobacteria	0.00	Mucilaginibacter	0.36	Exophiala	0.28
Oidiodendron	2	Sphingomonas	0.00	Thelephora	0.35	n/d Herpotrichiellaceae	0.25
BCP**	2	Acidothermus	0.00	Cladophialophora	0.35	BCP**	0.00
n/d Bacteria	2	Mucilaginibacter	0.00	Streptomyces	0.35	n/d Dermateaceae	0.00
Nocardia	2	Conexibacter	0.00	Coccomyxa subellipsoidea C-169	0.34	Pisolithus	0.00
Coccomyxa subellipsoidea C-169	2	n/d Chloroflexia	0.00	n/d Ktedonobacteria	0.33	Inocybe	0.00
Candidatus Solibacter	2	Streptomyces	0.00	Umbelopsis	0.33	n/d Gammaproteobacteria	0.00
Singulisphaera	2	Oidiodendron	0.00	Granulicella	0.33	n/d Bacteria	0.00
n/d Dermateaceae	1	n/d Dermateaceae	0.00	Vibrissea	0.29	Nocardia	0.00
Pisolithus	1	Pisolithus	0.00	Edaphobacter	0.29	Arthrobacter	0.00
Inocybe	1	Inocybe	0.00	n/d Deltaproteobacteria	0.29	n/d AD3	0.00
n/d Gammaproteobacteria	1	n/d Gammaproteobacteria	0.00	Sphingomonas	0.29	Thelephora	0.00
Arthrobacter	1	Arthrobacter	0.00	Acidothermus	0.28	Coccomyxa subellipsoidea C-169	0.00
n/d AD3	1	n/d AD3	0.00	Candidatus Solibacter	0.28	n/d Ktedonobacteria	0.00
Thelephora	1	Thelephora	0.00	Penicillium	0.28	Granulicella	0.00
n/d Ktedonobacteria	1	n/d Ktedonobacteria	0.00	Oidiodendron	0.28	Candidatus Solibacter	0.00
Granulicella	1	Granulicella	0.00	n/d Alphaproteobacteria	0.27	Penicillium	0.00
Penicillium	1	Penicillium	0.00	n/d Hyaloscyphaceae	0.25	n/d Hyaloscyphaceae	0.00
n/d Hyaloscyphaceae	1	n/d Hyaloscyphaceae	0.00	Singulisphaera	0.23	Singulisphaera	0.00
Acidibacter	1	Acidibacter	0.00	Acidibacter	0.22	Acidibacter	0.00
n/d Gemmatimonadetes	1	n/d Gemmatimonadetes	0.00	n/d Gemmatimonadetes	0.19	n/d Gemmatimonadetes	0.00

* *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, ** *Burkholderia-Caballeronia-Paraburkholderia*

Table S98. Field birch sampling site network parameters.

Degree		Betweenness Centrality		Closeness Centrality		Clustering Coefficient	
node	value	node	value	node	value	node	value
Pajaroellobacter	5	Singulisphaera	1.00	Singulisphaera	1.00	n/d Acidobacteriia	1.00
Terracidiphilus	4	Terracidiphilus	0.60	n/d Planctomycetacia	1.00	n/d Bacteroidia	1.00
Lactarius	4	Lactarius	0.54	Sphingomonas	1.00	Acidipila	1.00
n/d Herpotrichiellaceae	4	Pajaroellobacter	0.49	Actinospica	1.00	n/d Alphaproteobacteria	1.00
n/d Chloroflexia	4	Acidothermus	0.40	Chthoniobacter	1.00	Roseiarcus	1.00
n/d Bacteria	4	n/d Ktedonobacteria	0.28	n/d Verrucomicrobiae	1.00	n/d Saccharimonadia	1.00
n/d Fungi	4	n/d Herpotrichiellaceae	0.18	n/d Melainabacteria	1.00	n/d AD3	1.00
n/d Planctomycetacia	3	n/d Planctomycetacia	0.17	Terracidiphilus	0.83	n/d Planctomycetacia	0.67
Sphingomonas	3	Sphingomonas	0.17	n/d Acidobacteriia	0.75	Sphingomonas	0.67
n/d Chytridiomycota	3	Inocybe	0.15	n/d Bacteroidia	0.75	n/d Chloroflexia	0.67
Cladophialophora	3	n/d Chytridiomycota	0.10	Aquisphaera	0.67	n/d Bacteria	0.67
Acidipila	3	n/d Chloroflexia	0.07	n/d Thelephoraceae	0.67	Cladophialophora	0.67
n/d Alphaproteobacteria	3	n/d Bacteria	0.07	Acidothermus	0.63	Terracidiphilus	0.50
Roseiarcus	3	n/d Fungi	0.06	Acidipila	0.63	n/d Fungi	0.50
n/d Saccharimonadia	3	Cladophialophora	0.01	n/d Alphaproteobacteria	0.63	n/d Herpotrichiellaceae	0.33
Singulisphaera	2	Acidipila	0.00	Roseiarcus	0.63	n/d Chytridiomycota	0.33
Acidothermus	2	n/d Alphaproteobacteria	0.00	Lactarius	0.54	Pajaroellobacter	0.30
n/d Ktedonobacteria	2	Roseiarcus	0.00	Pajaroellobacter	0.52	Lactarius	0.17
Inocybe	2	n/d Saccharimonadia	0.00	n/d Herpotrichiellaceae	0.43	Singulisphaera	0.00
n/d AD3	2	n/d AD3	0.00	n/d Fungi	0.43	Actinospica	0.00
n/d Acidobacteriia	2	n/d Acidobacteriia	0.00	n/d Chytridiomycota	0.42	Chthoniobacter	0.00
n/d Bacteroidia	2	n/d Bacteroidia	0.00	n/d Phycisphaerae	0.42	n/d Verrucomicrobiae	0.00
Aquisphaera	1	Aquisphaera	0.00	n/d Ktedonobacteria	0.41	n/d Melainabacteria	0.00
Actinospica	1	Actinospica	0.00	n/d Chloroflexia	0.39	Aquisphaera	0.00
Chthoniobacter	1	Chthoniobacter	0.00	n/d Bacteria	0.39	n/d Thelephoraceae	0.00
Mucilaginibacter	1	Mucilaginibacter	0.00	Cladophialophora	0.38	Acidothermus	0.00
n/d Ascomycota	1	n/d Ascomycota	0.00	n/d Saccharimonadia	0.38	n/d Phycisphaerae	0.00
n/d Verrucomicrobiae	1	n/d Verrucomicrobiae	0.00	Inocybe	0.31	n/d Ktedonobacteria	0.00
n/d Melainabacteria	1	n/d Melainabacteria	0.00	n/d Ascomycota	0.31	Inocybe	0.00
n/d Phycisphaerae	1	n/d Phycisphaerae	0.00	n/d AD3	0.30	n/d Ascomycota	0.00
n/d Thelephoraceae	1	n/d Thelephoraceae	0.00	Mucilaginibacter	0.24	Mucilaginibacter	0.00

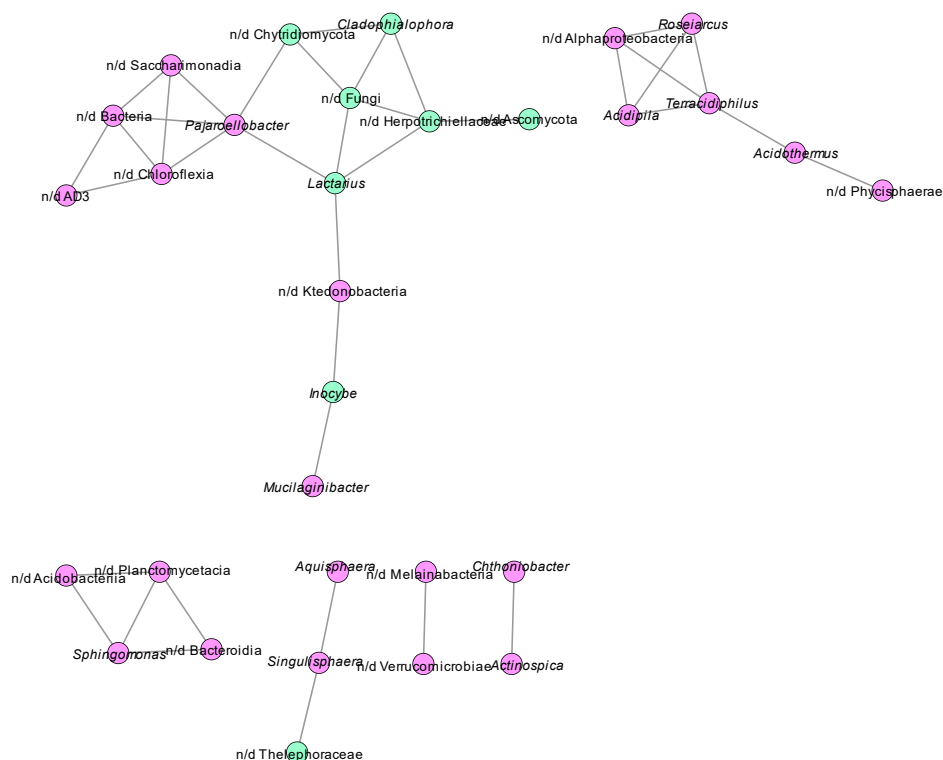


Figure S14. Field birch sampling site network. Each node represents bacterial or fungal ASVs assigned to genus level. Where ASVs could not be assigned to genus level, corresponding bacterial class or fungal family with the n/d were implemented. N/d Bacteria and n/d Fungi comprised all ASVs which could not be assigned for class or family level respectively. The colour of the node attributes assignment to bacteria (pink) or fungi (green).

Table S99. Field oak sampling site network parameters.

Degree		Betweenness Centrality		Closeness Centrality		Clustering Coefficient	
node	value	node	value	node	value	node	value
Haliangium	6	n/d Ascomycota	1.00	n/d Ascomycota	1.00	Mucilaginibacter	1.00
Phyllobacterium	6	Bryobacter	0.67	Bryobacter	1.00	BCP*	1.00
Plenodomus	6	Chryseolinea	0.67	Mucilaginibacter	1.00	n/d Bacteroidia	1.00
Trichoderma	6	Mesorhizobium	0.54	BCP*	1.00	Candidatus Xiphinematobacter	1.00
Bradyrhizobium	6	n/d Bacteria	0.53	n/d Bacteroidia	1.00	n/d Blastocatellia	1.00
n/d Thermoleophila	6	Pirellula	0.50	Candidatus Xiphinematobacter	1.00	n/d Fungi	1.00
n/d Bacteria	5	Variovorax	0.50	n/d Blastocatellia	1.00	Russula	1.00
Penicillium	5	Bdellovibrio	0.47	n/d Fungi	1.00	n/d Gammaproteobacteria	1.00
n/d Acidimicrobiia	4	Candidatus Udaeobacter	0.46	Russula	1.00	Pezoloma	1.00
IS-44	4	Penicillium	0.43	Solirubrobacter	1.00	Edaphobacter	1.00
Devosia	4	Flavisolibacter	0.36	Fimbrioglobus	1.00	Conexibacter	1.00
Chthoniobacter	4	Steroidobacter	0.25	n/d Planctomycetacia	1.00	Exophiala	1.00
Nordella	4	Bradyrhizobium	0.21	Hyphomicrobium	1.00	Gemmata	1.00
Steroidobacter	4	n/d Thermoleophila	0.21	Ferruginibacter	1.00	Acidibacter	1.00
Mucilaginibacter	3	Chthoniobacter	0.21	Lacunisphaera	1.00	n/d Deltaproteobacteria	1.00
BCP*	3	Nordella	0.21	Streptomyces	1.00	n/d Acidimicrobiia	1.00
n/d Bacteroidia	3	Serendipita	0.20	n/d Oxyphotobacteria	1.00	IS-44	1.00
Candidatus Xiphinematobacter	3	Haliangium	0.06	Rhodoplanes	1.00	Devosia	0.83
Edaphobacter	3	Phyllobacterium	0.06	n/d Verrucomicrobiae	1.00	Haliangium	0.80
Conexibacter	3	Plenodomus	0.06	Mycobacterium	1.00	Phyllobacterium	0.80
Exophiala	3	Trichoderma	0.06	Sphingomonas	1.00	Plenodomus	0.80
n/d Deltaproteobacteria	3	Devosia	0.04	Terrimonas	1.00	Trichoderma	0.80
Bryobacter	3	n/d Acidimicrobiia	0.00	Tardiphaga	1.00	Chthoniobacter	0.67
Mesorhizobium	3	IS-44	0.00	Bradyrhizobium	0.80	Nordella	0.67
Candidatus Udaeobacter	3	Mucilaginibacter	0.00	n/d Thermoleophila	0.80	n/d Bacteria	0.60
n/d Blastocatellia	2	BCP*	0.00	n/d Gammaproteobacteria	0.75	Bradyrhizobium	0.53
n/d Fungi	2	n/d Bacteroidia	0.00	Pezoloma	0.75	n/d Thermoleophila	0.53
Russula	2	Candidatus Xiphinematobacter	0.00	Penicillium	0.73	Steroidobacter	0.50
n/d Gammaproteobacteria	2	Edaphobacter	0.00	Chryseolinea	0.67	Penicillium	0.40
Pezoloma	2	Conexibacter	0.00	Devosia	0.67	Bryobacter	0.33
Gemmata	2	Exophiala	0.00	ANPR**	0.67	Mesorhizobium	0.33
Acidibacter	2	n/d Deltaproteobacteria	0.00	Ellin6067	0.67	n/d Ascomycota	0.00
n/d Ascomycota	2	n/d Blastocatellia	0.00	n/d Gemmatimonadetes	0.60	Solirubrobacter	0.00
Chryseolinea	2	n/d Fungi	0.00	Mesorhizobium	0.57	Fimbrioglobus	0.00
Pirellula	2	Russula	0.00	Pirellula	0.57	n/d Planctomycetacia	0.00
Variovorax	2	n/d Gammaproteobacteria	0.00	Variovorax	0.57	Hyphomicrobium	0.00
Bdellovibrio	2	Pezoloma	0.00	Steroidobacter	0.57	Ferruginibacter	0.00
Flavisolibacter	2	Gemmata	0.00	Nordella	0.57	Lacunisphaera	0.00
Serendipita	2	Acidibacter	0.00	n/d Bacteria	0.56	Streptomyces	0.00
Solirubrobacter	1	Solirubrobacter	0.00	Edaphobacter	0.53	n/d Oxyphotobacteria	0.00
Fimbrioglobus	1	Fimbrioglobus	0.00	Conexibacter	0.53	Rhodoplanes	0.00
n/d Planctomycetacia	1	n/d Planctomycetacia	0.00	Exophiala	0.53	n/d Verrucomicrobiae	0.00
Hyphomicrobium	1	Hyphomicrobium	0.00	Haliangium	0.50	Mycobacterium	0.00
Ferruginibacter	1	Ferruginibacter	0.00	Phyllobacterium	0.50	Sphingomonas	0.00
Lacunisphaera	1	Lacunisphaera	0.00	Plenodomus	0.50	Terrimonas	0.00
Streptomyces	1	Streptomyces	0.00	Trichoderma	0.50	Tardiphaga	0.00
n/d Oxyphotobacteria	1	n/d Oxyphotobacteria	0.00	Bdellovibrio	0.48	Chryseolinea	0.00
Rhodoplanes	1	Rhodoplanes	0.00	Candidatus Udaeobacter	0.47	ANPR**	0.00
n/d Verrucomicrobiae	1	n/d Verrucomicrobiae	0.00	Steroidobacter	0.47	Ellin6067	0.00
Mycobacterium	1	Mycobacterium	0.00	Gemmata	0.47	n/d Gemmatimonadetes	0.00
Sphingomonas	1	Sphingomonas	0.00	Acidibacter	0.47	Pirellula	0.00
Terrimonas	1	Terrimonas	0.00	n/d Deltaproteobacteria	0.44	Variovorax	0.00
Tardiphaga	1	Tardiphaga	0.00	Dongia	0.40	Bdellovibrio	0.00
ANPR**	1	ANPR**	0.00	n/d Subgroup 6	0.40	Candidatus Udaeobacter	0.00
Ellin6067	1	Ellin6067	0.00	Flavisolibacter	0.38	Dongia	0.00
n/d Gemmatimonadetes	1	n/d Gemmatimonadetes	0.00	n/d Acidimicrobiia	0.38	n/d Subgroup 6	0.00
Dongia	1	Dongia	0.00	IS-44	0.38	Flavisolibacter	0.00
n/d Subgroup 6	1	n/d Subgroup 6	0.00	Flavobacterium	0.33	Flavobacterium	0.00
Flavobacterium	1	Flavobacterium	0.00	n/d Phycisphaerae	0.33	n/d Phycisphaerae	0.00
n/d Phycisphaerae	1	n/d Phycisphaerae	0.00	Vicinamibacter	0.33	Vicinamibacter	0.00
Vicinamibacter	1	Vicinamibacter	0.00	Serendipita	0.30	Serendipita	0.00
n/d Holophagae	1	n/d Holophagae	0.00	n/d Holophagae	0.24	n/d Holophagae	0.00

* *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*

** *Burkholderia-Caballeronia-Paraburkholderia*

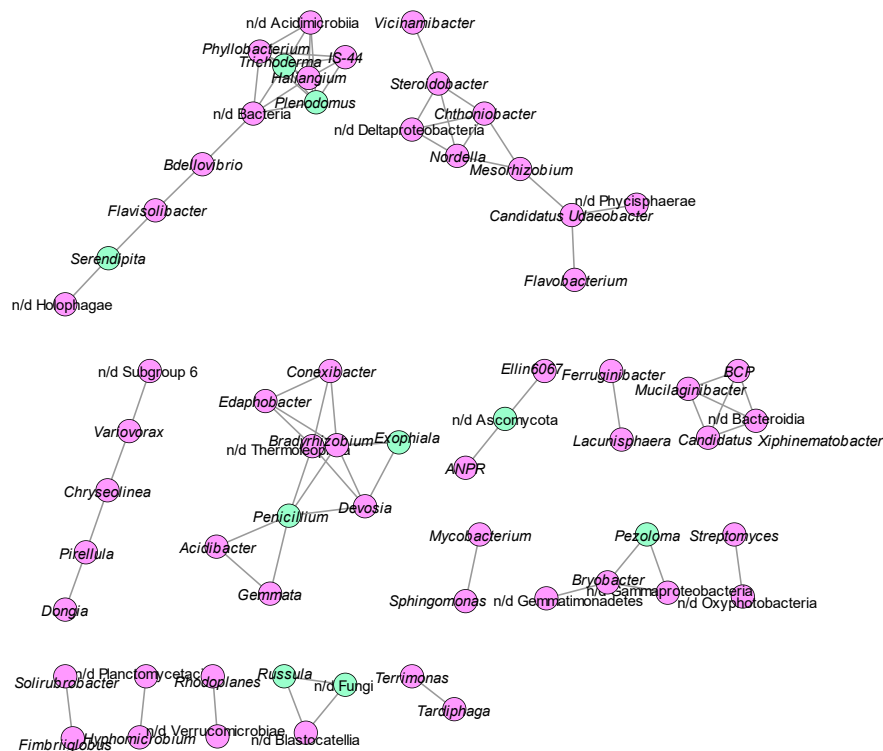


Figure S15. Field oak sampling site network. Each node represents bacterial or fungal ASVs assigned to genus level. Where ASVs could not be assigned to genus level, corresponding bacterial class or fungal family with the n/d were implemented. N/d Bacteria and n/d Fungi comprised all ASVs which could not be assigned for class or family level respectively. Here, abbreviation ANPR is used for a cluster *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, BCP is used for a cluster *Burkholderia-Caballeronia-Paraburkholderia*. The colour of the node attributes assignment to bacteria (pink) or fungi (green).

Table S100. Field pine sampling site network parameters.

Degree		Betweenness Centrality		Closeness Centrality		Clustering Coefficient	
node	value	node	value	node	value	node	value
n/d Planctomycetacia	6	Serendipita	1.00	Serendipita	1.00	ANPR*	1.00
Reyranella	6	Pezicula	0.55	n/d Acidimicrobiia	1.00	Mesorhizobium	1.00
Thelephora	6	n/d Subgroup 6	0.49	n/d Phycisphaerae	1.00	Sphingomonas	1.00
Bryobacter	6	n/d Chloroflexia	0.38	n/d Planctomycetacia	0.88	Flavisolibacter	1.00
n/d Thermoleophilii	6	Ellin6067	0.30	Reyranella	0.88	Haliangium	1.00
Sagenomella	6	Chthoniobacter	0.29	Thelephora	0.88	Mucilaginibacter	1.00
n/d Chloroflexia	6	n/d Oxyphotobacteria	0.22	Chthoniobacter	0.70	n/d Actinobacteria	1.00
ANPR*	5	Tomentella	0.22	ANPR*	0.70	Pezoloma	1.00
Mesorhizobium	5	n/d Blastocatellia	0.17	Mesorhizobium	0.70	n/d Gammaproteobacteria	1.00
Sphingomonas	5	Massilia	0.11	Sphingomonas	0.70	n/d Saccharimonadia	1.00
Mucilaginibacter	5	Bryobacter	0.11	n/d Alphaproteobacteria	0.67	Penicillium	1.00
n/d Actinobacteria	5	n/d Thermoleophilii	0.11	Cladophialophora	0.67	Skermanella	1.00
Pezoloma	5	Sagenomella	0.11	Pezicula	0.65	n/d Gemmatimonadetes	1.00
Pezicula	5	n/d Planctomycetacia	0.10	n/d Subgroup 6	0.60	n/d Planctomycetacia	0.80
n/d Oxyphotobacteria	5	Reyranella	0.10	Bryobacter	0.58	Reyranella	0.80
Tomentella	5	Thelephora	0.10	n/d Thermoleophilii	0.58	Thelephora	0.80
n/d Subgroup 6	5	n/d Verrucomicrobiae	0.05	Sagenomella	0.58	Bryobacter	0.80
Chthoniobacter	4	Flavitalea	0.04	n/d Chloroflexia	0.57	n/d Thermoleophilii	0.80
Massilia	4	n/d Anaeroliniae	0.02	n/d Oxyphotobacteria	0.55	Sagenomella	0.80
Flavisolibacter	3	ANPR*	0.00	Tomentella	0.55	n/d Verrucomicrobiae	0.67
Haliangium	3	Mesorhizobium	0.00	n/d Blastocatellia	0.50	Chthoniobacter	0.50
n/d Verrucomicrobiae	3	Sphingomonas	0.00	Massilia	0.48	Pezicula	0.40
Ellin6067	3	Mucilaginibacter	0.00	Flavisolibacter	0.48	n/d Oxyphotobacteria	0.40
Flavitalea	3	n/d Actinobacteria	0.00	Haliangium	0.48	Tomentella	0.40
n/d Blastocatellia	3	Pezoloma	0.00	n/d Verrucomicrobiae	0.46	Massilia	0.33
n/d Gammaproteobacteria	2	Flavisolibacter	0.00	Ellin6067	0.44	Ellin6067	0.33
n/d Saccharimonadia	2	Haliangium	0.00	Mucilaginibacter	0.44	Flavitalea	0.33
Penicillium	2	n/d Gammaproteobacteria	0.00	n/d Actinobacteria	0.44	n/d Subgroup 6	0.30
Skermanella	2	n/d Saccharimonadia	0.00	Pezoloma	0.44	n/d Chloroflexia	0.27
n/d Gemmatimonadetes	2	Penicillium	0.00	n/d Deltaproteobacteria	0.44	Serendipita	0.00
Serendipita	2	Skermanella	0.00	n/d Gammaproteobacteria	0.38	n/d Acidimicrobiia	0.00
n/d Anaeroliniae	2	n/d Gemmatimonadetes	0.00	n/d Saccharimonadia	0.38	n/d Phycisphaerae	0.00
n/d Acidimicrobiia	1	n/d Acidimicrobiia	0.00	Penicillium	0.38	n/d Alphaproteobacteria	0.00
n/d Phycisphaerae	1	n/d Phycisphaerae	0.00	n/d Anaeroliniae	0.38	Cladophialophora	0.00
n/d Alphaproteobacteria	1	n/d Alphaproteobacteria	0.00	Ferruginibacter	0.38	n/d Blastocatellia	0.00
Cladophialophora	1	Cladophialophora	0.00	Flavitalea	0.36	n/d Deltaproteobacteria	0.00
n/d Deltaproteobacteria	1	n/d Deltaproteobacteria	0.00	Skermanella	0.32	n/d Anaeroliniae	0.00
Ferruginibacter	1	Ferruginibacter	0.00	n/d Gemmatimonadetes	0.32	Ferruginibacter	0.00

* *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*

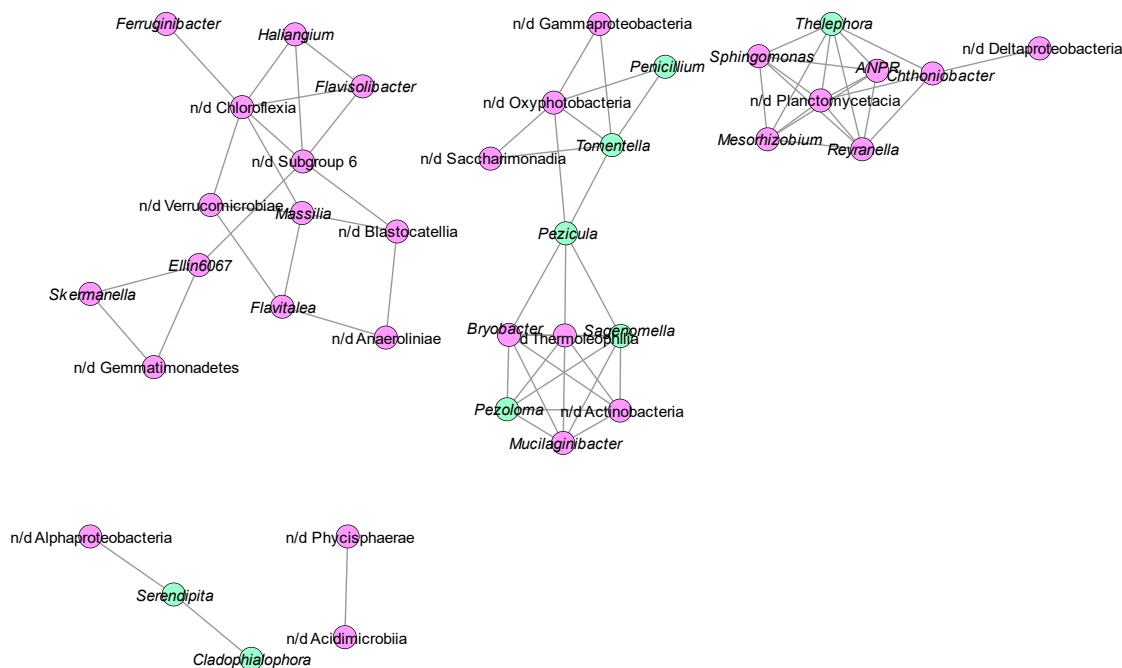


Figure S16. Field pine sampling site network. Each node represents bacterial or fungal ASVs assigned to genus level. Where ASVs could not be assigned to genus level, corresponding bacterial class or fungal family with the n/d were implemented. Here, abbreviation ANPR is used for a cluster *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*. The colour of the node attributes assignment to bacteria (pink) or fungi (green).

Table S101. Pot birch network parameters.

Degree		Betweenness Centrality		Closeness Centrality		Clustering Coefficient	
node	value	node	value	node	value	node	value
n/d Actinobacteria	15	n/d Gammaproteobacteria	0.57	Oidiodendron	1.00	n/d AD3	1.00
Pseudeurotium	11	n/d Acidobacteriia	0.44	Thelephora	1.00	Granulicella	1.00
Jatrophihabitans	10	n/d Alphaproteobacteria	0.39	n/d Actinobacteria	0.57	Candidatus Solibacter	1.00
n/d Armatimonadi	10	Nocardia	0.34	Pseudeurotium	0.52	n/d Gemmatimonadetes	1.00
Solicoccozyma	10	Acidipila	0.28	n/d Armatimonadi	0.49	Edaphobacter	1.00
n/d Gammaproteobacteria	10	n/d Actinobacteria	0.19	Rickenella	0.49	Acidibacter	1.00
Rickenella	10	Pseudeurotium	0.19	Solicoccozyma	0.48	Acidothermus	1.00
Mollisia	9	Jatrophihabitans	0.17	n/d Gammaproteobacteria	0.48	n/d Thermoleophilia	0.92
n/d Thermoleophilia	9	Ktedonobacter	0.12	Jatrophihabitans	0.46	n/d Bacteria	0.92
n/d Bacteria	9	n/d Herpotrichiellaceae	0.10	n/d Chloroflexia	0.46	n/d Fungi	0.92
n/d Fungi	9	Sphingomonas	0.09	Sphingomonas	0.45	n/d Deltaproteobacteria	0.87
Mycosphaerella	9	n/d Phycisphaerae	0.09	Mollisia	0.45	Occallatibacter	0.87
Sphingomonas	8	Solicoccozyma	0.07	Mycosphaerella	0.45	Terracidiphilus	0.87
Pezoloma	8	Catenulispora	0.07	n/d Herpotrichiellaceae	0.45	Rhodopila	0.87
Actinospica	7	Arthrobacter	0.06	n/d Acidimicrobiia	0.45	Mollisia	0.86
BCP*	7	Actinospica	0.06	Conexibacter	0.45	Mycosphaerella	0.86
Mucilaginibacter	7	BCP*	0.06	n/d Thermoleophilia	0.45	Actinospica	0.86
n/d Bacteroidia	7	Mucilaginibacter	0.06	n/d Bacteria	0.45	BCP*	0.86
Catenulispora	7	n/d Bacteroidia	0.06	n/d Fungi	0.45	Mucilaginibacter	0.86
n/d Acidimicrobiia	7	n/d Armatimonadi	0.05	Lactarius	0.43	n/d Bacteroidia	0.86
Conexibacter	7	Lactarius	0.05	Mortierella	0.43	Rickenella	0.80
Granulicella	6	Mortierella	0.05	Pezoloma	0.42	Umbelopsis	0.80
Candidatus Solibacter	6	n/d Hyaloscyphaceae	0.05	Umbelopsis	0.42	Bryobacter	0.80
Cladophialophora	6	Cladophialophora	0.04	n/d Acidobacteriia	0.42	n/d Ascomycota	0.80
n/d Ktedonobacteria	6	n/d Ktedonobacteria	0.04	n/d Hyaloscyphaceae	0.41	Jatrophihabitans	0.73
Lactarius	6	Placynthiella	0.04	n/d AD3	0.41	n/d Phycisphaerae	0.73
Mortierella	6	n/d Chloroflexia	0.03	n/d Phycisphaerae	0.41	Talaromyces	0.73
n/d Deltaproteobacteria	6	Mycosymbiotes	0.03	Placynthiella	0.40	n/d Acidimicrobiia	0.71
Occallatibacter	6	Penicillium	0.03	Mycosymbiotes	0.39	Conexibacter	0.71
Terracidiphilus	6	Pezoloma	0.02	Actinospica	0.38	n/d Armatimonadi	0.71
n/d Phycisphaerae	6	Rickenella	0.02	BCP*	0.38	Pezoloma	0.57
Rhodopila	6	n/d Deltaproteobacteria	0.02	Mucilaginibacter	0.38	Cladophialophora	0.53
n/d Gemmatimonadetes	6	Occallatibacter	0.02	n/d Bacteroidia	0.38	n/d Ktedonobacteria	0.53
Talaromyces	6	Terracidiphilus	0.02	Cladophialophora	0.38	Lactarius	0.47
Umbelopsis	6	Rhodopila	0.02	n/d Ktedonobacteria	0.38	Mortierella	0.47
Bryobacter	5	n/d Acidimicrobiia	0.01	Talaromyces	0.38	Catenulispora	0.43
n/d Ascomycota	5	Conexibacter	0.01	n/d Deltaproteobacteria	0.36	n/d Actinobacteria	0.41
n/d Herpotrichiellaceae	5	Mollisia	0.01	Occallatibacter	0.36	Solicoccozyma	0.40
Mycosymbiotes	5	Mycosphaerella	0.01	Terracidiphilus	0.36	n/d Chloroflexia	0.40
n/d Chloroflexia	5	Umbelopsis	0.01	Rhodopila	0.36	Penicillium	0.40
Penicillium	5	Talaromyces	0.00	Catenulispora	0.36	n/d Gammaproteobacteria	0.38
Acidipila	4	Bryobacter	0.00	Penicillium	0.35	Pseudeurotium	0.36
Edaphobacter	4	n/d Ascomycota	0.00	n/d Alphaproteobacteria	0.35	Sphingomonas	0.36
n/d AD3	3	n/d Thermoleophilia	0.00	Ktedonobacter	0.33	n/d Acidobacteriia	0.33
n/d Acidobacteriia	3	n/d Bacteria	0.00	Bryobacter	0.32	n/d Hyaloscyphaceae	0.33
n/d Hyaloscyphaceae	3	n/d Fungi	0.00	n/d Ascomycota	0.32	Placynthiella	0.33
Placynthiella	3	Granulicella	0.00	Granulicella	0.30	n/d Herpotrichiellaceae	0.30
Acidibacter	2	Candidatus Solibacter	0.00	Candidatus Solibacter	0.30	Mycosymbiotes	0.20
Acidothermus	2	n/d Gemmatimonadetes	0.00	n/d Gemmatimonadetes	0.30	Acidipila	0.17
Arthrobacter	2	Edaphobacter	0.00	Nocardia	0.29	Oidiodendron	0.00
Ktedonobacter	2	n/d AD3	0.00	Edaphobacter	0.28	Thelephora	0.00
n/d Alphaproteobacteria	2	Acidibacter	0.00	Arthrobacter	0.26	n/d Alphaproteobacteria	0.00
Nocardia	2	Acidothermus	0.00	Acidipila	0.25	Ktedonobacter	0.00
Mycobacterium	1	Mycobacterium	0.00	Mycobacterium	0.20	Nocardia	0.00
n/d Thelephoraceae	1	n/d Thelephoraceae	0.00	Acidibacter	0.20	Arthrobacter	0.00
Oidiodendron	1	Oidiodendron	0.00	Acidothermus	0.20	Mycobacterium	0.00
Thelephora	1	Thelephora	0.00	n/d Thelephoraceae	0.20	n/d Thelephoraceae	0.00

* *Burkholderia-Caballeronia-Paraburkholderia*

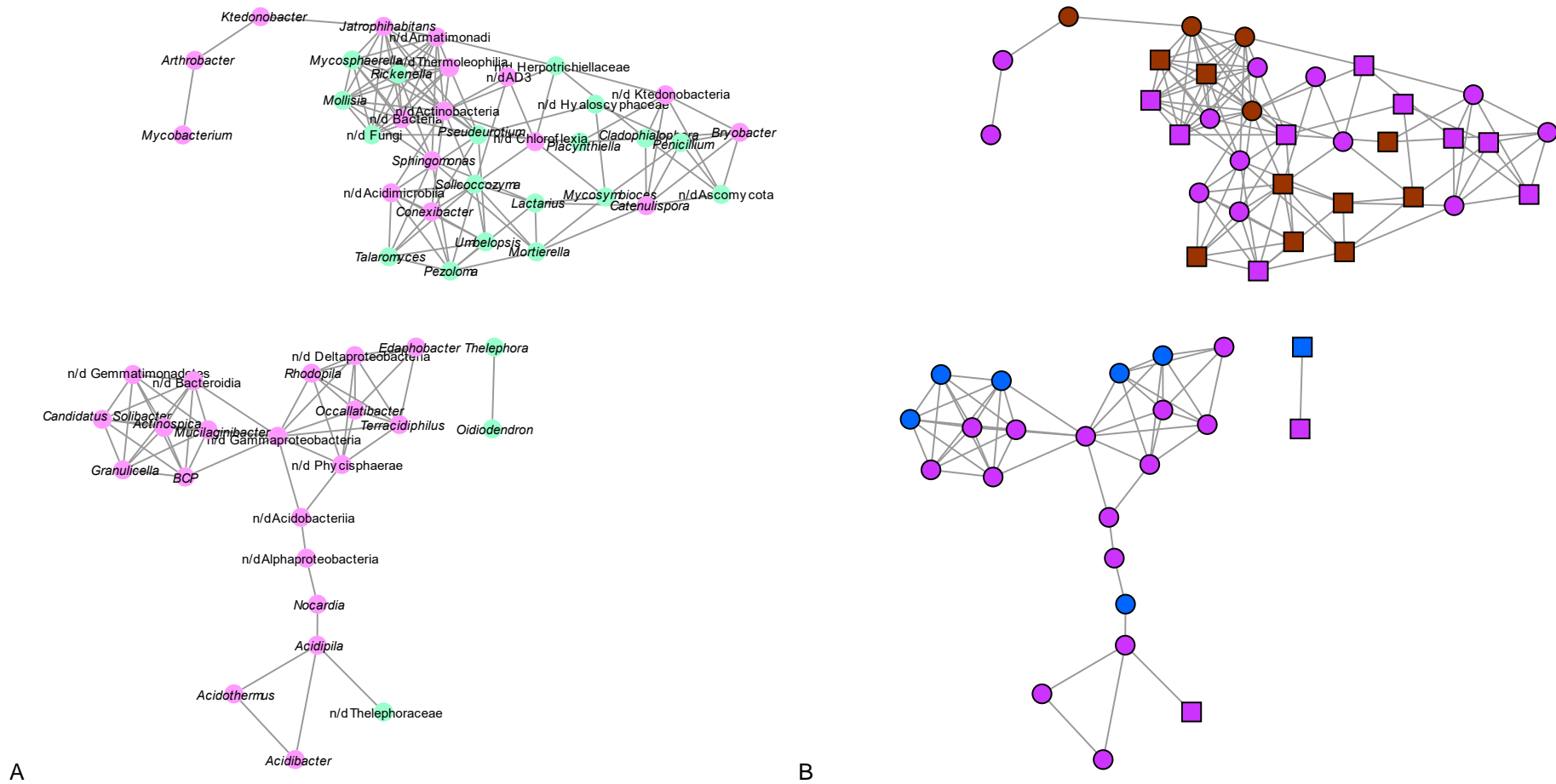


Figure S17. Pot birch network consists of pooled taxonomic data for pot birch mycorrhizosphere and control pot substrate. **A** – network of co-occurring species. Each node represents bacterial or fungal ASVs assigned to genus level. Where ASVs could not be assigned to genus level, corresponding bacterial class or fungal family with the n/d were implemented. Here, abbreviation *BCP* is used for a cluster *Burkholderia-Caballeronia-Paraburkholderia*. The colour of the node attributes assignment to bacteria (pink) or fungi (green). **B** – network of co-occurring generalists and specialists. The shape of the node attributes assignment to bacteria (round) or fungi (square). The colour of the node attributes ASVs occupancy preference: generalists found in mycorrhizosphere of pot birch as well as in control pot substrate (purple), specialists exhibited preference to pot birch mycorrhizosphere (blue).

Table S102. Pot oak network parameters.

Degree		Betweenness Centrality		Closeness Centrality		Clustering Coefficient	
node	value	node	value	node	value	node	value
n/d Acidimicrobiia	5	n/d Gemmatimonadetes	1.00	n/d Gemmatimonadetes	1.00	n/d Acidimicrobiia	1.00
Mycosphaerella	5	n/d Armatimonadi	1.00	n/d Armatimonadi	1.00	Mycosphaerella	1.00
n/d Fungi	5	n/d Acidimicrobiia	0.00	n/d Acidimicrobiia	1.00	n/d Fungi	1.00
Rickenella	5	Mycosphaerella	0.00	Mycosphaerella	1.00	Rickenella	1.00
Talaromyces	5	n/d Fungi	0.00	n/d Fungi	1.00	Talaromyces	1.00
Umbelopsis	5	Rickenella	0.00	Rickenella	1.00	Umbelopsis	1.00
Jatrophihabitans	4	Talaromyces	0.00	Talaromyces	1.00	Jatrophihabitans	1.00
Conexibacter	4	Umbelopsis	0.00	Umbelopsis	1.00	Conexibacter	1.00
Ktedonobacter	4	Jatrophihabitans	0.00	Jatrophihabitans	1.00	Ktedonobacter	1.00
Mollisia	4	Conexibacter	0.00	Conexibacter	1.00	Mollisia	1.00
n/d Bacteria	4	Ktedonobacter	0.00	Ktedonobacter	1.00	n/d Bacteria	1.00
Sphingomonas	3	Mollisia	0.00	Mollisia	1.00	Sphingomonas	1.00
Laccaria	3	n/d Bacteria	0.00	n/d Bacteria	1.00	Laccaria	1.00
Acidothermus	3	Sphingomonas	0.00	Sphingomonas	1.00	Acidothermus	1.00
n/d Basidiomycota	3	Laccaria	0.00	Laccaria	1.00	n/d Basidiomycota	1.00
n/d Hyaloscyphaceae	3	Acidothermus	0.00	Acidothermus	1.00	n/d Hyaloscyphaceae	1.00
Pisolithus	3	n/d Basidiomycota	0.00	n/d Basidiomycota	1.00	Pisolithus	1.00
Solicozozyma	3	n/d Hyaloscyphaceae	0.00	n/d Hyaloscyphaceae	1.00	Solicozozyma	1.00
Vibrissea	3	Pisolithus	0.00	Pisolithus	1.00	Vibrissea	1.00
n/d Gemmatimonadetes	2	Solicozozyma	0.00	Solicozozyma	1.00	Occallatibacter	1.00
n/d Armatimonadi	2	Vibrissea	0.00	Vibrissea	1.00	Actinospica	1.00
Occallatibacter	2	Occallatibacter	0.00	Occallatibacter	1.00	BCP*	1.00
Actinospica	2	Actinospica	0.00	Actinospica	1.00	Catenulispora	1.00
BCP*	2	BCP*	0.00	BCP*	1.00	Cladophialophora	1.00
Catenulispora	2	Catenulispora	0.00	Catenulispora	1.00	Mucilaginibacter	1.00
Cladophialophora	2	Cladophialophora	0.00	Cladophialophora	1.00	n/d Bacteroidia	1.00
Mucilaginibacter	2	Mucilaginibacter	0.00	Mucilaginibacter	1.00	n/d Deltaproteobacteria	1.00
n/d Bacteroidia	2	n/d Bacteroidia	0.00	n/d Bacteroidia	1.00	Oidiodendron	1.00
n/d Deltaproteobacteria	2	n/d Deltaproteobacteria	0.00	n/d Deltaproteobacteria	1.00	n/d Ascomycota	1.00
Oidiodendron	2	Oidiodendron	0.00	Oidiodendron	1.00	Penicillium	1.00
n/d Ascomycota	2	n/d Ascomycota	0.00	n/d Ascomycota	1.00	Placynthiella	1.00
Penicillium	2	Penicillium	0.00	Penicillium	1.00	n/d Gemmatimonadetes	0.00
Placynthiella	2	Placynthiella	0.00	Placynthiella	1.00	n/d Armatimonadi	0.00
Singulisphaera	1	Singulisphaera	0.00	Singulisphaera	1.00	Singulisphaera	0.00
Chthoniobacter	1	Chthoniobacter	0.00	Chthoniobacter	1.00	Chthoniobacter	0.00
n/d Gammaproteobacteria	1	n/d Gammaproteobacteria	0.00	n/d Gammaproteobacteria	1.00	n/d Gammaproteobacteria	0.00
Inocybe	1	Inocybe	0.00	Inocybe	1.00	Inocybe	0.00
n/d Herpotrichiellaceae	1	n/d Herpotrichiellaceae	0.00	n/d Herpotrichiellaceae	1.00	n/d Herpotrichiellaceae	0.00
Lactarius	1	Lactarius	0.00	Lactarius	1.00	Lactarius	0.00
n/d Subgroup 6	1	n/d Subgroup 6	0.00	n/d Subgroup 6	1.00	n/d Subgroup 6	0.00
Mycobacterium	1	Mycobacterium	0.00	Mycobacterium	1.00	Mycobacterium	0.00
Bryobacter	1	Bryobacter	0.00	Bryobacter	1.00	Bryobacter	0.00
n/d Oxyphotobacteria	1	n/d Oxyphotobacteria	0.00	n/d Oxyphotobacteria	1.00	n/d Oxyphotobacteria	0.00
n/d Acidobacteriia	1	n/d Acidobacteriia	0.00	n/d Acidobacteriia	1.00	n/d Acidobacteriia	0.00
n/d Thelephoraceae	1	n/d Thelephoraceae	0.00	n/d Thelephoraceae	1.00	n/d Thelephoraceae	0.00
Granulicella	1	Granulicella	0.00	Granulicella	1.00	Granulicella	0.00
Rhodopila	1	Rhodopila	0.00	Rhodopila	1.00	Rhodopila	0.00
Exophiala	1	Exophiala	0.00	Exophiala	0.67	Exophiala	0.00
n/d Phycisphaerae	1	n/d Phycisphaerae	0.00	n/d Phycisphaerae	0.67	n/d Phycisphaerae	0.00
n/d Alphaproteobacteria	1	n/d Alphaproteobacteria	0.00	n/d Alphaproteobacteria	0.67	n/d Alphaproteobacteria	0.00
Edaphobacter	1	Edaphobacter	0.00	Edaphobacter	0.67	Edaphobacter	0.00

* *Burkholderia-Caballeronia-Paraburkholderia*

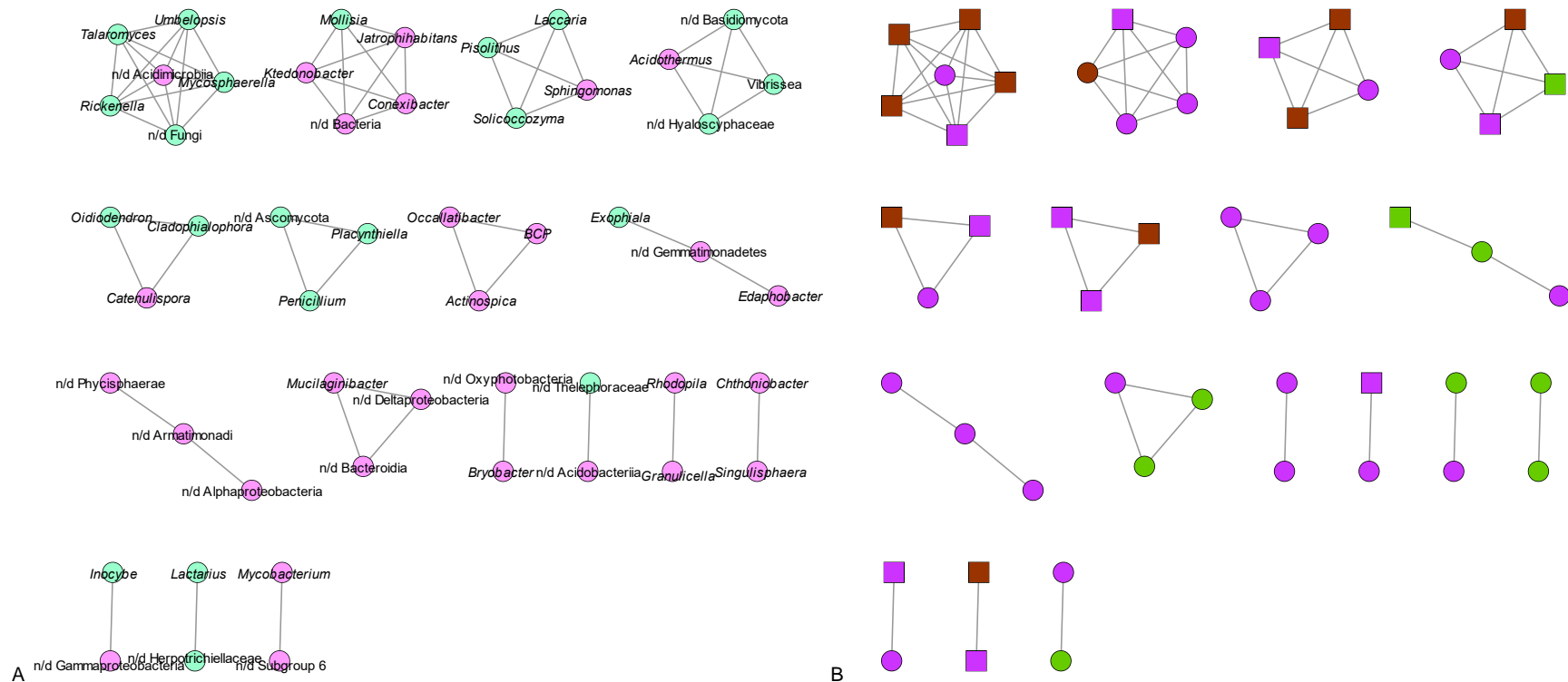


Figure S18. Pot oak network consists of pooled taxonomic data for pot oak mycorrhizosphere and control pot substrate. **A** – network of co-occurring species. Each node represents bacterial or fungal ASVs assigned to genus level. Where ASVs could not be assigned to genus level, corresponding bacterial class or fungal family with the n/d were implemented. Here, abbreviation *BCP* is used for a cluster *Burkholderia-Caballeronia-Paraburkholderia*. The colour of the node attributes assignment to bacteria (pink) or fungi (green). **B** – network of co-occurring generalists and specialists. The shape of the node attributes assignment to bacteria (round) or fungi (square). The colour of the node attributes ASVs occupancy preference: generalists found in mycorrhizosphere of pot oak as well as in control pot substrate (purple), specialists exhibited preference to pot oak mycorrhizosphere (green) or control pot substrate (red).

Table S103. Pot pine network parameters.

Degree		Betweenness Centrality		Closeness Centrality		Clustering Coefficient	
node	value	node	value	node	value	node	value
n/d Armatimonadi	16	Edaphobacter	0.18	Edaphobacter	0.57	Pezoloma	1.00
Pisolithus	16	n/d Gammaproteobacteria	0.14	n/d Gammaproteobacteria	0.57	Talaromyces	1.00
n/d Acidimicrobiia	15	Mycobacterium	0.13	BCP*	0.57	Acidibacter	1.00
Jatrophihabitans	15	n/d Thelephoraceae	0.11	Candidatus Solibacter	0.57	Mollisia	0.83
Conexibacter	15	n/d Hyaloscyphaceae	0.11	n/d Bacteroidia	0.57	n/d Bacteria	0.83
Rickenella	15	BCP*	0.11	n/d Deltaproteobacteria	0.57	Umbelopsis	0.83
Sphingomonas	15	Terracidiphilus	0.10	Mycobacterium	0.55	Granulicella	0.76
Solicocozyma	15	Coccomyxa subellipsoidea C-169	0.10	n/d Phycisphaerae	0.55	Mucilaginibacter	0.76
Mycobacterium	14	n/d Ascomycota	0.10	Granulicella	0.55	Arthrobacter	0.73
n/d AD3	14	Pseudeurotium	0.09	Mucilaginibacter	0.55	Bryobacter	0.73
n/d Thermoleophilia	13	n/d Phycisphaerae	0.09	Pseudeurotium	0.54	Penicillium	0.73
Mollisia	12	n/d AD3	0.07	n/d Chloroflexia	0.54	Laccaria	0.73
n/d Bacteria	12	Cladophialophora	0.06	n/d Herpotrichiellaceae	0.54	Candidatus Solibacter	0.71
Umbelopsis	12	n/d Basidiomycota	0.06	Lactarius	0.54	n/d Bacteroidia	0.71
Pseudeurotium	12	Actinospica	0.05	Sphingomonas	0.54	n/d Deltaproteobacteria	0.71
n/d Chloroflexia	11	Inocybe	0.05	Solicocozyma	0.54	n/d Acidimicrobiia	0.71
n/d Herpotrichiellaceae	11	n/d Chloroflexia	0.05	n/d Armatimonadi	0.54	Jatrophihabitans	0.71
Lactarius	11	n/d Herpotrichiellaceae	0.05	Pisolithus	0.54	Conexibacter	0.71
Arthrobacter	10	Lactarius	0.05	n/d Acidimicrobiia	0.54	Rickenella	0.71
n/d Ktedonobacteria	9	n/d Alphaproteobacteria	0.05	Jatrophihabitans	0.54	Occallatibacter	0.67
n/d Ascomycota	9	n/d Actinobacteria	0.05	Conexibacter	0.54	Rhodopila	0.67
n/d Actinobacteria	9	Granulicella	0.04	Rickenella	0.54	n/d Armatimonadi	0.64
Candidatus Solibacter	8	Mucilaginibacter	0.04	n/d AD3	0.53	Pisolithus	0.64
n/d Bacteroidia	8	Sphingomonas	0.03	n/d Thermoleophilia	0.53	Sphingomonas	0.64
n/d Deltaproteobacteria	8	Solicocozyma	0.03	Mollisia	0.52	Solicocozyma	0.64
n/d Fungi	8	n/d Thermoleophilia	0.03	n/d Bacteria	0.52	Edaphobacter	0.62
Mycosphaerella	8	Candidatus Solibacter	0.03	Umbelopsis	0.52	n/d Fungi	0.61
Pezoloma	7	n/d Bacteroidia	0.03	n/d Thelephoraceae	0.50	Mycosphaerella	0.61
Granulicella	7	n/d Deltaproteobacteria	0.03	Arthrobacter	0.50	n/d Thermoleophilia	0.60
Mucilaginibacter	7	n/d Armatimonadi	0.03	n/d Ascomycota	0.49	n/d Chloroflexia	0.60
Edaphobacter	7	Pisolithus	0.03	Terracidiphilus	0.49	n/d Herpotrichiellaceae	0.60
BCP*	7	Placynthiella	0.03	n/d Fungi	0.48	Lactarius	0.60
n/d Phycisphaerae	7	n/d Ktedonobacteria	0.02	Mycosphaerella	0.48	Actinospica	0.60
Mycosymbiocytes	7	n/d Fungi	0.02	Actinospica	0.47	Inocybe	0.60
Cladophialophora	7	Mycosphaerella	0.02	Inocybe	0.47	Placynthiella	0.60
n/d Gammaproteobacteria	7	n/d Acidimicrobiia	0.02	Bryobacter	0.46	BCP*	0.57
Bryobacter	6	Jatrophihabitans	0.02	Penicillium	0.46	n/d Phycisphaerae	0.57
Penicillium	6	Conexibacter	0.02	n/d Actinobacteria	0.45	Mycosymbiocytes	0.57
Laccaria	6	Rickenella	0.02	Placynthiella	0.45	Cladophialophora	0.52
Actinospica	5	Mycosymbiocytes	0.01	Cladophialophora	0.44	n/d Ktedonobacteria	0.50
Inocybe	5	Occallatibacter	0.01	n/d Ktedonobacteria	0.44	n/d Hyaloscyphaceae	0.50
Placynthiella	5	Rhodopila	0.01	Pezoloma	0.44	Pseudeurotium	0.48
Terracidiphilus	5	Mollisia	0.01	Mycosymbiocytes	0.43	Mycobacterium	0.47
Talaromyces	4	n/d Bacteria	0.01	Laccaria	0.43	n/d AD3	0.47
Occallatibacter	4	Umbelopsis	0.01	Coccomyxa subellipsoidea C-169	0.43	n/d Ascomycota	0.47
Rhodopila	4	Arthrobacter	0.01	Occallatibacter	0.40	n/d Actinobacteria	0.44
n/d Hyaloscyphaceae	4	Bryobacter	0.01	Rhodopila	0.40	n/d Gammaproteobacteria	0.43
Coccomyxa subellipsoidea C-169	3	Penicillium	0.01	n/d Hyaloscyphaceae	0.39	Terracidiphilus	0.40
n/d Alphaproteobacteria	3	Laccaria	0.01	n/d Alphaproteobacteria	0.39	Coccomyxa subellipsoidea C-169	0.33
n/d Thelephoraceae	3	Pezoloma	0.00	Talaromyces	0.36	n/d Alphaproteobacteria	0.33
Acidibacter	2	Talaromyces	0.00	Acidibacter	0.33	n/d Thelephoraceae	0.00
n/d Basidiomycota	2	Acidibacter	0.00	n/d Basidiomycota	0.29	n/d Basidiomycota	0.00
Acidothermus	1	Acidothermus	0.00	Acidothermus	0.23	Acidothermus	0.00

* *Burkholderia-Caballeronia-Paraburkholderia*

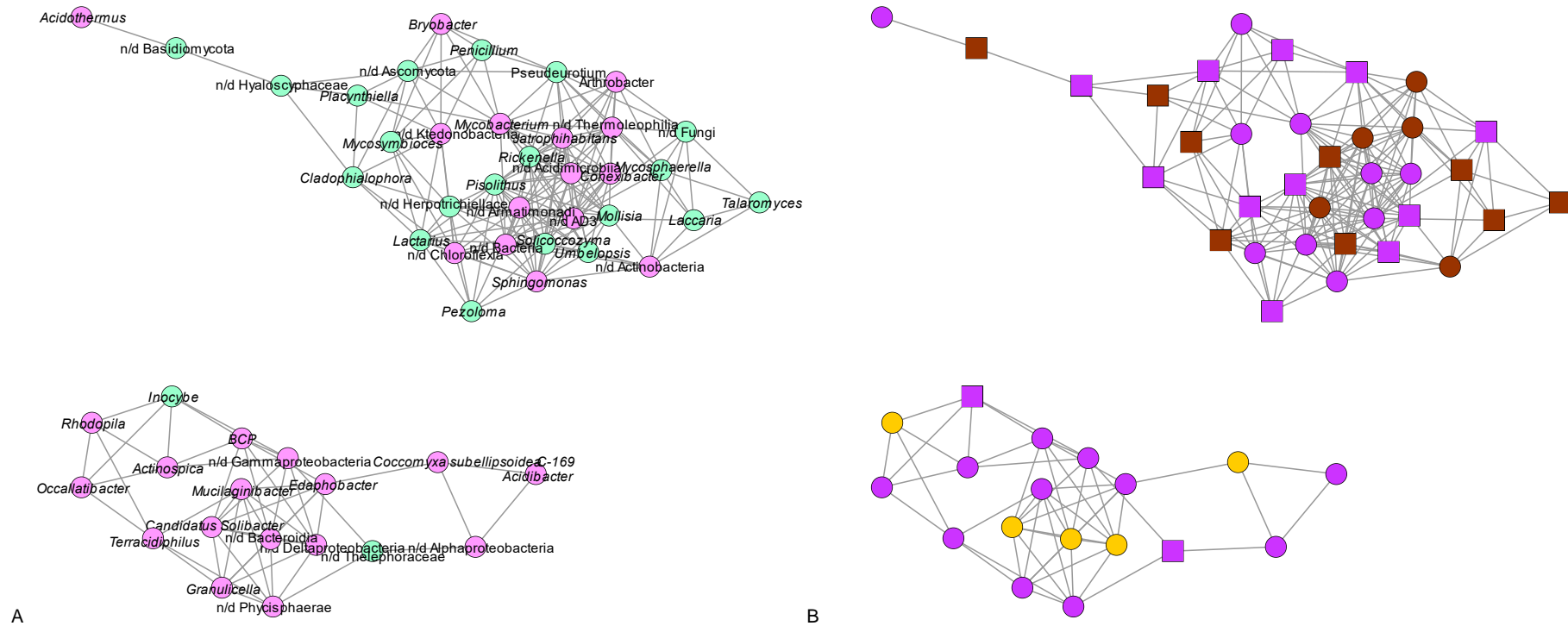
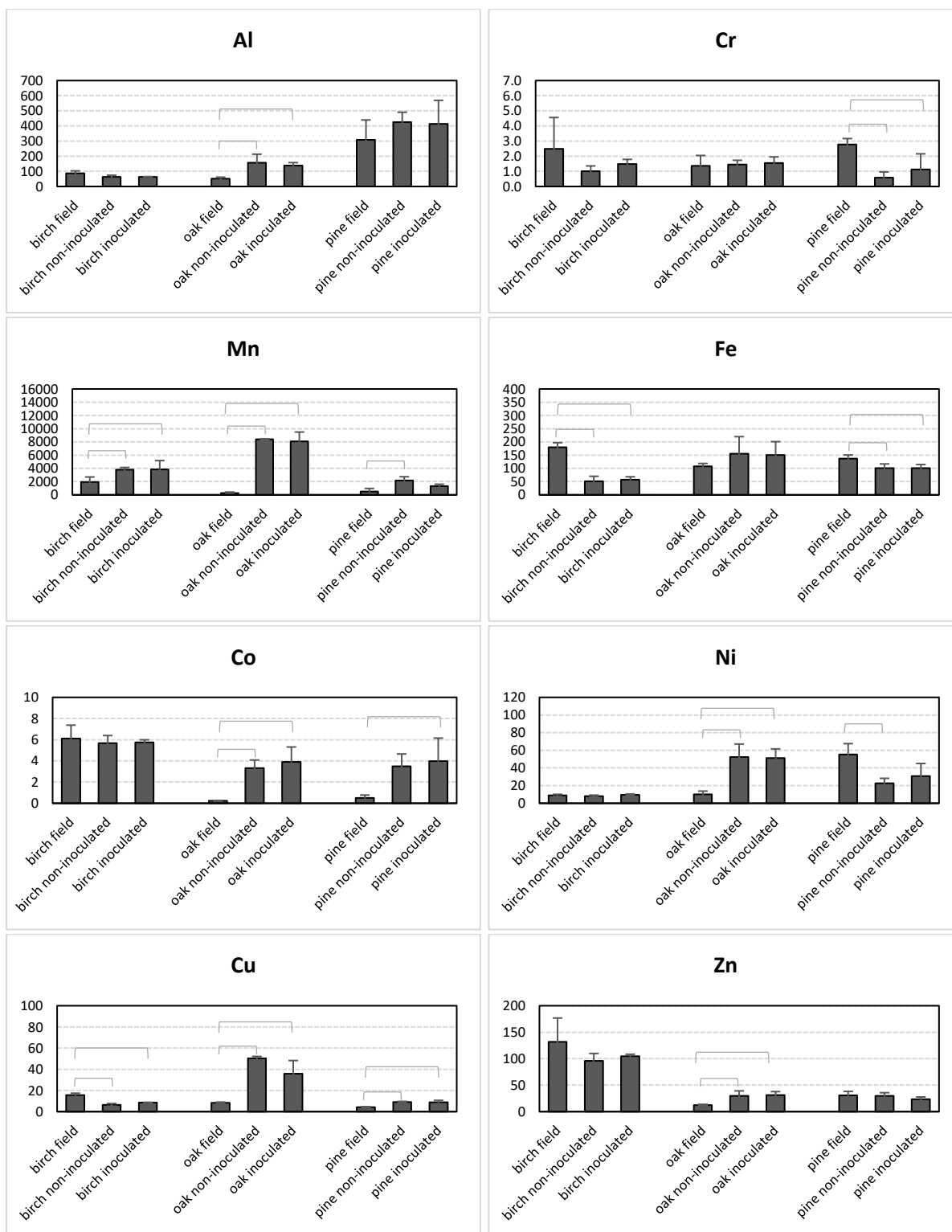


Figure S19. Pot pine network consists of pooled taxonomic data for pot pine mycorrhizosphere and control pot substrate. **A** – network of co-occurring species. Each node represents bacterial or fungal ASVs assigned to genus level. Where ASVs could not be assigned to genus level, corresponding bacterial class or fungal family with the n/d were implemented. Here, abbreviation *BCP* is used for a cluster *Burkholderia-Caballeronia-Paraburkholderia*. The colour of the node attributes assignment to bacteria (pink) or fungi (green). **B** – network of co-occurring generalists and specialists. The shape of the node attributes assignment to bacteria (round) or fungi (square). The colour of the node attributes ASVs occupancy preference: generalists found in mycorrhizosphere of pot pine as well as in control pot substrate (purple), specialists exhibited preference to pot pine mycorrhizosphere (yellow) or control pot substrate (red).

Table S104. Coefficients of correlation between soil characteristics and ectomycorrhizal morphotypes described for the field plants. Asterisks represent significant correlation ($p < 0.05$).

Soil characteristics	<i>Lactarius</i> sp.	<i>M. bicolor</i>	<i>Mallocybe</i> sp.	O_F_MT2	Helotiales	<i>Cortinarius</i> sp.	Thelephoraceae	<i>R. mohelnensis</i>	<i>T. argyraceum</i>
Al	0.76*	-0.36	0.79*	-0.69*	-0.44	-0.35	0.11	0.17	0.10
Co	-0.44	0.02	-0.40	0.19	0.26	0.21	0.26	0.45	0.38
Cu	0.75*	-0.49	0.75*	-0.42	-0.37	-0.41	-0.13	0.24	0.10
Fe	0.79*	-0.37	0.78*	-0.03	-0.13	0.10	-0.65*	-0.38	-0.17
Mn	-0.77*	0.11	-0.77*	0.15	-0.04	0.20	0.57*	0.45	0.38
Ni	-0.78*	0.23	-0.75*	0.20	0.25	0.10	0.53	0.45	0.38
Pb	-0.78*	0.21	-0.78*	-0.07	-0.04	0.06	0.79*	0.45	0.28
Sr	-0.76*	0.17	-0.76*	-0.06	-0.09	0.00	0.84*	0.24	0.38
Zn	-0.48	-0.11	-0.49	0.36	0.14	0.31	0.12	0.45	0.31
Cs	0.44	-0.02	0.45	-0.04	0.09	0.43	-0.52	0.00	-0.14
U	-0.02	-0.01	-0.01	-0.67*	-0.36	-0.50	0.82*	0.45	0.38
TC	-0.57*	0.34	-0.54*	0.61*	0.52	0.47	-0.20	-0.28	-0.03
TN	-0.54*	0.33	-0.50	0.54*	0.42	0.54*	-0.22	-0.24	-0.07
C/N	-0.61*	0.35	-0.61*	0.69*	0.44	0.35	-0.24	-0.38	-0.17
TP	0.17	0.03	0.22	0.58*	0.28	0.14	-0.84*	-0.24	-0.38
pH	0.14	-0.18	0.14	-0.64*	-0.39	-0.27	0.65*	0.45	0.38



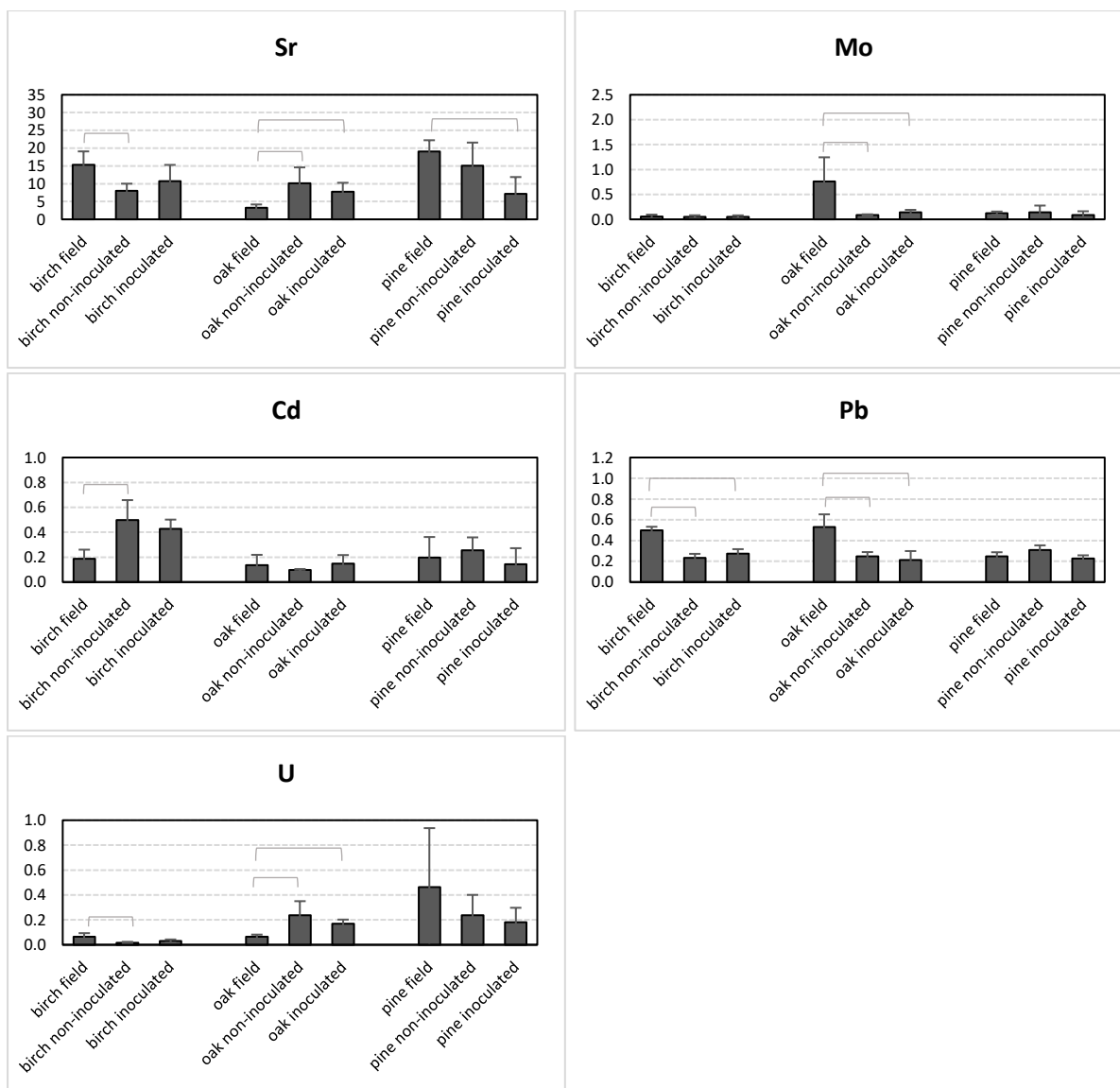


Figure S20. Content of toxic metals measured in aboveground plant biomass in variants of the experiment. y-axis represents concentration in µg/g. Brackets represent significant differences ($p < 0.05$).



Figure S21. Green algae-like patches on the surface of pot substrate.