Tree mycorrhizal type and tree diversity effects on the structure and functional potential of forest belowground microbial communities

Dissertation

zur Erlangung des

Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der

Naturwissenschaftlichen Fakultät I – Biowissenschaften –

der Martin-Luther-Universität

Halle-Wittenberg,

vorgelegt

von Herrn Bala Veera Venkata Apparao, Singavarapu

Reviewers

Prof. Dr. Helge Bruelheide

Martin Luther University Halle-Wittenberg, Institute of Biology / Geobotany and Botanical Garden; German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Germany

Prof. Dr. Sylvia Haider

Martin Luther University Halle-Wittenberg, Institute of Biology / Geobotany and Botanical Garden, Germany

Prof. Dr. Dominik Begerow

University of Hamburg, Institute of Plant Sciences and Microbiology, Hamburg, Germany

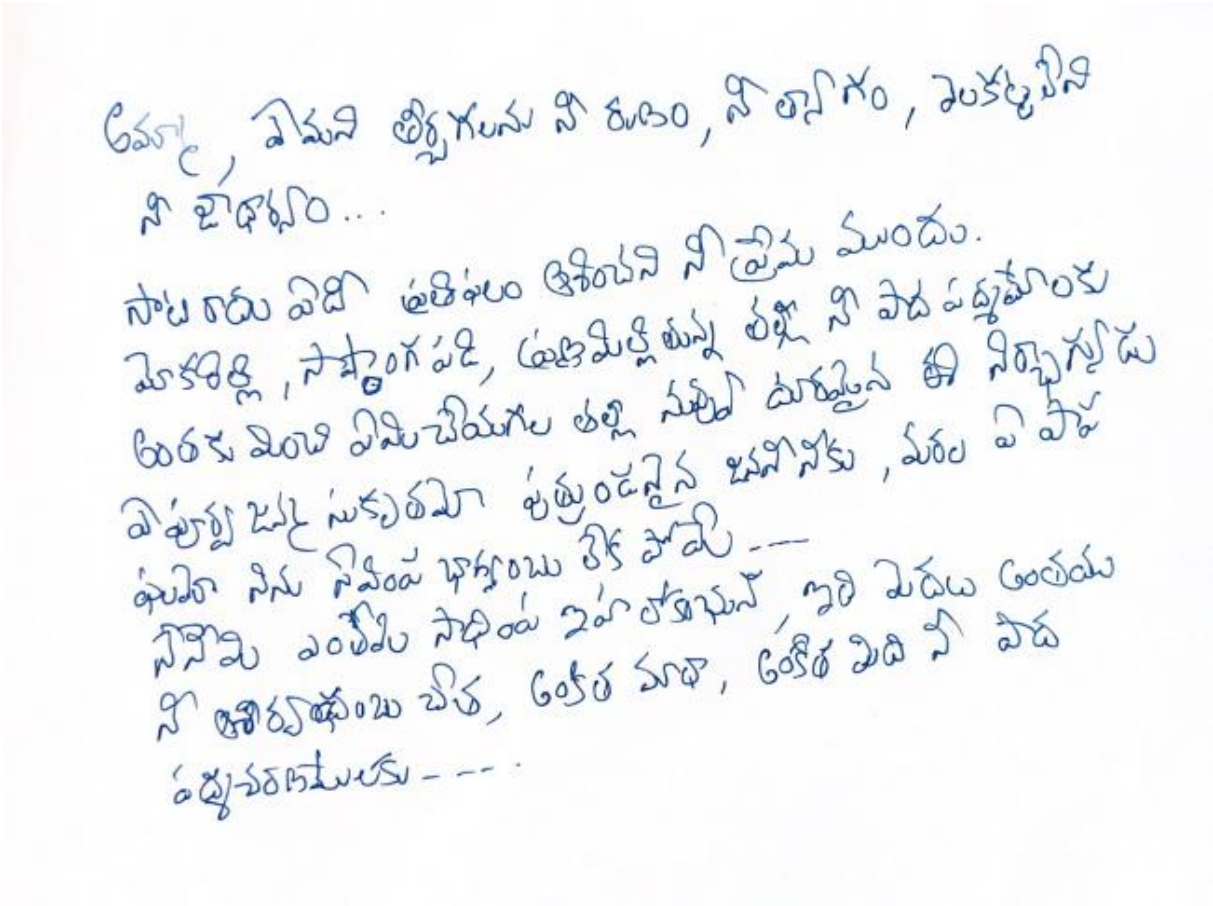
PhD Thesis defense date: 06 July 2023

Singavarapu B, Beugnon R, Bruelheide H, Cesarz S, Du J, Eisenhauer N, Guo L-D, Nawaz A, Wang Y, Xue K & Wubet T (2021). Tree mycorrhizal type and tree diversity shape the forest soil microbiota. Environmental Microbiology. doi.org/10.1111/1462-2920.15690.

Singavarapu B, Du J, Beugnon R, Cesarz S, Eisenhauer N, Xue K, Wang Y, Bruelheide H & Wubet T (2023). Functional Potential of Soil Microbial Communities and Their Subcommunities Varies with Tree Mycorrhizal Type and Tree Diversity. Microbiology spectrum, e0457822. https://doi.org/10.1128/spectrum.04578-22.

Singavarapu B, Haq Ul H, Darnstaedt F, Nawaz A, Beugnon R, Cesarz S, Eisenhauer N, Du J, Xue K, Wang Y, Bruelheide H & Wubet T (2024). Influence of tree mycorrhizal type, tree species identity, and diversity on forest root-associated mycobiomes. New Phytologist, 242, 1691–1703. doi.org/10.1111/nph.19722

This thesis is dedicated to my beloved mother Late Smt. Veera Mani Singavarapu



"***This is a story told by a few grams of soil, roots, and a bunch of human beings***."

- Bala Singavarapu, on this dissertation

"***What is research, but a blind date with knowledge.***"

- William J. Henry (1774 – 1836)

Table of Contents

Summary *1*

Zusammenfassung *3*

Chapter I: General Introduction *5*

Belowground microbial communities and their link with the environment **6**

Tree mycorrhizal type influence on the belowground microbial communities **9**

Tree diversity often but not always positively associated with belowground microbial communities **11**

Site-specific environmental impacts on the belowground microbial communities **12**

Why tree diversity and tree mycorrhizal type have to be studied together than separately? **14**

The aim of this thesis, objectives and outline **14**

Experimental design **16**

Chapter II: Tree mycorrhizal type and tree diversity shape the forest soil microbiota *20*

Summary **21**

Significance statement **21**

Introduction **22**

Materials and Methods **24**

Study site and experimental design 24

Soil sampling and processing 25

Soil chemical properties 25

DNA extraction, amplicon library preparation, and sequencing 26

Bioinformatics analysis 26

Statistical analysis 28

Results **30**

Sequence data processing 30

Tree mycorrhizal type and tree diversity effects on microbial alpha diversity 30

Tree mycorrhizal type and tree diversity effects on taxonomic and functional groups 32

Tree mycorrhizal type and tree diversity effects on microbial community composition 34

Random forest model based microbial predictors of tree mycorrhizal types across tree diversity levels 36

Interplay among environmental factors, tree mycorrhizal type and tree diversity in shaping soil microbial community composition 38

Discussion **39**

Tree mycorrhizal type affects fungal rather than bacterial alpha diversity 39

Tree diversity level affects fungal rather than bacterial alpha diversity **40**

Higher tree diversity levels neutralize the tree mycorrhizal type effects on soil microbial community composition 41
The role of classifier taxa in driving the differences between tree mycorrhizal types 43
The additional contribution of environmental factors explaining microbial community composition 44
Conclusions 45
Appendix S II 47
Chapter III: The functional potential of soil microbial communities and their sub-communities varies with tree mycorrhizal type and tree diversity 61
Abstract 62
Importance 62
Introduction 63
Material and methods 66
Bioinformatics analysis 66
Statistical analysis 67
Results 70
EcM and AM TSPs soil microbial inter-kingdom network characteristics 70
Sub-communities significantly responding to the soil environment 71
Tree mycorrhizal type and tree diversity level effects on the predicted functional potential of co-occurring bacterial and fungal communities 72
Pairwise comparison of functional abundances of EcM and AM TSPs soil microbial sub-communities 75
Differentially abundant taxa behind the observed functional abundance differences of EcM and AM TSPs soil microbial sub-communities 77
Discussion 79
EcM and AM TSPs soil microbial inter-kingdom networks and their sub-communities differ in their ecological properties 79
Functional potential of EcM and AM TSP soil co-occurring bacterial and fungal communities were strongly impacted by tree mycorrhizal type 81
Insights into the functional abundance differences of EcM and AM TSP soil co-occurring microbial sub-communities 82
Differentially abundant taxa and the top contributors to the functional abundance and nutrient cycling combinations 84
Conclusions 85
Appendix S III 87
Chapter IV: Phylogeny- and taxon abundance-based comparative analysis provides insights into the root-associated fungal communities of Arbuscular and Ectomycorrhizal tree species across forest tree diversity levels 104
Abstract 105
Introduction 105
Material and methods 108
Study site, experimental design and sampling 108

DNA extraction, amplicon library preparation, and sequencing 109
Bioinformatics analysis 110
Statistical analysis 110
Results 112
Observed alpha and phylogenetic diversity of root-associated fungi 112
Tree mycorrhizal type, tree species identity and tree diversity effects on root-associated fungal community composition 116
Tree species identity effects across the tree diversity levels 118
Root-associated fungal community taxonomic composition 119
Root-associated fungal community functional composition 120
Biotic and abiotic components shaping the root-associated fungal communities 122
Discussion 123
High tree diversity dilutes tree mycorrhizal type and tree species identity effects on the observed alpha and phylogenetic diversities of root-associated mycobiota 123
Phylogenetic relationships explain more variation in the root fungal community composition than taxon abundance 124
Differential contribution of biotic and abiotic factors in driving the assembly of root-associated fungal communities 127
Conclusions 128
Appendix S IV 129
Chapter V: General Discussion 131
Summary of Results 131
Discussion 133
Study limitations 138
Conclusions and future research perspectives 139
Literature Cited 141
Appendices 162
Author Contributions 162
Acknowledgements 163
Curriculum Vitae 165
Publications and Conference Contributions 166
Declaration of Independence / Eigenständigkeitserklärung 168

Summary

Forests provide multiple indispensable ecosystem services, such as timber, food, fuel, clean water, carbon storage, nutrient cycling and climate regulation. The belowground microbial communities, particularly soil and root-associated bacteria and fungi, play a crucial role in forest ecosystem functioning. Over 90% of terrestrial plants form symbiotic relationships with mycorrhizal fungi, the most common being arbuscular mycorrhiza (AM) and ectomycorrhizal (EcM). Plant communities in forests vary in richness and mycorrhizal associations, but the combined impact of tree mycorrhizal type and diversity on ecosystem services remains under-investigated. This thesis, examined the effects of tree mycorrhizal type and diversity, as well as site-specific environmental factors such as tree species identity, neighborhood, plot tree composition, soil, space, and topography, on the structure and functional potential of forest belowground microbial communities. The three main findings are summarized below:

- Chapter II found that the tree mycorrhizal type and species diversity influence the diversity and composition of soil bacterial and fungal communities, along with other biotic and abiotic factors. The tree mycorrhizal type had a greater effect on fungal communities than on bacterial communities, and both soil communities converged as tree diversity increased. Spatial variables impacted microbial communities of both mycorrhizal types regardless of tree diversity, while soil, tree community and topography related variables showed varying effects. The number of environmental factors affecting soil fungal and bacterial communities was fewer in multi-species tree mixtures than in low-diversity stands, suggesting a complex interplay of factors.

- Chapter III utilized a network approach to examine the genomic functional potential of co-occurring soil bacterial and fungal communities for cycling carbon (C), nitrogen (N), and phosphorus (P) and their combinations. The tree mycorrhizal type had a strong impact on the assembly and organization of co-occurring microbial taxa. Microbial sub-communities were identified that respond to soil characteristics and provide similar nutrient cycling functions. The tree diversity levels were linked to different sets of nutrient cycling enzymes. In high-diversity plots with different mycorrhizal types, the functional potential of microbial communities converged, indicating stable microbiome functioning.

- Chapter IV examined the influence of fungal phylogeny on root-associated fungal communities and the effects of experimental factors. The variation in root-associated fungal community composition was explained better by taxa phylogenetic relationships weighted by abundance differences, rather than by simple taxon relative abundances. The composition of fungal communities for both AM and EcM tree mycorrhizal types became similar in multi-species mixtures. The fungal community composition was mainly influenced by tree species composition and spatial variables. In multi-species tree mixtures, dual mycorrhization was observed, suggesting rapid shifts in plant-microbe relationships induced by ecological interactions.

This thesis highlighted the complex interplay between tree mycorrhizal type, species diversity, and site-specific factors on the structure and function of belowground microbiota in forests. This understanding is crucial for gaining insight into the ecological and evolutionary processes maintaining these microbial communities. The findings suggest that diversifying tree species with different mycorrhizal partners can lead to a more robust belowground microbial community with rich genomic functional potential, providing valuable information for forest management practices.

Zusammenfassung

Wälder erbringen zahlreiche unverzichtbare Ökosystemleistungen wie Holz, Nahrung, Brennstoff, sauberes Wasser, Kohlenstoffspeicherung, Nährstoffkreislauf und Klimaregulierung. Unterirdische mikrobielle Gemeinschaften, insbesondere boden- und wurzelassoziierte Bakterien und Pilze, spielen eine entscheidende Rolle für das Funktionieren des Waldökosystems. Über 90 % der Landpflanzen gehen symbiotische Beziehungen mit Mykorrhizapilzen ein, wobei die arbuskuläre Mykorrhiza (AM) und die Ektomykorrhiza (EcM) am häufigsten vorkommen. Pflanzengemeinschaften in Wäldern variieren in ihrem Mykorrhiza-Reichtum und ihren Mykorrhiza-Assoziationen, aber die kombinierten Auswirkungen von Mykorrhiza-Typ und -Vielfalt auf Ökosystemdienstleistungen sind noch nicht ausreichend erforscht. In dieser Arbeit wurde der Einfluss von Mykorrhiza-Typ und -Vielfalt sowie standortsspezifischen Umweltfaktoren wie Baumartenidentität, Nachbarschaft, Bestandeszusammensetzung, Boden, Raum und Topographie auf die Struktur und das funktionelle Potenzial mikrobieller Gemeinschaften in Wäldern untersucht. Die drei wichtigsten Ergebnisse werden im Folgenden zusammengefasst:

In Kapitel II wurde festgestellt, dass der Mykorrhizatyp und die Baumartenvielfalt zusammen mit anderen biotischen und abiotischen Faktoren die Vielfalt und Zusammensetzung der Bakterien- und Pilzgemeinschaften im Boden beeinflussen. Der Baummykorrhizatyp hatte einen stärkeren Einfluss auf die Pilzgemeinschaften als auf die Bakteriengemeinschaften, und beide Bodengemeinschaften konvergierten mit zunehmender Baumartenvielfalt. Räumliche Variablen wirkten sich unabhängig von der Baumdiversität auf die mikrobiellen Gemeinschaften beider Mykorrhizatypen aus, während boden-, baumgemeinschafts- und topographiebezogene Variablen unterschiedliche Effekte zeigten. Die Anzahl der Umweltfaktoren, die sich auf die Pilz- und Bakteriengemeinschaften im Boden auswirkten, war in artenreichen Baummischungen geringer als in Beständen mit geringer Diversität, was auf ein komplexes Zusammenspiel von Faktoren hindeutet.

In Kapitel III wurde mit Hilfe eines Netzwerkansatzes das genomische Funktionspotenzial der gemeinsamen bakteriellen und pilzlichen Bodengemeinschaften für den Kohlenstoff-

(C), Stickstoff- (N) und Phosphor(P)-Kreislauf sowie deren Kombinationen untersucht. Der Mykorrhizatyp des Baumes hatte einen starken Einfluss auf die Zusammensetzung und Organisation der gemeinsam auftretenden mikrobiellen Taxa. Es wurden mikrobielle Untergemeinschaften identifiziert, die auf Bodeneigenschaften reagieren und ähnliche Funktionen im Nährstoffkreislauf erfüllen. Der Grad der Baumdiversität wurde mit unterschiedlichen Enzymsätzen für den Nährstoffkreislauf in Verbindung gebracht. In Parzellen mit hoher Diversität und verschiedenen Mykorrhizatypen konvergierte das funktionelle Potenzial der mikrobiellen Gemeinschaften, was auf eine stabile Funktion des Mikrobioms hinweist.

Kapitel IV untersuchte den Einfluss der Pilzphylogenie auf die wurzelassoziierten Pilzgemeinschaften und den Einfluss experimenteller Faktoren. Die Unterschiede in der Zusammensetzung der wurzelassoziierten Pilzgemeinschaften konnten besser durch die phylogenetischen Beziehungen zwischen den Taxa, gewichtet nach den Unterschieden in der Häufigkeit, als durch die einfachen relativen Häufigkeiten der Taxa erklärt werden. Die Zusammensetzung der Pilzgemeinschaften für AM- und EcM-Baummykorrhizatypen war in den interspezifischen Mischungen ähnlich. Die Zusammensetzung der Pilzgemeinschaften wurde hauptsächlich durch die Baumartenzusammensetzung und räumliche Variablen beeinflusst. In Baummischungen mit mehreren Baumarten wurde eine doppelte Mykorrhizierung beobachtet, was auf schnelle Veränderungen in den Beziehungen zwischen Pflanzen und Mikroben aufgrund ökologischer Interaktionen hinweist.

In dieser Arbeit wurde das komplexe Zusammenspiel zwischen Baummykorrhizatyp, Artenvielfalt und standortspezifischen Faktoren auf die Struktur und Funktion der unterirdischen Mikrobiota in Wäldern aufgezeigt. Dieses Verständnis ist entscheidend, um Einblicke in die ökologischen und evolutionären Prozesse zu gewinnen, die diese mikrobiellen Gemeinschaften erhalten. Die Ergebnisse deuten darauf hin, dass die Diversifizierung von Baumarten mit unterschiedlichen Mykorrhizapartnern zu einer robusteren unterirdischen mikrobiellen Gemeinschaft mit reichem genomischen Funktionspotenzial führen kann, die wertvollen Informationen für die Waldbewirtschaftung liefert.

Chapter I: General Introduction

Forests are vital ecosystems on our planet providing multiple essential ecosystem services. Briefly, forests provide *provisioning services* such as timber, food, clean water, and fuel; *regulating services* such as water, and climate regulation; *supporting services* such as biomass production, nutrient cycling, and soil formation; and *cultural services* such as educational, recreation, and tourism (Balloffet et al., 2012; Jenkins and Schaap, 2018). Despite the profound importance, deforestation and forest degradation remain ongoing at perturbing rates, for instance, since 1990, around 420 million hectares of forests were lost (UNEP, 2020). This significantly increases the risk of the loss of valuable forest-provided multifunctional ecosystem services and promptly calls for increased forest restoration efforts. High tree species diversity is reported to have positive effects on forest ecosystem functioning (Paquette et al., 2018). It is also reported that more than 90% of terrestrial plants have symbiotic associations with mycorrhizal fungi, predominantly with arbuscular and ectomycorrhiza (Brundrett and Tedersoo, 2018). Hence, it is important to know the role of the combination of tree diversity and tree mycorrhizal type play in relation to ecosystem services for much-needed afforestation and reforestation practices.

Most of the biodiversity hosted by forests which accounts for more than 80% of living terrestrial species is found belowground (Stohr, 2013; Jenkins and Schaap, 2018). This belowground soil biodiversity is crucial in providing the forest ecosystem services including carbon storage and nutrient cycling etc. (Stohr, 2013; FAO et al., 2020). Microorganisms such as soil and root-associated bacteria and fungi not only contribute to the soil functions but also promote the growth and health of the plants and positively influence the plant productivity and community functioning (Jansson and Hofmockel, 2020; Almario et al., 2022). Concurrently, plant-related biotic variables and abiotic variables such as soil characteristics, space and the site topography can affect the belowground microbes which in turn impact the forest ecosystem services. However, we still have a limited understanding of how the combination of tree mycorrhizal type and tree diversity affects the forest belowground microbial communities.

In this work, along with my colleagues, I investigated the tree mycorrhizal type and tree diversity effects in association with site-specific environmental factors on the structure and

functional potential of forest belowground bacterial and fungal communities. This introductory chapter provides the background for this work, specifies the research aim and objectives, provides the structural outline of the thesis, and also describes the experimental design.

Belowground microbial communities and their link with the environment

The diversity and abundance of living species found under our feet are tremendous. Majorly, the soil organisms, based on size were categorized into *Microbes* (e.g., viruses, bacteria, fungi; 20 nm to 10 μm), *Microfauna* (e.g., protozoa, nematodes; 10 μm to 0.1 mm), *Mesofauna* (e.g., mites, springtails; 0.1 mm to 2 mm), *Macrofauna* (e.g., earthworms, ants, termites; 2 mm to 20 mm) and *Megafauna* (moles, voles, gophers; >20 mm) (Swift et al., 1979). It was predicted that the earth is estimated to harbor about 10^{11}–10^{12} microbial species (Locey and Lennon, 2016). Out of microbes, bacteria and fungi are highly diverse and the most abundant microorganisms found in soil with around 10^{2}–10^{4} times more biomass than the other major soil microbiota such as archaea and viruses (Fierer, 2017).

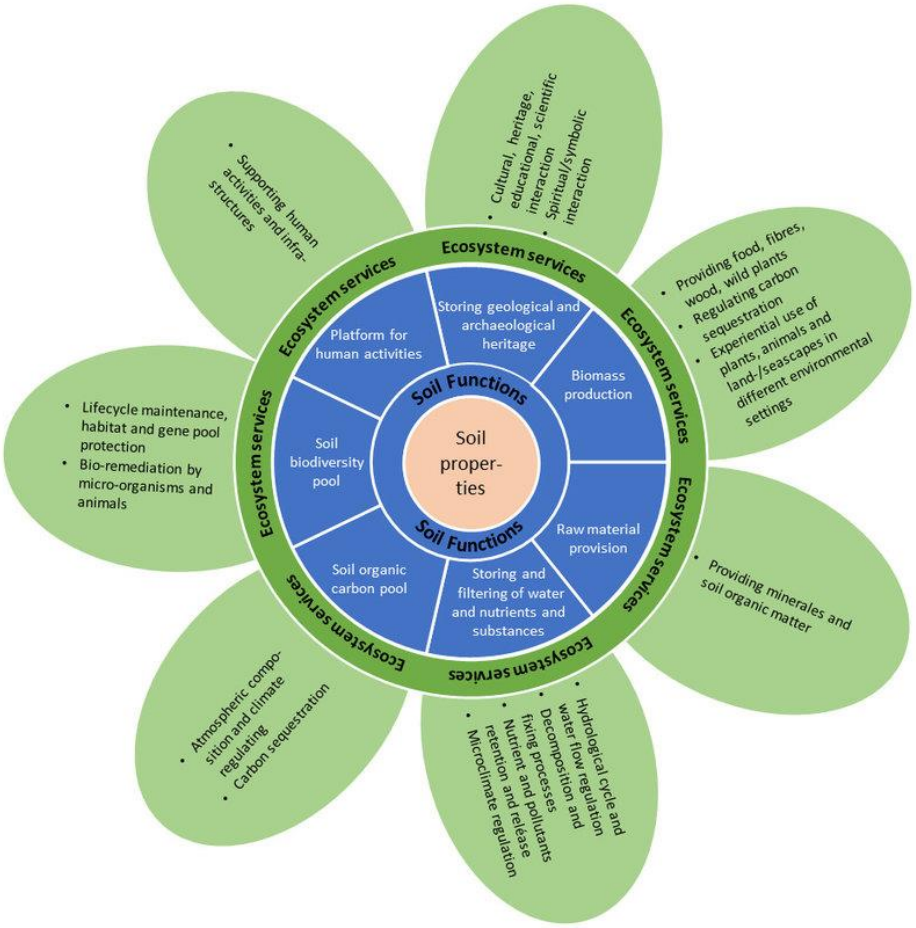


Figure I.1 Diagram describing the functions and ecosystem services provided by the soil arising from the inherent soil properties. From Gregor et al. (2018)

Bacteria along with archaea were the oldest *prokaryotic* (single cell without a nucleus and other membrane-bound organelles) living microbes that evolved around 3.5 billion years ago (Woese et al., 1990). Recent evidence showed that bacteria were involved in biogeochemical cycling on land even around 3.2 billion years ago (Homann et al., 2018). Fungi are *eukaryotic* (cells with membrane-bound organelles including a nucleus) microbes that evolved later around 1.5 billion years ago (Wang et al., 1999). It is believed that the colonization of the early terrestrial plants on land approximately 450 million years ago was assisted by the symbiotic soil fungi (Humphreys et al., 2010). To sum up, below-ground bacteria and fungi played crucial roles in shaping the 'Nature' on our planet that we see today.

Soil acts as a platform for delivering many of the forest and other ecosystem-related services including provisioning, regulating, supporting and cultural services (Figure I.1) and to do so, the role of soil microbes like bacteria and fungi is essential. For instance,

bacteria and fungi are major engines of the biogeochemical cycles on Earth by transforming organic substances (e.g., by decomposition) and also key in soil carbon sequestration (e.g., fugal mycelial network and microbial biomass) (Clemmensen et al., 2013; Graham et al., 2016; Li et al., 2019). They also greatly influence the soil structure, fertility and water quality and thereby the related ecosystem services (Sylvia et al., 2005; Bender et al., 2016; Nagy et al., 2017). Bacteria and fungi are vital for plant nutrition (e.g., nitrogen-fixing bacteria, mycorrhizal fungi), affect the diversity and functioning of the plants, and hence, enable ecosystem services (Baldrian, 2017). Recent research showed that soil microbial diversity significantly contributes to the ecosystem multifunctionality, underlining the importance to study the drivers of the belowground microbial diversity, their community structure and functions (Wagg et al., 2014; Delgado-Baquerizo et al., 2016; Wagg et al., 2019).

The patterns of belowground microbial diversity can be explained by ecological theories which pivot broadly on three aspects of environmental factors comprising above and belowground resource availability, soil nutrient stoichiometry and abiotic factors (Bardgett and Van Der Putten, 2014; Delgado-Baquerizo et al., 2019). Generally, belowground microbial diversity is expected to increase to a certain extent with increasing resource availability, for instance, provided by the aboveground (vegetation) litter inputs and soil organic matter. Furthermore, soil nutrient stoichiometry which describes the relative abundance of elements like carbon (C), nitrogen (N) and phosphorus (P) in the soil can potentially regulate the microbial communities as these elemental ratios can affect the processes such as litter decomposition, mineralization and nutrient immobilization (Hooper et al., 2000; Wardle et al., 2004). Last but not least, abiotic factors such as soil pH, topography and spatial distance were shown to strongly shape the belowground microbial communities (Fierer and Jackson, 2006; Tedersoo et al., 2014). Based on ecological theories, previous and ongoing research has demonstrated various mechanisms to explain belowground microbial community patterns operating at different scales from fine local to broad continental and global scales. Below, I describe the important biotic and abiotic factors that are studied in my thesis with respect to forest belowground microbial communities.

Tree mycorrhizal type influence on the belowground microbial communities

Mycorrhizas are symbiotic associations between plants and some specialized soil fungi which take place at the plant root tissues. Broadly, there are four types of mycorrhizas categorized based on the anatomy and taxonomic identity of the plant and fungal partners, namely, arbuscular mycorrhiza (AM), ectomycorrhiza (EcM), ericoid mycorrhiza (ErM) and orchid mycorrhiza (OrM) (Brundrett and Tedersoo, 2018). Plant/tree mycorrhizal type is a functional trait usually determined based on the plant's association with its major mycorrhizal partners, and nearly 80% of all vascular plants were reported to be either arbuscular mycorrhizal (AM plants) or ectomycorrhizal (EcM plants) (Aerts, 2003; Blackwell, 2011). Ectomycorrhizal fungi colonize the plant root tissue with a thick mantle of hyphae around the root tip and forms hartig net around the epidermal cells. In contrast, hyphae of arbuscular mycorrhizal fungi reach the root inner cortex and forms vesicular structures called arbuscules which are the key points of symbiotic nutrient exchange (Figure I.2, Bonfante and Genre (2010).

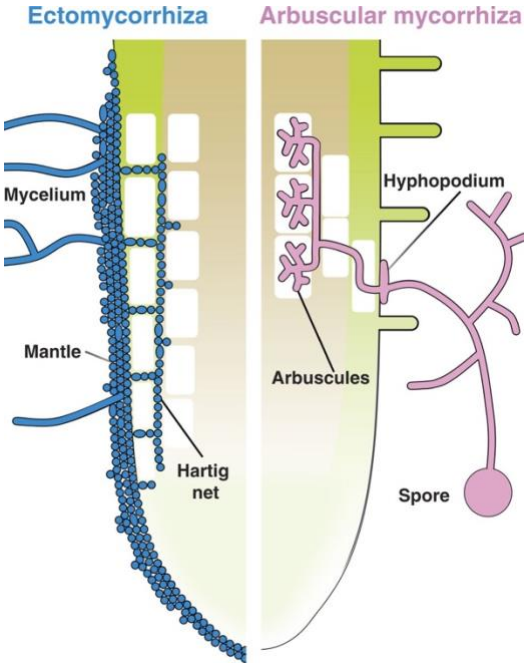


Figure I.2 Sketch showing root colonization structural differences in ectomycorrhizal (blue) and arbuscular mycorrhizal (pink) interactions. Adapted from Bonfante and Genre, 2010

Within this symbiosis, plant carbon is exchanged for nutrients such as phosphorus and nitrogen provided by mycorrhizal fungi (Brundrett and Tedersoo, 2018). The area of soil under the combined influence of the plant root and the mycorrhizal fungi is referred to as

the mycorrhizosphere (Johansson et al., 2004). EcM and AM plants differ in their resource acquisition, allocation strategies, and plant-soil feedback relations (Aerts, 2003; Phillips et al., 2013; Bennett et al., 2017; Kadowaki et al., 2018). For example, EcM fungal partners can efficiently mobilize organic compounds, whereas AMF are efficient in mobilizing inorganic compounds (Read and Perez-Moreno, 2003; Smith and Read, 2008). These contrasting processes may cause differences in the habitat within and around the mycorrhizosphere, such as abiotic conditions and resource quality and composition, and thus strongly influence the assembly of other microbes. For example, AM fungi have been reported to support nitrogen-fixing bacteria through enhanced phosphorus acquisition (Amora-Lazcano et al., 1998; Püschel et al., 2017). Conversely, ectomycorrhizal fungi can select for bacterial taxa with high weathering potential through efficient carbon transfer (Uroz et al., 2007; Churchland and Grayston, 2014). These differences in community composition between EcM and AM systems further lead to contrasts in belowground microbial functionality, such as nutrient cycling. (Cheeke et al., 2017).

After the advent of molecular methods such as polymerase chain reaction (PCR) and DNA sequencing using molecular phylogenetic markers like small subunit ribosomal RNA genes, studies on the diversity and composition of mycorrhizal communities have become possible (Taylor and Bruns, 1997; Lee Taylor and Bruns, 1999). Across different forest biomes comprising boreal, temperate, tropical and sub-tropical forests, the relationships between the host plant (EcM or AM type) and its root-associated fungal communities, including the mycorrhizal symbionts, were investigated (Öpik et al., 2008; Wubet et al., 2009; Tedersoo et al., 2012; Gao et al., 2013; Toju et al., 2014; Davison et al., 2020). Positive relationships between the abundance of plants with a given mycorrhizal type and that of their symbiotic mycorrhizal communities, i.e., EcM plants with EMF and AM plants with AMF in both soil and roots (Gao et al., 2013; Neuenkamp et al., 2018; Weißbecker et al., 2018) were reported. Furthermore, it was shown that the plant mycorrhizal partners can significantly influence the diversity and composition of the opposing root-associated fungal communities (Toju et al., 2014; Ferlian et al., 2021; Heklau et al., 2021), highlighting the role of plant mycorrhizal type in the assembly of microbial communities. Despite the research advancement, studies of plant mycorrhizal type effects on belowground bacterial communities are still scarce.

Tree diversity often but not always positively associated with belowground microbial communities

The prevailing pattern shows a positive correlation between plant diversity and multiple ecosystem functions. For example, diverse plant mixtures are shown to be more productive than their respective conspecific stands (Tilman, 1999; E. M. et al., 2000). In addition, a meta-analysis combining multiple experimental grassland studies reported that plant diversity effects on soil organisms became significant over time (Eisenhauer et al., 2012). Further, the study indicated that positive facilitative net effects by soil biota like AMF and rhizobacteria promote plant growth in species-rich grasslands. Similarly, for trees with increasing diversity, different tree-tree interactions develop, and so does the complexity of the associated tree-tree, tree-microbe, and microbe-microbe interactions (Bonfante and Anca, 2009; Schuldt et al., 2017). Positive tree diversity effects were also revealed both on soil (Barberan et al., 2015; Hiiesalu et al., 2017) and root microbial communities (Gao et al., 2013; Ferlian et al., 2021), suggesting a strong link between above- and belowground compartments.

These positive plant diversity effects could be caused by several underlying mechanisms, and out of those, two contrasting explanations are mainly debated (Tilman et al., 2014). One mechanism are sampling effects or selection effects: simply put, diverse mixtures are more likely to have a few species with strong effects on ecosystem function (in this case, e.g. high microbial diversity), which drive the positive outcome of the community but are not brought about by species diversity *per se* (Huston, 1997; Cardinale et al., 2006). The other mechanism can be described by complementarity effects, which in short are the results of the increased complementarity between species, consequently allowing better resource (e.g., nutrient, water, space, etc.) availability and usage (Loreau and Hector, 2001; Trogisch et al., 2021). For example, Brassard et al. (2013) showed evidence for the below-ground species complementarity in evenly mixed forest stands by demonstrating increased root productivity by efficiently filling and exploiting the soil environment compared to single-species dominated stands. Also, in a global meta-analysis positive effects of plant diversity on soil fungal and bacterial biomass and respiration were shown as a result of plant species complementarity by factoring out the selection effects related to plant species composition (Chen et al., 2019a).

Nevertheless, plant species diversity effects on soil and root-associated microbial communities are not without contradiction. For instance, the absence of any significant effects of plant richness on root-associated fungal communities was also reported (Navratilova et al., 2019; Otsing et al., 2021). In addition, no or negligible effects of tree diversity on soil microbial diversity were also presented (McGuire et al., 2012; Rivest et al., 2019). Tree species richness effects may develop or be observed in some cases and may not in others. There could be many unaccounted factors leading to these inconsistent findings, resulting from a strong context-dependency, such as the ecosystem's age (Eisenhauer et al., 2012) and the convergent effect of environmental factors on both plant and belowground microbial communities (Tedersoo et al., 2016). This highlights the need for controlled experimental settings and to consider the effects of multiple factors in studying plant diversity effects.

Site-specific environmental impacts on the belowground microbial communities

At large, the relationships between belowground microbial communities and the environmental variables at global/continental scales were reported to be primarily influenced by climatic factors such as temperature and precipitation (Zhou et al., 2016; Bahram et al., 2018). In contrast, at the local scale, biotic factors such as plant species composition along with site-specific abiotic factors such as soil, spatial and topographic parameters generally become more prominent (Nielsen et al., 2010; Urbanová et al., 2015; Weißbecker et al., 2018; Tajik et al., 2020). Moreover, soil characteristics such as pH and C: N ratio were shown to be ubiquitously important factors in shaping belowground microbial communities from the global to the local scale, with, however, stronger effects of pH on bacterial than on fungal communities (Rousk et al., 2010; Glassman et al., 2017; Bahram et al., 2018). Tree species identity, for instance, can shape the belowground soil microbiota by influencing the soil physico-chemical properties (Tedersoo et al., 2016; Baldrian, 2017) and root-associated microbiota through carbon inputs (Eisenhauer et al., 2017). Similarly, relationships between plant aboveground and microbial belowground community composition were reported, indicating the strong effects of aboveground vegetation (Barberan et al., 2015; Nguyen et al., 2016b; Weißbecker et al., 2018). In addition, plant neighborhood i.e., the immediate surrounding plants of a target plant in a plot, can also substantially impact the associated belowground assemblages. In a recent

subtropical forest study, (Cheng and Yu, 2020) reported that the density of conspecific neighboring trees positively affected the relative abundance of root-associated phytopathogens, while the heterospecific neighbors had a significant negative impact on phytopathogen richness.

Community tree diversity and neighborhood tree diversities are correlated, i.e., higher plot tree diversity is likely to result in higher neighborhood tree diversity and vice versa. Similar relationships can be imagined between tree diversity and plot tree species composition. Nevertheless, the immediate neighborhood is expected to have relatively stronger effects on belowground microbial communities than community composition at a broader spatial scale. (Barberan et al., 2015; Mony et al., 2021). Furthermore, plants and microbes share evolutionary relationships that can be studied by phylogenetics which is the discipline of determining evolutionary relationships among or within populations of organisms. Such relationships are inferred from heritable traits, such as DNA sequences, and represented in phylogenetic trees (Semple and Steel, 2003). Two organisms are more phylogenetically related if they exhibit greater similarity in their observed heritable traits or have a more recent common ancestor. This means, phylogenetically related plants tend to possess similar characteristics, and thus may have a similar impact on belowground microbial communities (Koyama et al., 2019). Some studies reported that the plant phylogeny effect was stronger than spatial and soil variables on both soil and root-associated microbes (Wehner et al., 2014; Barberan et al., 2015; Wang et al., 2019).

In addition, abiotic soil properties can determine the observed plant diversity for example through nutrient availability (Laliberté et al., 2013; Xu et al., 2016). Vice versa, plant diversity can alter soil properties through various inputs from litter and rhizodeposits (Lange et al., 2015; Huang et al., 2017). Furthermore, plant mycorrhizal type was shown to affect the plant community structure via plant-soil feedbacks, often mediated by modulating resource competition (Jiang et al., 2017; Jiang et al., 2020). Moreover, it was reported that, in general, EcM trees produce low-quality litter (e.g., high C: N) compared to AM trees. Thus, besides differences in their resource preferences (organic vs inorganic), mycorrhizal type can influence soil properties (Midgley et al., 2015). Taken together, these findings highlight the inter-relationships between biotic factors and between soil and biotic variables that shape the belowground microbial communities. Nevertheless, comprehensive studies considering both abiotic and biotic factors in studying the belowground microbial

communities are seldom, and particularly, the interplay among environmental factors and tree mycorrhizal type and tree diversity are yet to be studied.

Why tree diversity and tree mycorrhizal type have to be studied together than separately?

As described above, we know that most terrestrial plants have symbiotic associations with mycorrhizal fungi (Brundrett and Tedersoo, 2018). Forest plant communities exhibit varying levels of richness and mycorrhizal associations, yet studies examining the impact of both tree diversity and mycorrhizal type on belowground microbial community patterns are scarce. Whether including tree mycorrhizal type as a factor in tree diversity experiments can address some of the inconsistencies in tree diversity effects on the belowground microbial communities is yet to be explored in detail. Furthermore, the tree mycorrhizal type effect was mainly studied for belowground fungal communities leaving a knowledge gap on the bacterial communities. The relationships between belowground bacterial and fungal communities can be studied using co-occurrence network analysis (Faust and Raes, 2012). This approach represents taxa as nodes and their interactions as edges, with the strength of the edges determined by the frequency of co-occurrence in the samples. Interactions can be intra-kingdom (e.g., between bacteria or between fungi) or inter-kingdom (e.g., between bacteria and fungi). The structure and patterns in these networks provide insight into the underlying mechanisms driving community assembly, and clusters of tightly connected species might suggest the presence of functional groups or guilds (Röttjers and Faust, 2018). To date, there has been a lack of research on how the mixing of different mycorrhizal type tree species in varying diversity levels affects the belowground fungal and bacterial community structure, including their inter-kingdom co-occurrence network patterns, sub-communities, and functional potential. Filling this knowledge gap is crucial to better understand the community assembly of forest soil and root-associated bacterial and fungal communities, and consequently, their functional roles in ecosystem functioning such as nutrient cycling. Such deep mechanistic understanding would also be vital for managing forest soils to provide multiple ecosystem services.

The aim of this thesis, objectives and outline

In this thesis, I have attempted to address the above-mentioned research gaps. This thesis aims at studying the effects of tree mycorrhizal type and tree diversity in association with

site-specific environmental factors on the structure and functional potential of forest belowground bacterial and fungal communities. To achieve the purpose, I focused on three main research objectives that are presented in three chapters (II, III, IV) as outlined below (Figure I.3).

In chapter II, I sought to characterize the soil microbiota and study the effects of experimental and site-specific environmental factors on the microbial communities. To do so, I tested the effects of tree mycorrhizal type and tree species diversity on the diversity and composition of the soil bacterial and fungal communities. Additionally, the effects of other site-specific biotic (plant-related variables such as tree species identity, plot tree species composition and neighborhood of the focal trees) and abiotic environmental factors (soil characteristics, spatial distance and topographic parameters) were also assessed.

In chapter III, I investigated how the co-occurring soil bacterial and fungal communities were structured into sub-communities and which soil characteristics drive their composition under different mycorrhizal type tree species across the tree diversity levels. Besides, I determined the genomic functional potential of those communities and sub-communities with regard to the cycling of three major nutrients carbon (C), nitrogen (N) and phosphorus (P), and their combinations. The effects of tree mycorrhizal type and tree species diversity on the genomic functional diversity and composition were tested. Furthermore, differences in their genomic functional abundances were evaluated within the tree diversity levels and the microbial taxa were identified that drove these differences.

In chapter IV, I employed a comparative approach using taxon relative abundance and phylogeny-based analyses to account for evolutionary relationships in addition to the ecological forces operating on the root-associated fungal communities. I studied the tree mycorrhizal type, tree species identity and tree species diversity effects on the observed alpha and phylogenetic diversities of root-associated fungi. I tested how much these factors affected the fungal community composition based on phylogeny compared to that based on simple relative abundance of taxa. In addition, the variation explained by site-specific biotic and abiotic environmental factors was partitioned to assess their relative contribution to the root-associated fungal community assembly.

In Chapter V, I reviewed and discussed the main results obtained from the above-mentioned research objectives. Here, I synthesized the key findings, provided implications, discussed the limitations of the study and laid out the future research perspectives.

Experimental design

The studies of my thesis were conducted at the subtropical biodiversity-ecosystem functioning tree experiment BEF-China located in southeast China, which is the largest tree diversity experiment worldwide (Bruelheide et al., 2014). It has two experimental forest sites ("Site A" and "Site B") and my studies were performed at Site A (Xingangshan, Jiangxi Province, 29.08-29.11° N, 117.90-117.93° E) which was established in 2009 on a total area of 18.4 ha of sloped terrain (Figure I.4A). We employed tree species pair (TSP) concept focusing on tree-tree interactions so that to understand their contribution to the observed tree diversity effects on key ecosystem aspects (Trogisch et al., 2021). The site comprises naturally occurring tree species with a richness gradient ranging from 1, 2, 4, 8, 16 and 24 species in a total of 271 plots of size 25.8 m x 25.8 m. Each plot was planted with 400 trees (20 x 20 individuals) with a horizontal planting distance of 1.29 m and the ten surrounding trees of a focal TSP (i.e., two adjacent trees) were considered as the immediate neighborhood (Figure I.4B).

The subtropics harbor abundantly both AM and EcM forming tree species which is an advantage to studying the tree mycorrhizal type as an experimental factor. To study the combination of tree mycorrhizal type and tree diversity and their interaction, I developed a factorial crossed study design. Tree diversity was categorized into three levels, namely monocultures or monospecific stands (richness=1), two-species mixtures (richness=2) and multi-species mixtures (richness ≥4). As for tree mycorrhizal type, 6 EcM and 6 AM TSPs were considered at each tree diversity level (Table I.1, Figure I.4C). In chapter II, to study the heterotypic combination of mycorrhizal type (i.e., combination of EcM tree and AM tree), six pairs of EcM and AM tree species were included in the multi-species mixtures as Mycomix-TSPs. For chapters III and IV, only conspecific TSPs were considered as there were no significant differences found in the microbiota analyses between con- and heterospecific TSPs from chapter II. For each TSP, three replicates were randomly collected across the plots in monocultures, two- and multi-tree species mixtures (one replicate in each 4, 8 and 16 or 24 plot tree species richness). Soil and root samples were collected from mid-August to the end of September 2018 as depicted in Figure I.4D. The

horizontal axis between the two TSP partners was treated as the tree-tree interaction zone. Composite soil samples were collected from the pool of four soil cores taken along the tree-tree interaction zone. Also, fine root samples were collected from each of the two trees of a TSP in the interaction zone.

Table I.1 List of tree species and their mycorrhizal types studied in this thesis

Tree Species	Mycorrhizal type	Reference
Castanea henryi	EcM	(Wang and Qiu, 2006; Soudzilovskaia et al., 2020)
Castanopsis sclerophylla	EcM	(Haug et al., 1994); Soudzilovskaia et al., 2020
Cyclobalanopsis glauca	EcM	Haug et al., 1994; Soudzilovskaia et al., 2020
Lithocarpus glaber	EcM	Haug et al., 1994; Soudzilovskaia et al., 2020
Quercus fabri	EcM	Wang and Qiu, 2006; Soudzilovskaia et al., 2020
Quercus serrata	EcM	Wang and Qiu, 2006; Soudzilovskaia et al., 2020
Sapindus mukorossi	AM	Wang and Qiu, 2006; Soudzilovskaia et al., 2020
Sapium sebiferum	AM	Wang and Qiu, 2006; Soudzilovskaia et al., 2020
Choerospondias axillaris	AM	Wang and Qiu, 2006; Soudzilovskaia et al., 2020
Koelreuteria bipinnata	AM	Wang and Qiu, 2006; Soudzilovskaia et al., 2020
Liquidambar formosana	AM	Haug et al., 1994; Soudzilovskaia et al., 2020
Nyssa sinensis	AM	Haug et al., 1994; Soudzilovskaia et al., 2020

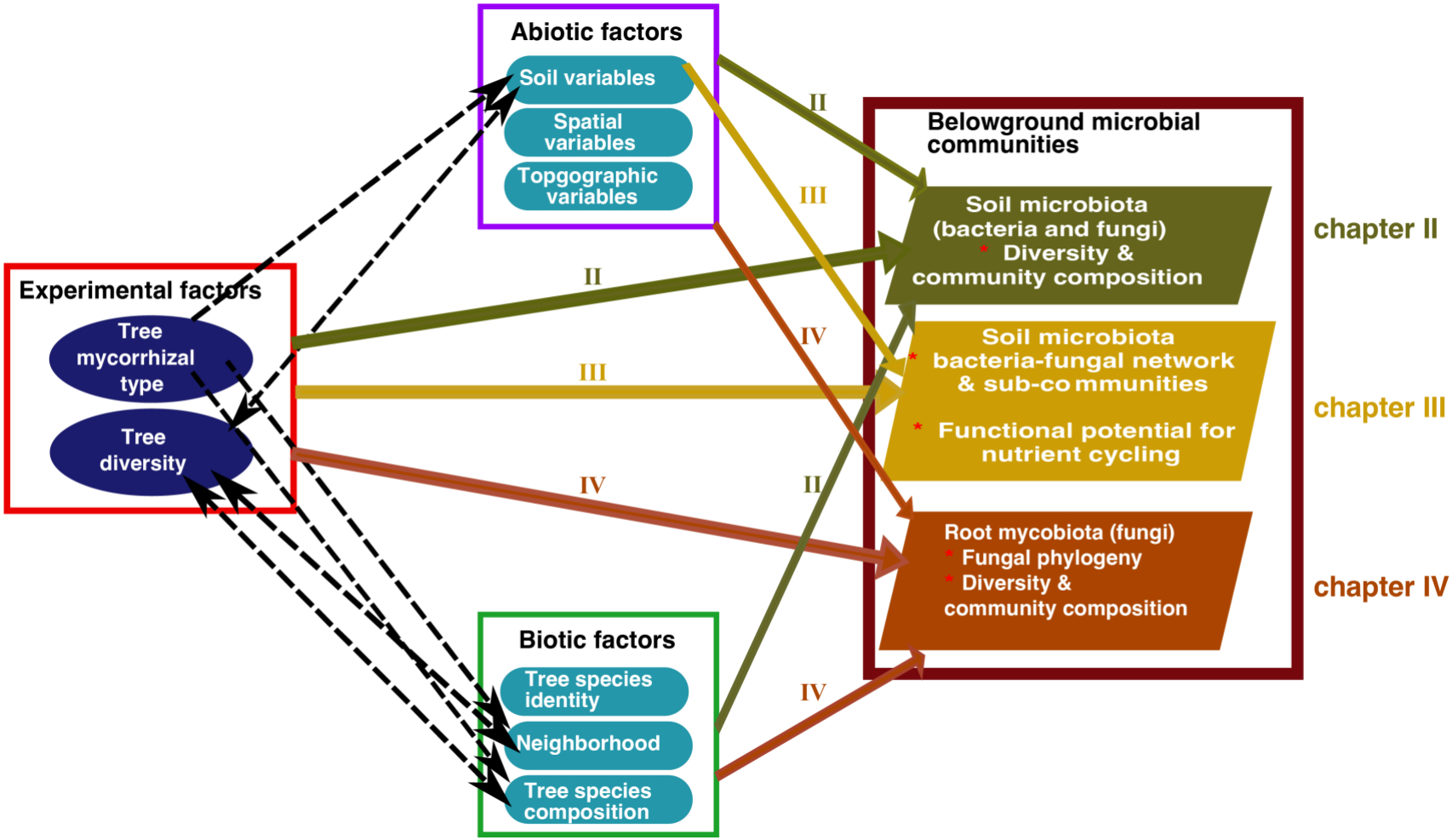


Figure I.3 Conceptual figure showing the relationships between the variables. Different solid-colored arrows represent the relationships investigated for the research objectives in chapters II to IV. Dotted black arrows indicate the expected or known relationships.

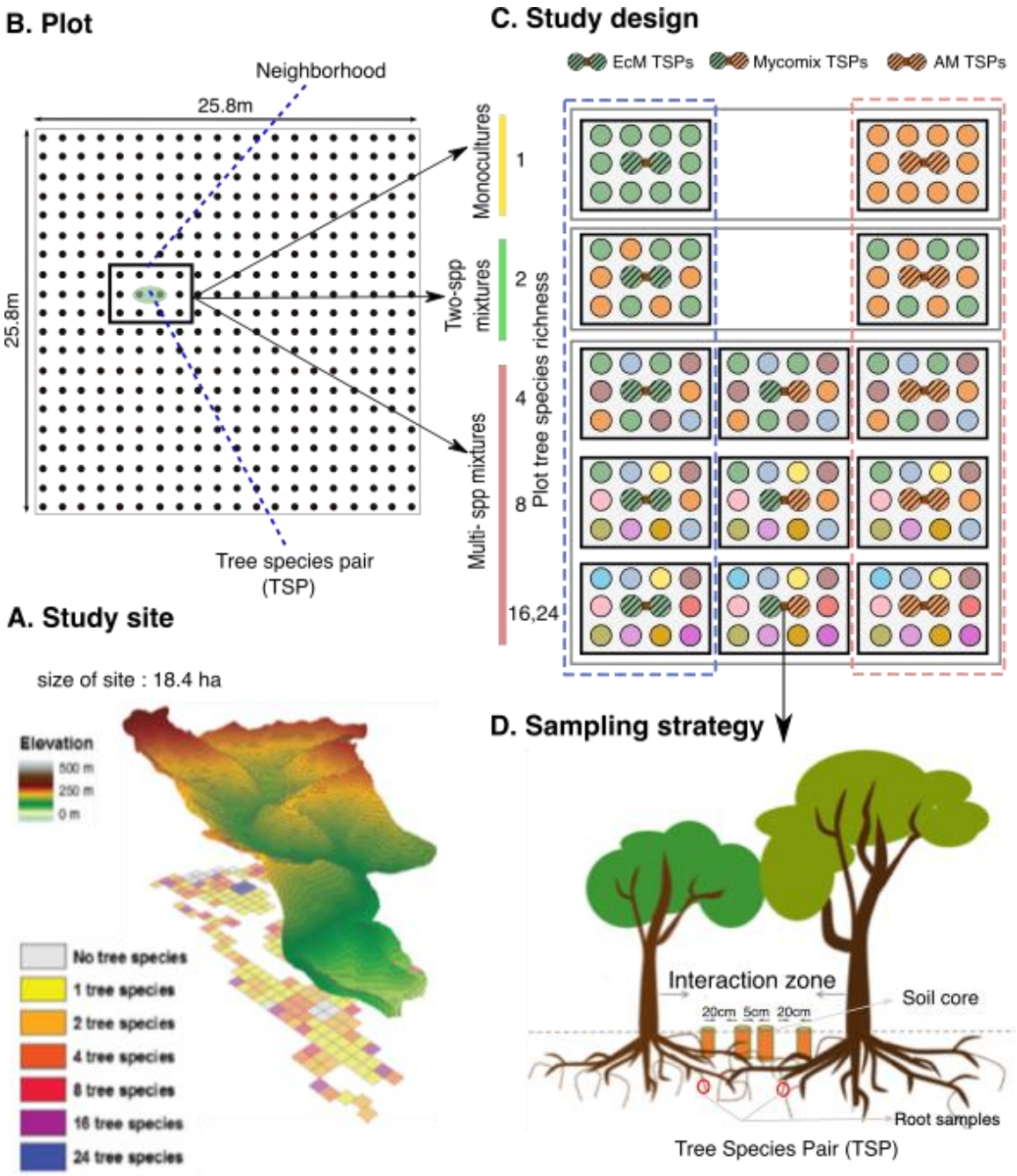


Figure I.4 A schematic diagram of the study site, plot, study design and sampling strategy. **A**. Study site showing the topography and arrangement of the plots (modified from Bruelheide et al., 2014, Fig. 4). **B**. A schematic of a plot within the site showing individual trees, tree species pair (TSP) and its neighborhood. **C**. An illustration of study design with tree species pairs (TSPs) categorized based on their mycorrhizal type shown at their neighborhood level. The vertical dotted line (blue color – EcM; red color – AM) in this schematic depicts the comparison of one EcM and one AM TSP across tree diversity. Mycomix-TSPs i.e., a pair of one EcM and one AM tree were shown in the multi-species mixtures. **D**. A cartoon portraying the soil and root sampling from the interaction zone of TSPs.

Chapter II: Tree mycorrhizal type and tree diversity shape the forest soil microbiota

This chapter is a modified version of the publication. This chapter has been published in the ***Environmental Microbiology*** journal as

Singavarapu, B., Beugnon, R., Bruelheide, H., Cesarz, S., Du, J., Eisenhauer, N., Guo, L.-D., Nawaz, A., Wang, Y., Xue, K. and Wubet, T. (2022), Tree mycorrhizal type and tree diversity shape the forest soil microbiota. Environ Microbiol, 24: 4236-4255.

https://doi.org/10.1111/1462-2920.15690

Summary

There is limited knowledge on how the association of trees with different mycorrhizal types shapes soil microbial communities in the context of changing tree diversity levels. We used arbuscular (AM) and ectomycorrhizal (EcM) tree species as con- and heterospecific tree species pairs (TSPs), which were established in plots of three tree diversity levels including monocultures, two-species mixtures, and multi-tree species mixtures in a tree diversity experiment in subtropical China. We found that the tree mycorrhizal type had a significant effect on fungal but not bacterial alpha diversity. Furthermore, only EcM but not AM TSPs fungal alpha diversity increased with tree diversity, and the differences between AM and EcM TSPs disappeared in multi-species mixtures. Tree mycorrhizal type, tree diversity and their interaction had significant effects on fungal community composition. Neither fungi nor bacteria showed any significant compositional variation in TSPs located in multi-species mixtures. Accordingly, the most influential taxa driving the tree mycorrhizal differences at low tree diversity were not significant in multi-tree species mixtures. Collectively, our results indicate that tree mycorrhizal type is an important factor determining the diversity and community composition of soil microbes, and higher tree diversity levels promote convergence of the soil microbial communities.

Significance statement

More than 90% of terrestrial plants have symbiotic associations with mycorrhizal fungi which could influence the coexisting microbiota. Systematic understanding of the individual and interactive effects of tree mycorrhizal type and tree species diversity on the soil microbiota is crucial for the mechanistic comprehension of the role of microbes in forest soil ecological processes. Our tree species pair (TSP) concept coupled with random sampling within and across the plots, allowed us the unbiased assessment of tree mycorrhizal type and tree diversity effects on the tree-tree interaction zone soil microbiota. Unlike in monocultures and two-species mixtures, we identified species-rich and converging fungal and bacterial communities in multi-tree species mixtures. Consequently, we recommend planting species-rich mixtures of EcM and AM trees, for afforestation and reforestation regimes. Specifically, our findings highlight the significance of tree mycorrhizal type in studying ‘tree diversity – microbial diversity – ecosystem function’ relationships.

Introduction

Soil microorganisms, predominantly fungi and bacteria, are highly abundant and diverse living entities on earth (Fierer, 2017). Both fungi and bacteria play key roles in a wide range of processes like biogeochemical cycles and regulate plant diversity and productivity (Van Der Heijden et al., 2008; Bender and van der Heijden, 2015; Delgado-Baquerizo et al., 2016; Kappler and Bryce, 2017; Wei et al., 2019).

Notably, the diversity and composition of microbial communities are essential for the multifunctionality of ecosystems (Wagg et al., 2014; Delgado-Baquerizo et al., 2016). As an essential part of soil microbial communities, mycorrhizal fungi, form symbiotic associations with more than 90% of terrestrial plant species. Within this symbiosis, plants exchange carbon with mycorrhizal fungi to support their nutrient uptake, pathogen defense, and environmental stress tolerance (Wang and Qiu, 2006; Smith and Read, 2010; Brundrett and Tedersoo, 2018). There are two dominant mycorrhizal types, namely ectomycorrhiza (EcM) and arbuscular mycorrhiza (AM), that are associated with approximately 80% of all vascular plants. Ecto and arbuscular mycorrhizal fungi differ in resource acquisition, allocation strategies, and plant-soil feedback relations (Aerts, 2003; Phillips et al., 2013; Bennett et al., 2017; Kadowaki et al., 2018). For example, ectomycorrhizal fungi has relatively greater access to the organic nitrogen in the soil than arbuscular mycorrhizal fungi (Tedersoo and Bahram, 2019). The fungal mycorrhizal partners can mediate the interactions between plants and the soil microbial community through the mycorrhizosphere (i.e., the area of soil under the combined influence of the plant root and the mycorrhizal fungal community) and the hyphosphere (i.e., the soil zone under the influence of mycorrhizal extraradical hyphae) (Rambelli, 1973; Buee et al., 2009; Churchland and Grayston, 2014). The extraradical hyphae can form belowground networks connecting numerous plant roots, known as hyphal networks or common mycorrhizal networks (Simard et al., 2012). In addition, free-living soil fungi and bacteria respond to changes in the mycorrhizosphere and surrounding soil processes such as rhizodeposition and organic matter decomposition (Fitter and Garbaye, 1994; Johansson et al., 2004; Bardgett and Wardle, 2010). In an observational study from boreal and temperate regional sites, (Bahram et al., 2020) described differentiated microbial communities between sites dominated by AM and EcM type plants. In addition, (Weißbecker et al., 2018) reported a significant correlation of EcM fungal community structure with EcM type trees. Despite

these studies, the influence of a plant's mycorrhizal type on the diversity and composition of soil microbial communities, including bacteria, remains unclear, especially at the local scale.

As tree diversity increases, different tree-tree interactions develop, and so does the complexity of the associated plant-plant, plant-microbe, and microbe-microbe interactions (Bonfante and Anca, 2009; Schuldt et al., 2017). Previous research has shown positive tree diversity effects on soil microbial diversity (Gao et al., 2013; Barberan et al., 2015; Hiiesalu et al., 2017) but also no or small effects were reported (McGuire et al., 2012; Rivest et al., 2019). Tree species richness effects may develop in some cases and may not in others. These inconsistent findings might also result from a strong context-dependency of tree diversity effects on the soil microbial community (Tedersoo et al., 2016), which calls for an experimental setting with a controlled environmental context. Such controlled settings facilitate the systematic testing of how the tree mycorrhizal type in tree-tree interactions affects soil microbiota in forest ecosystems and how these relations are shaped by different levels of tree species diversity. In this way, context-dependency can be reduced to diversity effects, in addition to the effects of the identity of the target tree species and their neighbors, and environmental variation that differs between sampling locations. While in a field experiment, environmental variation cannot be fully excluded, it can be accounted for when being measured. The knowledge of how the tree mycorrhizal type of focal trees, their neighbor tree species and tree species diversity affect the soil microbiota of the tree-tree interaction zone would shed light on microbial community assembly and ecosystem functioning.

To address this knowledge gap, we used the BEF-China experimental research platform, where trees were grown with tree diversity levels of 1, 2, 4, 8, 16 and 24 species (Bruelheide et al., 2014). We employed the tree-species pair (TSP) concept wherein, two adjacent trees were selected as a target sampling unit (Trogisch et al., 2021). The TSP design provides a focal TSP partner and also facilitates uniform soil sampling to capture the focal tree-tree soil interaction zone. Combined with random sampling, this would further facilitate the unbiased identification and comparison of tree mycorrhizal type effects on the soil microbiota across tree diversity levels. The interaction zone soil microbial communities were assessed using paired-end Illumina sequencing targeting the bacterial 16S (V4 region) and the fungal internal transcribed spacer (ITS2) regions.

We hypothesized that: (H1) soil microbial alpha diversity is affected by the tree mycorrhizal type and that within the mycorrhizal type of EcM and AM TSPs, microbial alpha diversity increases with increasing tree species diversity. Given the anatomical and ecophysiological differences of the two mycorrhizal types (Bonfante and Genre, 2010), their effect on the soil nutrient cycling (Cheeke et al., 2017), and differential capability to mobilize organic (EcM fungi) and inorganic (AM fungi) compounds (Read and Perez-Moreno, 2003; Smith and Read, 2008), we expected a lower microbial diversity in EcM TSPs than in AM TSPs. Furthermore, since higher plant diversity can enrich the microbial communities through increased carbon inputs into the rhizosphere (Lange et al., 2015; Eisenhauer et al., 2017), we expect an increase in microbial diversity with increasing tree diversity in both AM and EcM TSPs. Likewise, we hypothesized that (H2) tree mycorrhizal type, tree diversity levels and the site-specific environmental conditions influence the microbial community composition. Through promoting the diversity of nutrient resources and increasing microhabitat complexity (Hooper et al., 2000; Prober et al., 2015) a high plant diversity facilitates the co-existence of diverse microbial communities. More specifically, we tested the hypothesis that (H2a) microbial community composition depends on tree mycorrhizal type, because different mycorrhizal type trees provide different types of resources (Tedersoo and Bahram, 2019). Furthermore, we expected (H2b) microbial communities to become more similar with increasing tree diversity because the more diverse resources provided by the host species should allow the co-existence of a larger part of the total pool of bacteria and fungi (Lange et al., 2015; Kaspari et al., 2017). Consequently, with increasing tree diversity, we expected that the most influential microbial taxa driving the differences between mycorrhizal types would be reduced. Besides, since plant diversity influences the local edaphic and microclimatic environment (Bruelheide et al., 2014), while some environmental variation (such as topography) is independent of plant diversity, we expected (H2c) abiotic and biotic environmental factors to contribute to shaping the soil microbial community composition in addition to tree diversity and the mycorrhizal type effects.

Materials and Methods

Study site and experimental design

For details on study site and experimental design, please refer to the 'Experimental design' section of Chapter I (Table I.1, Figure I.4), as well as Singavarapu et al., 2021 (Appendix

S1). Briefly, six EcM and six AM TSPs were randomly selected across 57 plots, with each three replicates in monocultures (denoted by "1") and two-species mixtures (denoted by "2"), and one replicate in each 4, 8, and 16 or 24 multi-tree species mixtures (denoted by "≥4"). We obtained the following six combinations: 'EcM|1'(n=18), 'EcM|2'(n=18), 'EcM|≥4'(n=18), 'AM|1'(n=18), 'AM|2'(n=18) and 'AM|≥4'(n=18). Besides, to study the heterotypic combination of mycorrhizal type (i.e., combination of EcM tree and AM tree), we included six pairs of AM and EcM tree species, each with three replicates (n=18) as heterotypic pairs (referred to as 'Mycomix-TSPs') only in the multi-tree species mixtures but not in the two-species mixtures. This resulted in a total of 126 soil samples.

Soil sampling and processing

Soil samples were randomly collected from mid-August to the end of September. Before taking soil cores, litter and any other debris were cleared from the soil surface. Four cores of each 10 cm depth and 5 cm diameter were collected along the horizontal axis of the two partner trees of a TSP with distances of 5 cm from the center for the first two cores and further 20 cm away for the other two cores (Chapter I: Figure I.4D). The obtained four soil cores were pooled, mixed, and root fragments were removed by sieving the mixed soil through 2-mm mesh size sieves to yield a composite soil sample. These soil samples were then aliquoted for soil chemistry (50 g) and microbiota analyses (30 g) into sampling tubes and immediately placed on dry ice in a cool box and transported to the field lab. Then the samples for the microbiota analysis were freeze-dried (Weißbecker et al., 2017) and stored at -80°C until further analyses.

Soil chemical properties

Each soil sample was divided into two parts used in the analysis of soil moisture and soil nutrients, respectively. For the first portion, soil moisture was measured by recording the mass lost after drying the soil at 105°C for 24 h. The other sub-sample was air-dried. NH_4^+ and NO_3^- were extracted with 2 M KCl and measured by the colorimetric method with a Smart Chem 200 Discrete Auto Analyzer (AMS, Italy) (Talbot et al., 2014). Soil total organic carbon (TOC) was measured by a TOC Analyzer (Liqui TOC II; Elementar Analysensysteme GmbH, Hanau, Germany). Soil total nitrogen (TN) was measured on an auto-analyzer (SEAL Analytical GmbH, Norderstedt, Germany) using the Kjeldahl method (Bradstreet, 1954). Soil total phosphorus (TP) was measured after wet digestion with

H_2SO_4 and $HClO_4$ by a UV-VIS spectrophotometer (UV2700, SHIMADZU, Japan). Soil pH was measured in a 1:2.5 soil-water solution (pH meter Thermo Scientific Orion Star A221) after air-drying the soil at 40°C for two days.

DNA extraction, amplicon library preparation, and sequencing

Microbial genomic DNA was extracted from freeze-dried soil samples using PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, United States) according to the manufacturer's instructions. DNA concentrations were measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany), and the extracts were adjusted to 10–15 ng/µl template concentration. The bacterial and fungal amplicon libraries were prepared as previously described (Schöps et al., 2018; Nawaz et al., 2019). Briefly, the V4 region of the bacterial 16S rRNA gene was amplified using the universal primer pair 515f and 806r (Caporaso et al., 2011) with Illumina adapter sequence overhangs. Semi-nested PCR was performed for fungi to amplify the ITS2 rDNA region using the initial primer combination of ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) followed by the primer pair fITS7 (Ihrmark et al., 2012) and ITS4 containing the Illumina adapter sequences. The amplicon libraries were purified with Agencourt AMPure XP beads (Beckman Coulter, Krefeld, Germany). Illumina Nextera XT Indices were added to both ends of the bacterial and fungal fragments in the indexing PCR. The indexed products were purified again with AMPure beads and then quantified by PicoGreen assay. The amplicon libraries were pooled equimolarly to a final concentration of 4 nM each for fungi and bacteria. Then fungal and bacterial libraries were pooled in 1:3 ratio to make the final library and paired-end sequencing of 2x300 bp was performed on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, United States) using MiSeq Reagent kit v3 at the Department of Environmental Microbiology, UFZ, Leipzig, Germany.

Bioinformatics analysis

Bioinformatic analysis was performed to filter out high-quality reads from the raw reads generated by the Illumina MiSeq Sequencing platform using the Quantitative Insights into Microbial Ecology – QIIME 2 2020.2 (Bolyen et al., 2019) software. The forward and reverse reads were demultiplexed according to the index combinations, primer sequences were trimmed, followed by sequence denoising and grouping into Amplicon Sequence Variants (ASVs) using cut-adapt (Martin, 2011) (q2-cutadapt) and DADA2 (Callahan et

al., 2016) (via q2-dada2), respectively. Taxonomy was assigned to 16S bacterial ASVs using the q2-feature-classifier (Bokulich et al., 2018), using the classify-sklearn naive Bayes taxonomy classifier against the silva-132-99-515-806-nb-classifier. The fungal ITS dataset was analyzed using the q2-ITSxpress Qiime2 plugin (Rivers et al., 2018), where the ITS2 fungal sequences were identified and trimmed, followed by denoising and grouping into Amplicon sequence variants (ASVs) using the DADA2. Taxonomy was assigned to fungal ITS ASVs using the q2-feature-classifier (Bokulich et al., 2018), using the classify-sklearn naive Bayes taxonomy classifier against the unite-ver8-99-classifier-04.02.2020.

The respective fungal and bacterial ASV matrices, taxonomic tables and representative sequences were imported into R (version 4.0.2) using the phyloseq package (McMurdie and Holmes, 2013) for further statistical analysis. The fungal and bacterial ASVs were filtered, and the ASV matrices were rarefied to 16,542 and 28,897 reads per sample, respectively. Furthermore, to avoid the potentially spurious taxa and to reduce the noise, taxa that were not present in at least 5% of the samples were removed in both fungal and bacterial datasets (Cao et al., 2021). Liner regression ('lm' function) and Mantel tests ('mantel' function in vegan) were used to test the effect of removal of low abundant taxa on alpha diversity indices and microbial community composition analyses, respectively. Fungal and bacterial ASVs were annotated for potential functional groups using FUNGuild (Nguyen et al., 2016a) and FAPROTAX (Louca et al., 2016), respectively. Saprotrophs and pathotrophs were considered one functional group each. Symbiotrophs were further classified to distinguish ectomycorrhizae and arbuscular mycorrhizae as functional groups, and the remaining guilds of symbiotrophs were named as 'other symbiotrophs'. The fungal taxa with more than one trophic mode were classified as 'others'. We assigned the putative bacterial functional groups to broad ecological processes, namely carbon cycle, nitrogen cycle, and sulphur cycle. Bacterial taxa were assigned to the respective aforementioned nutrient cycles if that particular taxon was assigned to at least one functional group within that particular category. If a taxon was associated with two or more functional groups belonging to different nutrient cycles (e.g., carbon cycle and nitrogen cycle), then it was assigned to a combined category (e.g., Carbon & Nitrogen cycle). Functional groups that did not fall under these preceding categories were grouped as 'other'.

Statistical analysis

All the statistical analyses were done in R (version 4.0.2) using the phyloseq package (McMurdie and Holmes, 2013). In both fungal and bacterial datasets, sequencing data of each replicate collected from 4, 8, and 16 or 24 tree species richness levels were combined into a 'multi-tree species mixtures' group after checking for the homogeneity of variance of sequence library sizes (Levene's test; for Fungi p=0.42; for Bacteria p=0.22). EcM and AM TSPs (n=108) were used in the following statistical analyses of mycorrhizal type across tree diversity levels. The heterotypic TSP combination, i.e., Mycomix-TSPs (n=18), were included in the analyses only to compare different mycorrhizal types (EcM, AM, and Mycomix) at tree diversity level of multi-tree species mixtures unless otherwise stated. The ecosystem state variables, i.e., observed richness, Shannon diversity, Pielou evenness, and Gini dominance were calculated as measures of alpha diversity using the microbiome package (Lahti et al., 2017). Wilcoxon rank sum-tests were used to test for group differences in alpha diversity. The interaction between mycorrhizal type and tree diversity was tested for fungi with two-way ANOVA, and for this purpose, the data was tested for normality and homogeneity of variance using Shapiro-Wilk test and Levene's test, respectively. Fungal observed richness and Pielou evenness, each was Box-Cox transformed with a lambda value of 1.45 to meet the normality and homogeneity of variance assumptions using the car package (Fox and Weisberg, 2018). Taxonomic and assigned functional group relative abundances were calculated and visualized with bar charts. Distance-based ordination (dbRDA) constrained by tree mycorrhizal type and tree diversity was done with the 'capscale' function in the vegan package (Oksanen et al., 2019), using Bray-Curtis distance to test and visualize the patterns in microbial community compositions. The differences in compositions were tested for the effect of mycorrhizal type and tree diversity with permutational analysis of variance (PERMANOVA) using the vegan package. Multivariate homogeneity of variances of groups was checked with 'betadisper' function before PERMANOVA. Pairwise community compositional differences were tested using the function 'pairwise.adonis' from the pairwiseAdonis package (Arbizu; Martinez Arbizu, 2017).

We used random forest (RF) models to determine the most influential microbial taxa driving the differences between tree mycorrhizal types. RF is a robust machine-learning tool with high prediction accuracy befitting for the microbiome data (Statnikov et al., 2013;

Kim et al., 2020). All taxa with an abundance of >3% mean total sequencing reads and a frequency of at least 2/3rd of the samples (≥33%) were considered for RF analysis in both fungal (728 taxa) and bacterial (798 taxa) datasets. The fungal and bacterial ASV relative abundance matrices were z-score standardized and then RF classification models were constructed over 3001 decision trees using the rfPermute package (Archer, 2016). The RF models were assessed for statistical significance with 999 permutations using 'rf.significance' function in the rfUtilities package (Evans and Murphy, 2015). Further, the significance of the importance metrics of each microbial taxon was measured using 999 permutations of the response variable in the 'rfPermute' function in the rfPermute package. The microbial taxa responsible for significant (p<0.05) mean decrease in accuracy and mean decrease in Gini impurity index of the RF models were selected as the most influential microbial taxa (here, referred to as classifier taxa). The top ten taxa in RF models with high mean decrease in accuracy were identified as the top classifier taxa and their relative abundances among EcM and AM TSPs were visualized with heatmaps. Subsequently, the performance of the RF models was evaluated by the receiver operating characteristic curve (ROC curve) and Area under the ROC Curve (AUC) metrics using ROCR package (Sing et al., 2005).

Significant biotic and abiotic factors associated with the microbial (fungi and bacteria) community compositions were selected using distance-based redundancy analysis (dbRDA) models based on the Bray–Curtis distance ('capscale' function in vegan). Explanatory variables were standardized to a constant mean and standard deviation ('decostand' function in vegan). Prior to variable selection, multi co-linearity was checked using the 'vifstep' function in usdm package (Naimi et al., 2014), and then stepwise model selection ('ordistep' function in vegan) was carried out with permutation tests. Four groups of environmental components were considered for analysis, including tree community variables (tree community composition, tree species pair identity, tree and shrub species richness, tree Shannon and Simpson diversity indices, abundance and richness of tree neighborhood, abundance and richness of neighbor AM and EcM TSPs) as biotic factors, soil parameters (C, N, P, C/N, C/P, N/P, TOC, SOM, NH_4^+, NO_3^-, pH and moisture) and topographical variables (altitude, slope, northness, and eastness) as abiotic factors and sampling locations (latitude and longitude) as a spatial component. Vectors from principal coordinates of neighborhood matrices (PCNM) (Dray et al., 2006) were used to represent the spatial component (vegan package). Tree community composition and TSP identity

were characterized by principal components ('prcomp' function) on the Hellinger-transformed incidence data. Subsequently, the selected variables were used in the dbRDA models and their significance was tested with permutational test ('anova.cca' function in vegan).

Results

Sequence data processing

From 4,648,777 and 11,720,448 raw sequencing reads, after quality filtering through denoising, merging, chimera and non-target taxa removal, we obtained 3,678,803 (79.1%) ITS and 8,939,606 (76.3%) 16S sequence reads, which were then clustered into 12,813 fungal and 25,928 bacterial amplicon sequence variants (ASVs), respectively. Rarefaction followed by removing low abundant and potentially spurious ASVs in both fungal and bacterial datasets at a threshold of 5% sample abundance, resulted in 8,041 fungal and 15,913 bacterial taxa, respectively. The alpha diversity indices after removal of low abundant taxa were well fitted (adj.R^2 values range: 0.98 - 1) with that of the indices before filtering (Figure S II.1). Also, the Mantel tests using Bray-Curtis distance on data matrices before and after removal of low abundant taxa showed high congruence (for fungi R=1, p=0.001; for bacteria R=0.99, p=0.001), therefore suggesting that the removal of rare taxa had no significant impact on the microbial community analysis. Thus, we used the latter dataset to test our hypotheses.

Tree mycorrhizal type and tree diversity effects on microbial alpha diversity

The alpha diversity measures observed richness, Shannon diversity, Pielou's evenness, and Gini dominance indices showed significant differences between tree mycorrhizal types for fungal but not for soil bacterial communities (Figure II.1). Further, Wilcoxon rank-sum tests within the tree diversity levels revealed that for fungal communities, the differences between mycorrhizal types were present in monocultures and two-species mixtures but were absent at multi-tree species mixtures (Figure II.1B, D, F, H). A two-way ANOVA analysis on fungal alpha diversity metrics showed strong effects of tree mycorrhizal type and significant interaction with tree diversity levels (Table S II.1). Furthermore, pairwise analysis of EcM and AM TSP soil fungal communities along the tree diversity levels also confirmed that the fungal alpha diversity increased only for EcM TSPs, and the differences

between EcM and AM TSPs disappeared at multi-tree species mixtures (Figure S II.2). In contrast, within the tree diversity levels, no significant differences were found in bacterial communities except for Pielou's evenness in two species mixtures (Figure II.1J, L, N, P). Comparison of the effect of tree mycorrhizal types at the multi-tree species mixtures, including also the Mycomix-TSPs along with EcM and AM TSPs, showed no significant differences among these different types (Figure S II.3).

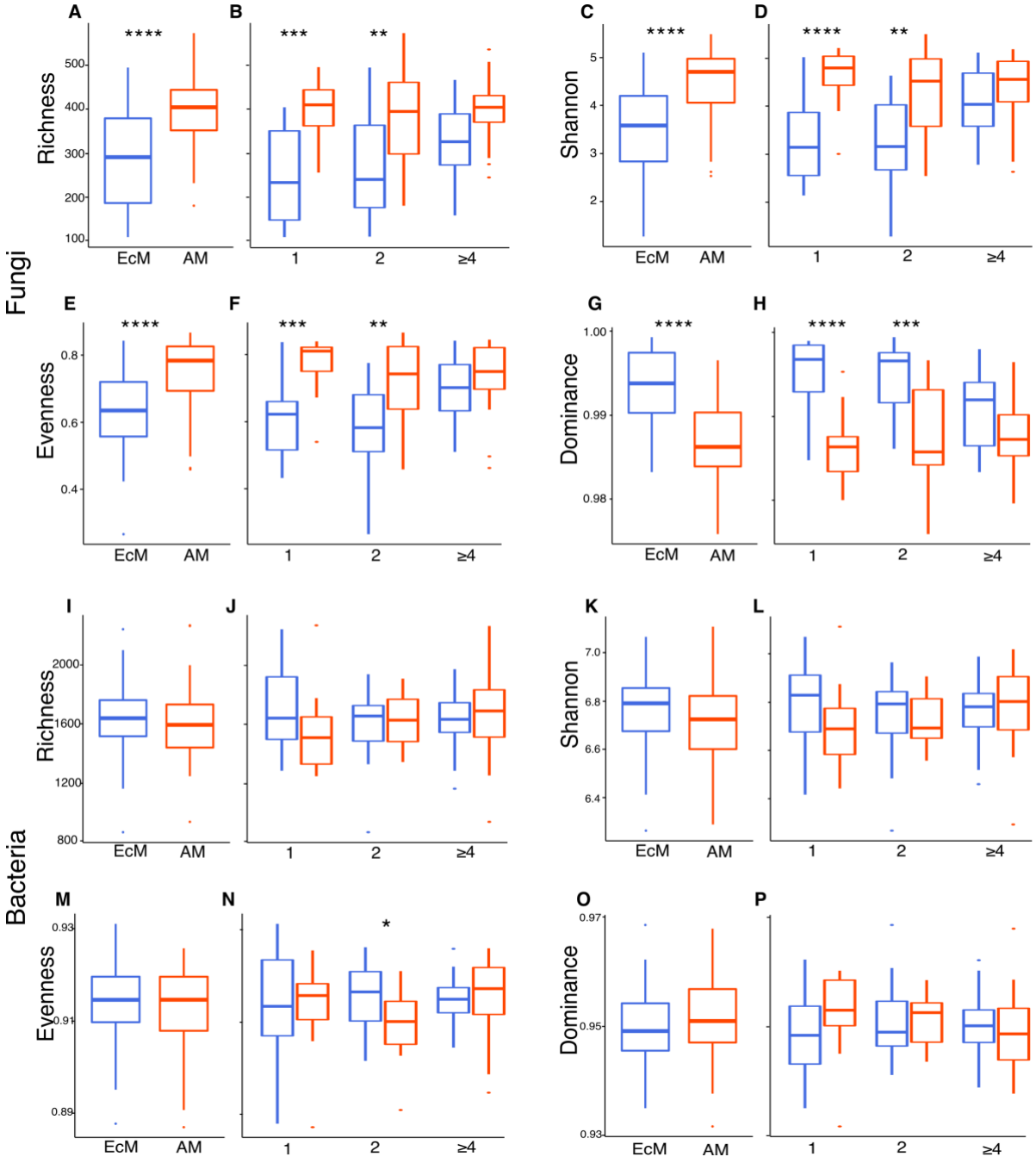


Figure II.1 Fungal and bacterial alpha diversity indices namely observed ASV richness (i.e., "Richness"), Shannon diversity (i.e., "Shannon"), Pielou evenness (i.e., "Evenness") and Gini dominance (i.e., "Dominance"). On the x-axis EcM (blue color) and AM TSPs

(red color) and the tree diversity levels (1- monocultures, 2 - two-species mixtures and ≥4 - multi-tree species mixtures). A, C, E, G: Comparison of soil fungal alpha diversity between all EcM and AM TSPs. B, D, F, H: Within the tree diversity level differences between EcM and AM TSPs for the respective fungal alpha diversity measures. I, K, M, O: Comparison of soil bacterial alpha diversity between all EcM and AM TSPs. J, L, N, P: Within the tree diversity level differences between EcM and AM TSPs for the respective bacterial alpha diversity measures. The asterisks show the p-value significance level, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p< 0.0001$

Tree mycorrhizal type and tree diversity effects on taxonomic and functional groups

Fungal communities were dominated by Basidiomycota in EcM TSPs, while both Ascomycota and Basidiomycota were in nearly equal proportions in AM TSPs. In contrast, bacterial communities were dominated by the phylum Acidobacteriota followed by Proteobacteria, although these proportions were not distinctly different between EcM and AM TSPs (Figure S II.4). Visualization of the taxonomic compositions at the order level indicated that the soil fungal communities differed in their relative abundances of taxa between tree mycorrhizal types and along the tree diversity levels (Figure II.2A, B), whereas bacterial communities displayed relatively less conspicuous differences (Figure II.2E, F). For instance, in fungal communities, Cantharellales with a major proportion of ectomycorrhizal fungi (Table S II.2) were distinctive in EcM TSPs but minuscule in AM TSPs. In contrast, Glomerales were relatively less abundant in EcM than in the AM TSPs. The relative abundances of Thelephorales and Sebacinales were decreased in EcM TSPs of multi-tree species mixtures compared to monocultures, while these taxa were trivial in AM tree monocultures. Whereas, in bacterial communities, the EcM TSPs of EcM tree monocultures had a higher relative abundance of Acidobacteriota|Subgroup_7, Chloroflexi|SBR1031, Gemmatimonadales, Rokubacteriales, and Vicinamibacterales than that of multi-tree species mixtures.

Analysis of the functional group abundances of the soil fungal communities showed distinct patterns between the EcM and AM TSPs and among the different tree diversity levels. The EcM TSPs were dominated by symbiotrophs, mainly by ectomycorrhizal fungi (e.g., the genera *Inocybe, Russula, Clavulina*). In comparison, the AM TSPs were dominated by saprotrophs and displayed a lower proportion of symbiotrophs, mainly by arbuscular mycorrhizal fungi (e.g., the genera *Glomus, Rhizophagus, Diversispora*) in the monocultures and an increasing proportion of ectomycorrhiza and other symbiotrophs in

the two and multi-tree species mixtures (Figure II.2C, D). The bacterial functional groups, however, showed no clear pattern between the tree mycorrhizal types and the diversity levels in both EcM and AM TSPs (Figure II.2G, H).

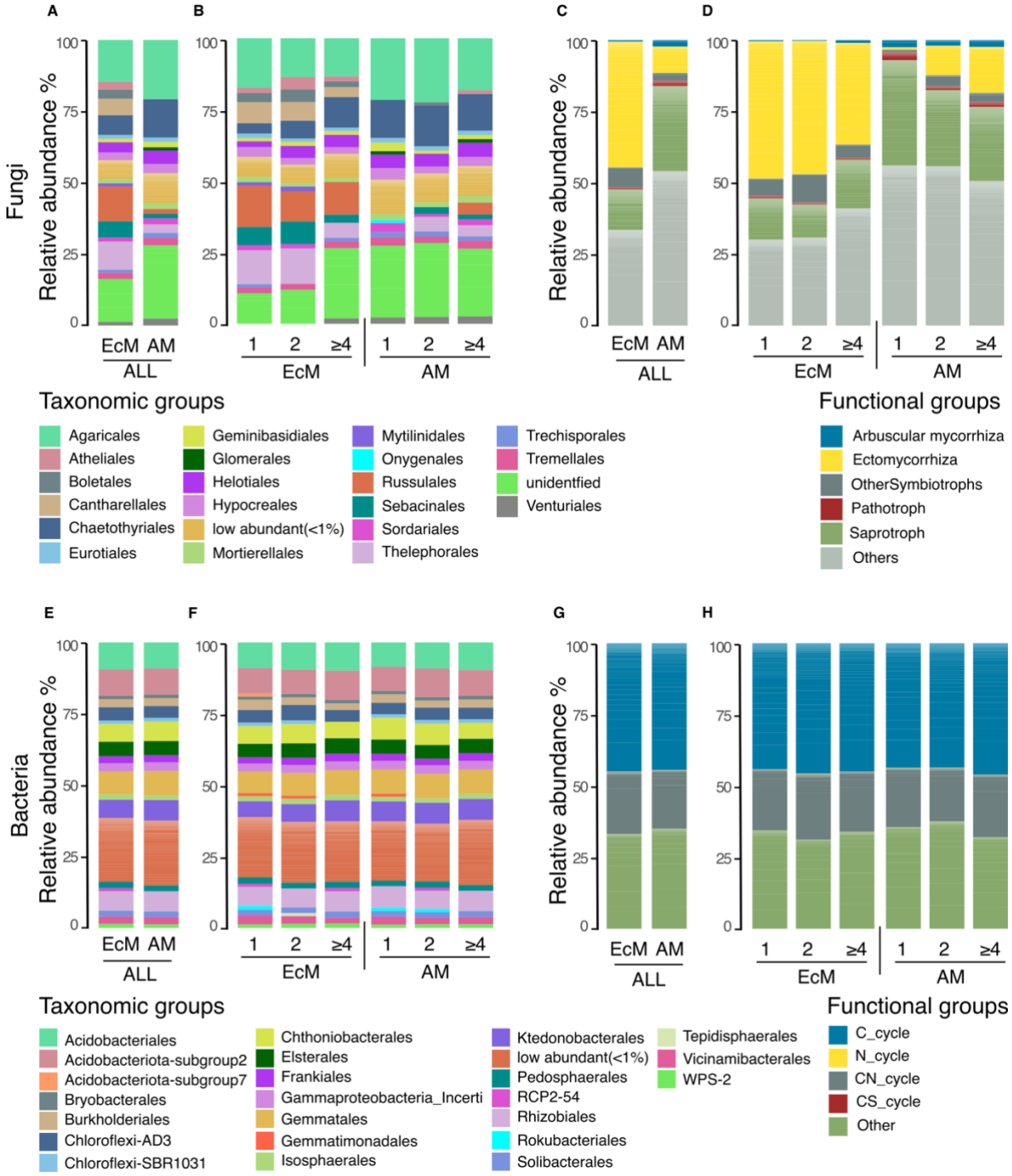


Figure II.2 Taxonomic and functional group composition of soil fungal and bacterial communities. On the x-axis EcM and AM TSPs and the tree diversity levels (All- combined dataset irrespective of tree diversity,1- monocultures, 2- two-species mixtures and ≥4 - multi-tree species mixtures). Assigned functional groups were only shown here. Order-level taxonomic composition of fungal communities of (A) EcM and AM TSPs and (B) across diversity levels. Functional group composition of fungal communities of (C) EcM

and AM TSPs and (D) across diversity levels. Order-level taxonomic composition of bacterial communities of (E) EcM and AM TSPs and (F) across diversity levels. Functional group composition of bacterial communities of (G) EcM and AM TSPs and (H) across diversity levels.

Tree mycorrhizal type and tree diversity effects on microbial community composition

The dbRDA-based ordination analysis showed that EcM and AM TSPs soil fungal communities were significantly more distant in monocultures than in two-species mixtures, while they clustered closely together in the multi-tree species mixtures (Figure II.3A, B). In contrast, bacterial communities were relatively less distinct between EcM and AM TSPs and showed differences in the tree diversity levels, wherein the tree mycorrhizal types clustered closely in multi-tree species mixtures (Figure II.3C, D). The PERMANOVA test also confirmed the significant main and interaction effects of tree mycorrhizal type and tree diversity levels on fungal community composition (explained variance = 6.9%). In contrast, there was only a significant main effect of tree diversity (explained variance = 2.8%) in bacterial communities (Table II.1). The analysis of multivariate homogeneity of the groups' dispersion confirmed that the variances within groups did not differ among groups, thus indicating that the significant differences between group means as revealed by the PERMANOVA were not an artifact of heterogeneity among groups (Fungi, F= 1.39, p=0.22; Bacteria, F= 0.18, p=0.98).

Furthermore, pairwise comparisons along the tree diversity levels revealed that the EcM TSPs soil fungal communities differed in their composition between monocultures and multi-tree species mixtures and between two and multi-tree species mixtures (Table S II.3). In contrast, no such differences were encountered for AM TSPs soil fungal communities between tree diversity levels. Differences between EcM and AM TSPs soil fungal communities along tree diversity levels were found in monocultures and two-species mixtures, but they disappeared at the multi-tree species level (Table S II.3). For bacterial communities, the only significant difference was detected between the EcM tree monocultures and EcM multi-tree species mixtures. Comparison of the tree mycorrhizal types at the multi-tree species mixtures, including also the Mycomix-TSPs along with EcM and AM TSPs, showed no significant differences for both fungal and bacterial community compositions (Fungi, F= 0.91, p=0.78; Bacteria, F= 0.88, p=0.63).

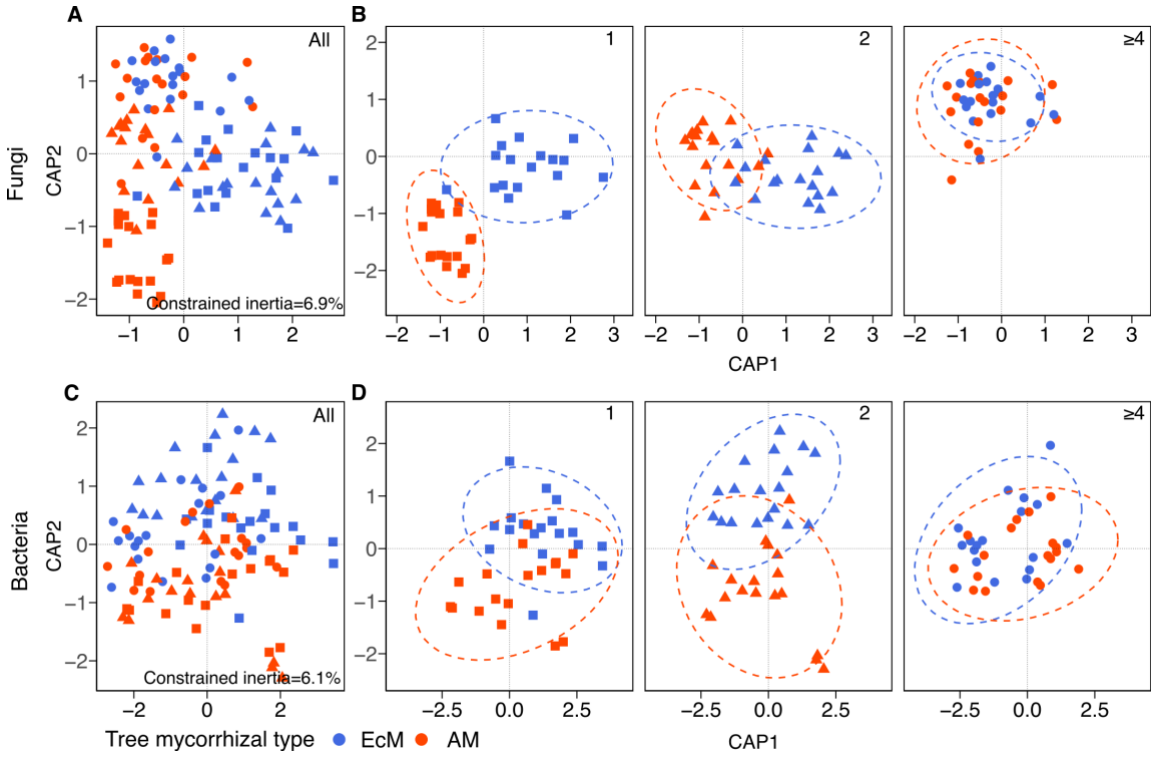


Figure II.3 Distance-based RDA (dbRDA) ordination plots constrained on the mycorrhizal type and tree diversity levels. EcM samples -blue color and AM samples -red color. A) Fungal communities - combined dataset (both factors significant including the interaction between mycorrhizal type and tree diversity (permutest, p=0.01)). C) Bacterial communities - combined dataset (only tree diversity significant (permutest, p=0.03)). B and D: ordination of fungal and bacterial communities faceted across mono (1), two (2), and multi-tree species mixtures (≥4), respectively. Ellipses represent 95% confidence intervals around mycorrhizal group centroids.

Table II.1:
Effects of tree mycorrhizal type and tree diversity level on the compositional differences of soil fungal and bacterial communities based on PERMANOVA with 999 permutations

	Fungal communities				Bacterial communities			
	df	F	R^2	p	df	F	R^2	p
Mycorrhizal type (M)	1	2.522	0.023	**0.001*****	1	1.318	0.012	0.123
Tree diversity level (L)	2	1.228	0.022	**0.015***	2	1.529	0.028	**0.030***
Interaction (MxL)	2	1.290	0.024	**0.010****	2	1.111	0.020	0.236
Residual	102		0.931		102		0.939	

*All significant p values are highlighted in bold followed by significance level codes, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$.*

Furthermore, to evaluate the effects of TSPs within the mycorrhizal type on the microbial community variation, PERMANOVA analysis was performed. The EcM TSPs (F= 1.202, R^2= 11.13%, p =0.009) and the AM TSPs (F= 1.263, R^2= 11.63%, p =0.001) had a similar

effect on fungal community composition. Post-hoc pairwise analysis revealed that AM TSPs including *Nyssa sinensis*, *Liquidambar formosana*, *Choerospondias axillaris* and *Koelreuteria bipinnata* had significant TSP effects (Table S II.4). While EcM TSPs did not show any significant effects in post-hoc pairwise analyses. Similar to fungi, both EcM TSPs (*F= 1.494, R^2= 13.47%, p =0.005*) and AM TSPs *(F= 1.423, R^2= 12.96%, p =0.025*) had a comparable strong effect on bacterial communities. Further post-hoc pairwise analyses revealed marginal significant effects (p =0.053) only for EcM TSPs that included *Quercus fabri, Castanopsis sclerophylla* and *Cyclobalanopsis glauca*.

Random forest model based microbial predictors of tree mycorrhizal types across tree diversity levels

Random forest models further revealed the effects of tree mycorrhizal type and tree diversity by identifying the most influential microbial taxa (classifier taxa), differentiating the tree mycorrhizal types across the tree diversity levels except in multi-species mixtures (Figure II.4). The soil fungal communities exhibited a higher number (90) of classifier taxa for tree mycorrhizal type irrespective of the tree diversity (RF model, p<0.001, AUC = 0.75) (Figure II.4, Figure S II.5 A, G). The number of classifier fungal taxa was reduced to 53 and 27 in monocultures (RF model, p=0.005, AUC = 0.78) and two-species mixtures (RF model, p=0.008, AUC = 0.74), respectively (Figure II.4, Figure S II.5 B, C, G), while the RF model was not significant in multi-tree species mixtures (p=0.247). In case of bacteria, the number of classifier taxa showed little variation among all TSPs in the combined dataset (RF model, p=0.001, AUC = 0.75), monocultures (RF model, p=0.003, AUC = 0.78) and two-species mixtures (RF model, p=0.001, AUC = 0.74) (Figure II.4, Figure S II.5 D-F, G). Similar to fungi, the RF model for bacteria was also not significant in multi-tree species mixtures (p=0.701). Furthermore, including also the Mycomix-TSPs along with EcM and AM TSPs at multi-tree species mixtures, as well resulted in no significant RF models for both fungi and bacteria (Fungi, p=0.179; Bacteria, p=0.529).

The majority of the top fungal classifier taxa belonged to ectomycorrhiza and saprotrophs, which consisted of 4, 8, and 6 ASVs out of the top ten ASVs in the combined dataset, monocultures and two-species mixtures, respectively (Figure II.4A, B, C). Among the top fungal classifier taxa, all ectomycorrhizal ASVs (e.g., *Tomentella* fASV2714 and *Byssocorticium* fASV3237, fASV3238) had comparatively higher relative abundances in EcM TSPs than in AM TSPs in the overall dataset and across the monocultures and two-

species mixtures. In contrast, saprotrophs did not show any distinct abundance pattern. For example, fASV0289 had a higher relative abundance in monocultures of AM TSPs, while fASV3950 had a higher abundance in EcM TSPs. In the case of top bacterial classifier taxa, the ASVs belonging to Bacteroidota (*Puia* bASV01352, bASV01341 and bASV01235) and Proteobacteria (Elsterales bASV02827, bASV03024, bASV02960 and *Burkholderia* bASV04648) had comparatively higher relative abundances in EcM TSPs than AM TSPs. In contrast, the ASVs belonging to the family Ktedonobacteraceae of the phylum Chloroflexi were relatively highly abundant in AM TSPs (Figure II.4D, E, F).

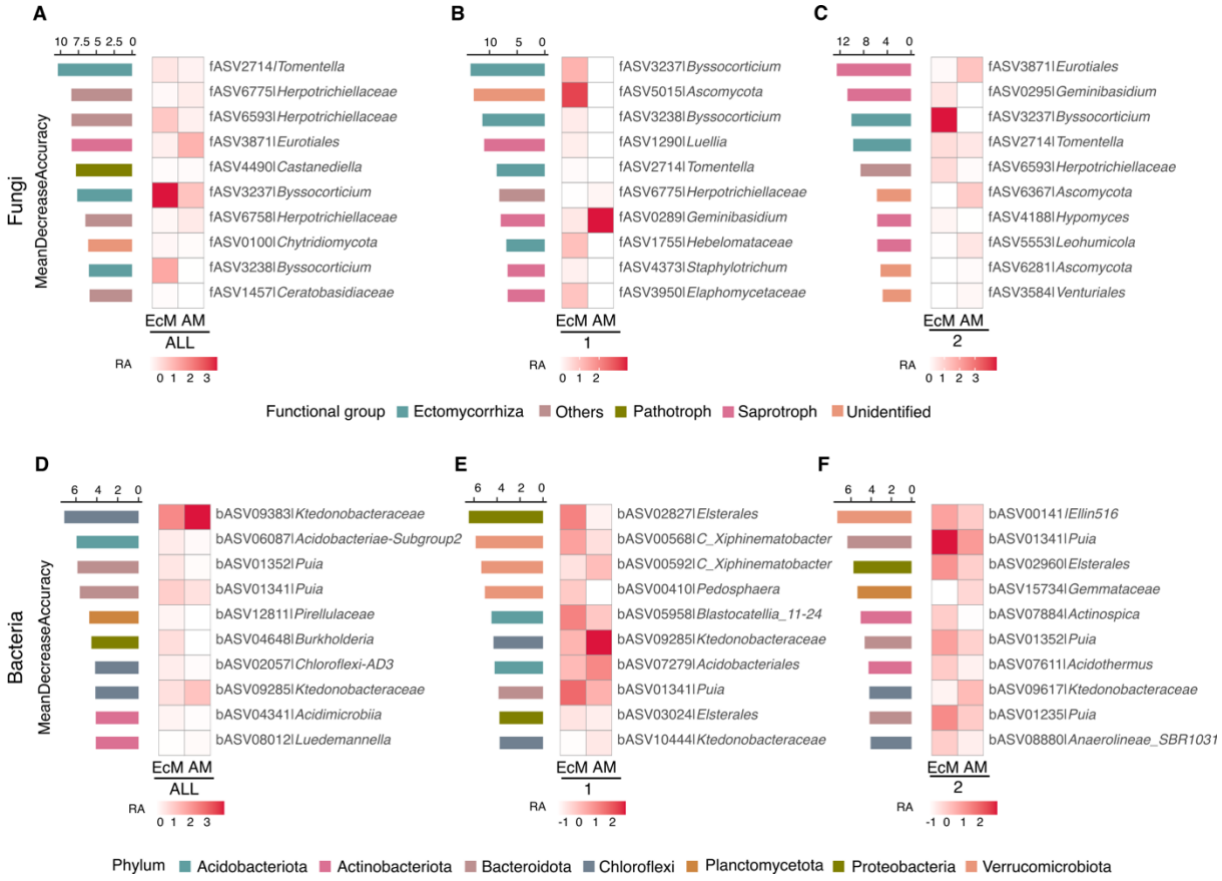


Figure II.4 Topmost influential soil microbial taxa driving the differences between tree mycorrhizal types. On the x-axis, EcM and AM TSPs and the tree diversity levels (All-combined dataset irrespective of tree diversity,1- monocultures, 2- two-species mixtures) Random forest (RF) model determined top 10 microbial taxa (ASVs) arranged in descending order of the mean decrease in accuracy. The left side panel of each subplot (i.e., A, B, C, D, E, F) shows the mean decrease in accuracy in bar graphs colored by functional groups for Fungi (i.e., A, B, C) and phylum for Bacteria (i.e., D, E, F). Right side panel is the heatmap representation of the z-standardized percentage relative abundances (RA) of the respective taxa in EcM and AM TSPs. The taxa were named by respective ASV followed by its lowest taxonomic level up to the genus. (A) Combined fungal dataset of all TSPs (B) Fungi in monocultures (C) Fungi in two-species mixtures (D) Combined bacterial dataset of all TSPs (E) Bacteria in monocultures (F) Bacteria in two-species mixtures. The RF models were not significant in multi-tree species mixtures

Interplay among environmental factors, tree mycorrhizal type and tree diversity in shaping soil microbial community composition

Analysis of the role of soil, plant, topography, and spatial variables in shaping the soil microbiota using the dbRDA model revealed that the fungal communities of both EcM and AM TSPs were associated with a common set of environmental conditions (Table S II.5). P, NO_3^-, NH_4^+, and pH were the significant edaphic variables along with topographic, spatial, and tree community variables that mainly influenced the variation in fungal community composition. However, across the tree diversity levels, the environmental factors associated with EcM and AM TSPs fungal communities varied to some extent (Table S II.6). In general, AM TSPs fungal communities were significantly associated with a greater number of environmental variables measured in this study. In monocultures, pH and TSP identity were common edaphic and tree variables, respectively, that were significantly associated with both the EcM and AM TSPs fungal communities, while P, NH_4^+, and tree community composition were only related to AM TSPs fungal communities. In two-species mixtures, pH (F=1.547, p<0.001) and N (F=1.317, p=0.034) were significant edaphic factors related to the AM TSPs fungal communities, while P (F=1.576, p=0.009) was the only significant soil factor related to EcM TSPs fungal communities. In multi-tree species mixtures, a relatively smaller number of environmental variables had significant associations with the variation in fungal community composition. NO_3^-, altitude, and slope were common variables related to both EcM and AM TSPs fungal community composition. In addition to AM TSPs (F=1.478, p=0.027), fungal communities under the Mycomix-TSPs (F=1.620, p=0.001) in multi-tree species mixtures were significantly related to the tree community composition (Table S II.6).

Bacterial communities of both EcM and AM TSPs were also associated with a common set of environmental variables, including NO_3^-, pH, moisture, and tree community composition along with topographical and spatial variables (Table S II.5). Soil pH (F=10.05, p<0.001) was the most influential factor for bacterial communities, irrespective of the tree mycorrhizal type and tree diversity level. In monocultures, pH (*F=2.980, p<0.001*) was the only soil variable significantly associated with EcM TSPs bacterial communities, while AM TSPs bacterial communities in addition to pH (*F=4.961, p<0.001*), were also affected by P (*F=2.113, p=0.031*) (Table S II.7). In two-species mixtures, a relatively greater number of environmental variables displayed significant associations with bacterial

community composition compared to monocultures and multi-tree species mixtures. In multi-tree species mixtures, AM TSPs bacterial communities were significantly related to NO_3^- (*F=1.700, p=0.041*) and moisture (*F=1.913, p=0.029*), as well as to pH (*F=3.492, p<0.001*). Similar to fungi, bacterial communities under the Mycomix-TSPs in multi-tree species mixtures were in addition significantly related to the tree community variables (tree community composition: *F=2.317, p=0.001;* TSP identity: *F=1.784, p=0.039*).

Discussion

Tree mycorrhizal type affects fungal rather than bacterial alpha diversity

We found that the mycorrhizal type of the TSPs affected the fungal alpha diversity confirming our hypothesis (H1). The soil fungal alpha diversity of EcM TSPs was significantly lower than that of AM TSPs in terms of taxa richness, evenness, and diversity. These consistent differences in various aspects of fungal alpha diversity indicate an important role of the mycorrhizal partner of EcM and AM TSPs in the recruitment of the co-occurring fungal community. These results are in line with the negative impact of higher EcM plant abundance in the soil fungal richness reported in boreal and temperate sites, underlining the differences between EcM and AM tree dominated forests (Bahram et al., 2020). This is mainly because EcM and AM fungal partners of the EcM and AM TSPs differ in their resource acquisition, allocation and plant-soil feedback strategies, which affect the recruitment of the different microbes into their respective mycorrhizospheres (Bonfante and Anca, 2009). EcM fungi were reported to have slower decomposition rates and could limit the abundance of saprotrophs and other free-living fungi through competitive interactions for organic nutrients (Moore et al., 2015; Bödeker et al., 2016; Bahram et al., 2020). In contrast, AM fungal partners rely on co-existing saprophytic fungal partners to facilitate decomposition and nutrient cycling in AM tree dominated habitats (Midgley et al., 2015; Jacobs et al., 2018; Tedersoo and Bahram, 2019). Accordingly the high fungal diversity and relative abundances of saprotrophs under AM TSPs, irrespective of the tree diversity levels considered, indicate the taxonomic and functional contribution of saprotrophic fungi in AM dominated systems (Beidler and Pritchard, 2017). The overall soil bacterial alpha diversity of EcM and AM TSPs, however, was not significantly different, indicating no strong impact by the mycorrhizal type in this early-successional forest ecosystem. (Bahram et al., 2020) documented that sites in which EcM plants dominated had significantly lower soil bacterial taxonomic richness. However, they found

a small difference in the bacterial richness among the sites dominated by deciduous EcM plants, as well as among both coniferous and deciduous AM plants dominated sites. It is known that there are only a few strong drivers of the soil bacterial diversity, mainly soil pH (Fierer and Jackson, 2006; Delgado-Baquerizo and Eldridge, 2019). One of the possible explanations for non-significant differences in soil bacterial alpha diversity between EcM and AM TSPs could be that the differences in environmental conditions brought about by the experimental treatments, such as tree diversity and tree species composition, were not large enough to result in large differences as were reported in other studies.

Tree diversity level affects fungal rather than bacterial alpha diversity

As part of our hypothesis (H1), we had postulated that the soil microbial alpha diversity of the EcM and AM TSPs increases with the increasing tree species diversity. Tree diversity *per se* had no significant effect, neither on the overall fungal richness nor on bacterial richness. Nevertheless, we found that EcM TSPs soil fungal alpha diversity but not that of AM TSPs increased with tree species diversity. Furthermore, we found significant interactions between tree mycorrhizal type and tree diversity levels, indicating that the tree mycorrhizal type effect on soil fungal communities was dependent on the tree diversity level. Previous research was inconclusive about the tree diversity effect on soil fungal communities. For instance, in their global observational study on soil fungi, (Tedersoo et al., 2014) found no significant relationship between plant diversity and fungal richness, except for ectomycorrhizal fungi. Recently, a 7-year-old tree diversity experiment with temperate mixed deciduous trees (Rivest et al., 2019) could not demonstrate any effect of tree diversity on the fungal alpha diversity. Conversely, studies in grassland (Lange et al., 2015; Chen et al., 2017), temperate (Hiiesalu et al., 2017), sub-tropical (Gao et al., 2013; Chen et al., 2019b; Weißbecker et al., 2019), and tropical (Peay et al., 2013) ecosystems have reported positive relationships between tree diversity and fungal alpha diversity. Instead, our findings underline the need to consider tree mycorrhizal type as an important factor in studying 'tree diversity – soil microbial diversity' relationships. Previous studies described plant diversity and guild-specific fungal relationships, especially the positive relationship of ectomycorrhizal fungi with plant richness, while non-significant or rather weak effects were reported in the case of saprotrophs (Peay et al., 2013; Nguyen et al., 2016b). We, however, found contrasting patterns for EcM fungal relative abundance in the

EcM and AM TSPs with increasing diversity levels which could be justified based on the knowledge that EcM fungi competitive interactions (Moore et al., 2015; Bödeker et al., 2016; Bahram et al., 2020) and AM fungi co-operative interactions (Beidler and Pritchard, 2017) with other fungal communities in resource acquisition. The predominance of ectomycorrhizal fungi in monocultures and two-species mixtures of EcM TSPs compared to that of multi-tree species mixtures might be an explanation for the higher alpha diversity in the latter. In both AM and EcM TSPs, the relative contribution of the EcM and saprotrophic fungi decreases with increasing tree diversity as the alpha diversity of other fungal groups increases.

In contrast to our expectation, the bacterial alpha diversity did not significantly increase with tree diversity. In a 10-year-old tropical tree experimental site, (Yamamura et al., 2013) were not able to detect any significant differences in bacterial richness among plots with differing tree species richness. Likewise, no significant relationship between plant alpha diversity and bacterial alpha diversity was reported in grasslands (Prober et al., 2015). Evidence shows that the plant diversity effects are relatively stronger for fungi than that of bacteria (Lange et al., 2015; Eisenhauer et al., 2017; Vieira et al., 2020), probably as a result of their morphological and ecophysiological differences (Barberan et al., 2015; Dassen et al., 2017) which could be a possible reason for the observed non-significant differences in bacterial diversity in our study. Alternatively, the effect of tree diversity on soil bacterial diversity as well as on the fungal diversity of AM trees at our study site might become more important in the long term (Eisenhauer et al., 2010; Chen et al., 2019a; Xu et al., 2020). A noteworthy outcome of the positive tree diversity effects was the absence of soil microbial diversity differences in multi-tree species mixtures as a result of less diverging communities, irrespective of which tree species were involved.

Higher tree diversity levels neutralize the tree mycorrhizal type effects on soil microbial community composition

Mycorrhizal fungi are known to influence the surrounding soil microbiota composition through the mycorrhizosphere and extraradical mycelium by controlling resource allocation and chemical signalling (Wallander et al., 2006; Finlay, 2008; Tedersoo et al., 2009; Tedersoo et al., 2020a). We had hypothesized (H2a) that the microbial community composition depends on tree mycorrhizal type, and in line with this expectation, the tree mycorrhizal type had a significant effect on the fungal community composition. In contrast,

bacterial community composition was not significantly impacted by the tree mycorrhizal type. Likewise, (Bahram et al., 2020) also reported that bacterial community composition was not driven by the tree mycorrhizal type. Our data showed a strong impact of the environmental variables, such as soil chemistry, topographical variables, and spatial variables, on the bacterial community compositional differences rather than by tree community variables, which explains the relatively weaker effect of the tree mycorrhizal type.

Both soil fungal and bacterial community compositions of the EcM and AM TSPs became less dissimilar with increasing tree species diversity, confirming the second statement of our second hypothesis (H2b) tested by PERMANOVA and dbRDA analyses. Tree species (host) can select the soil microbiota, for instance, by the effects of tree species identity (Wubet et al., 2009; Weißbecker et al., 2018) and the genotype (Karliński et al., 2020). These effects can be mediated through modulating the soil chemistry resources (Urbanová et al., 2015; Wu and Yu, 2019). Assuming that each tree species to some extent can have species-specific and generalist soil microbial communities, one would expect an increasing number of microbial species with increasing tree diversity covering more and more taxa of the local microbial species pool. In addition, plants can both recruit from and contribute to the surrounding soil microbial species pool (Compant et al., 2019), and therefore, may explain the more similar microbial community composition in multi-tree species mixtures in this study. This view is supported by the ASV richness patterns both in fungi (here in particular under the EcM TSPs) and in bacteria (here in particular under the AM TSPs). However, it is important to consider that the observed neutralizing effect at higher tree diversity level is driven by either tree diversity regardless of the tree mycorrhizal type in bacterial communities, or the presence of different mycorrhizal type trees in the high diversity plots in the case of fungal communities. The fungal taxonomic and functional group relative abundance distributions of both EcM and AM TSPs in multi-tree species mixtures resembles a ‘give-and-take’ relationship (for example, Chaetothyriales abundance got increased in EcM TSPs of multi-tree species mixtures which were relatively abundant in AM TSPs, while ditto was the case for Thelephorales in AM TSPs of multi-tree species mixtures which were relatively abundant in EcM TSPs). These patterns might explain the maintenance of the local soil microbial species reservoir at the higher tree diversity levels.

The role of classifier taxa in driving the differences between tree mycorrhizal types

The discriminatory power of random forest models confirmed the second statement of our second hypothesis (H2b), as the most influential microbial taxa driving the differences between tree mycorrhizal types were reduced to non-significant at the multi-tree species mixtures for both fungi and bacteria. This finding shows that at high tree species richness the presence of strong indicator taxa does not exclude the presence of other strong indicator taxa, thus allowing their coexistence. This is in concordance with the results from ordination and PERMANOVA analyses, as with lower dissimilarity in the microbial community composition also fewer microbial taxa should determine the differences. Moreover, random forest models highlighted the differences between monocultures and two-species mixtures in bacterial communities, which were not reflected by the PERMANOVA. We observed that the bulk of the top fungal classifier taxa belonged to ectomycorrhiza and saprotrophs. This can not only be expected with regards to their respective relative abundance distributions under EcM and AM TSPs but also, more importantly, manifests the differential patterns in their nutrient acquisition and processing strategies (Tedersoo and Bahram, 2019). We found higher relative abundances of ectomycorrhiza as top fungal classifier taxa in EcM TSPs compared to that of AM TSPs, which was expected, but interestingly, saprotrophs did not show a similar pattern. Some saprotrophic taxa (e.g., fASV3871, fASV1290) were relatively either abundant or rare under AM TSPs, while the same was the case for other saprotrophic taxa under EcM TSPs (e.g., fASV3950, fASV5553). This pattern suggests that some saprotrophic taxa have an exclusive preferential association with either EcM or AM TSPs, which might indicate the role of tree mycorrhizal partners in the assembly of other taxonomic groups by modulating the microenvironment surrounding the hyphosphere. In a study by (Liu et al., 2018) characterizing relationships between macro-fungi and bacteria, the authors reported more Bacteroides in ectomycorrhizal hyphosphere soils, whereas they found more Chloroflexi in hyphosphere soils of saprotrophic fungi. We noticed a similar preferential pattern also in the top bacterial classifier taxa in which the ASVs belonging to Bacteroidota were relatively abundant in EcM than AM TSPs. Whereas, Ktedonobacteraceae of the phylum Chloroflexi were relatively abundant in AM than EcM TSPs. This pattern highlights the essential role of fungal-bacterial interactions in the soil interaction zone of trees in forest ecosystems.

Recently, microbial taxa have been more frequently used as potential predictors of various aspects of ecosystem status like pathogen suppression (Trivedi et al., 2017) and soil quality and physicochemical variables (Hermans et al., 2020). Similarly, we presented the soil microbial classifier taxa for EcM and AM mycorrhizal type TSPs at the local scale.

The additional contribution of environmental factors explaining microbial community composition

Investigation of the environmental factors across tree diversity levels revealed their significant contribution in shaping the microbial communities besides the tree mycorrhizal type and tree diversity level, confirming the last part of our expectation (H2c). Furthermore, we found that most of the edaphic and tree community variables selected by our models were common ones, except neighborhood abundance, for both AM and EcM TSPs soil fungal communities, while bacterial communities were differentially regulated by total organic carbon (TOC) and TSP identity. Nevertheless, AM TSPs soil fungal communities were more strongly affected by the topographic and spatial variables compared to that of EcM TSPs. These results are in accordance with earlier reports on the impact of common edaphic, floristic and spatial variables on fungal communities and their differential effect on different taxonomic and functional groups such as saprotrophs, ectomycorrhiza, and arbuscular mycorrhizal fungi (Tedersoo et al., 2014; Nguyen et al., 2016b; Weißbecker et al., 2018)

We found soil pH (Rousk et al., 2010; Tedersoo et al., 2020b) and host identity (Tedersoo et al., 2016), which were known to impact fungal communities, were important factors in both EcM and AM TSPs in monocultures. Also, in bacterial communities, soil pH known as the strong factor driving bacterial community composition in different ecosystems (Fierer and Jackson, 2006; Delgado-Baquerizo and Eldridge, 2019; Jiao and Lu, 2020), was found to have a consistently strong effect irrespective of the mycorrhizal type and tree diversity levels. Since our study site is a tree diversity experiment in which tree composition was manipulated, the variation in soil conditions may have been caused, at least in part, by the differences between EcM and AM trees. This variation could be induced by different mechanisms such as litter inputs and mycorrhizal partner-mediated microbe-microbe interactions. It was reported that generally, AM trees produce high-quality litter (e.g., low C:N) and higher nutrient content compared to EcM trees (Midgley et al., 2015). This was evident, for example, that EcM TSPs bacterial communities were significantly impacted by

TOC and the fungal communities of AM TSPs in monoculture were significantly impacted by NH_4^+. We observed in the multi-tree species mixtures for both fungi and bacteria that the number of significantly associated environmental factors decreased in comparison to lower diversity tree stands. This is expected as with the increasing tree diversity, the co-occurrence of tree species increases, yielding more similar environmental conditions. Microbes can also change the soil environment through their interactions by promoting or impeding processes like mineralization or nitrification. Soil chemical properties, including NO_3^-, N, pH, and moisture, were the significant factors in the multi-tree species mixtures, whose significance might imply the microbe-regulated processes like mineralization or nitrification at higher tree diversity levels. Altogether, our findings confirm a tripartite interplay of tree mycorrhizal type, tree diversity and environmental factors in modulating the microbiota of the tree-tree soil interaction zone. Nevertheless, there might be potential unknown legacy effects at the site from the previous conifer plantations, which would be very difficult to quantify. However, the experiment was already 10 years old at the time of sampling, making legacy effects of the previous vegetation on the microbial community less likely.

Conclusions

Here, we provided unprecedented empirical evidence for the interactive effects of the tree mycorrhizal type and tree diversity on the soil fungal and bacterial communities. We also demonstrated that these effects varied with environmental conditions. Furthermore, differences in microbial species composition disappeared with increasing tree species richness. For bacterial communities, this effect was caused by the different tree species irrespective of their mycorrhizal type, while for fungal communities the effect was the result of the interactive effects of the coexistence of tree species of different mycorrhizal types at higher tree species richness. Overall, this led us to the generalized conclusion that microbial community differences among tree mycorrhizal types disappear in multi-tree species mixtures. Our results show that tree mycorrhizal type is an important factor to disentangle the mechanisms underlying positive, negative and/or neutral effects of tree diversity on soil microbial diversity in tree diversity experiments. This knowledge is crucial in light of the ongoing and much-needed research on the biodiversity-ecosystem function (BEF) relationships. Moreover, we encourage further research to get a deeper understanding of the causal relationships among environmental variables, tree mycorrhizal

type, soil microbial communities, and the forest ecosystem functioning using controlled experiments. It is known that higher fungal and bacterial diversity enhances the soil ecosystem functioning (Wagg et al., 2019), but context-dependent effects need further exploration (Eisenhauer et al., 2019). Finally, using the tree species pair approach, we have identified that planting AM and EcM mycorrhizal type trees together in higher tree diversity levels may promote high soil microbial diversity with converging community composition, which in turn might contribute to the stable and better forest soil ecosystem functioning.

Appendix S II

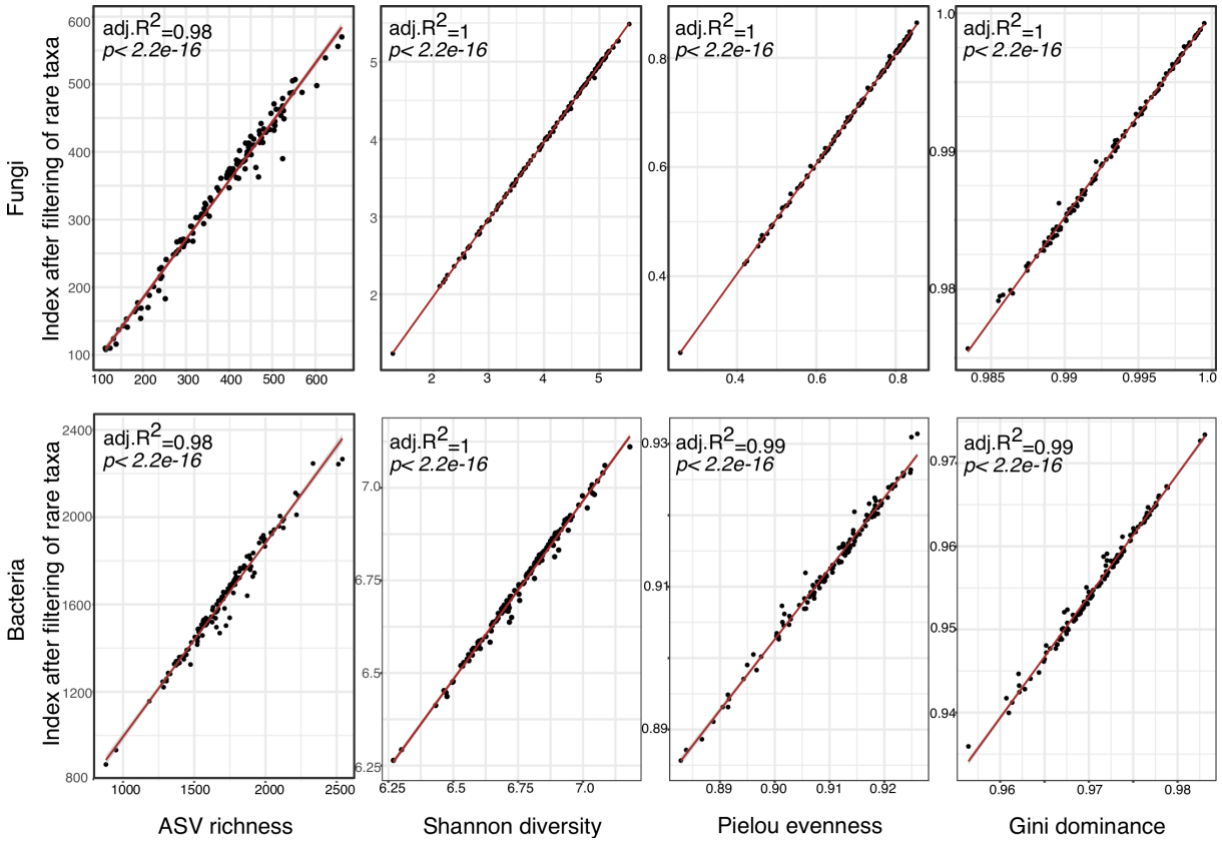


Figure S II.1 | Fungal and bacterial alpha diversity indices after filtering the low abundant (rare taxa) regressed on the diversity indices before removal of the low abundant taxa (X-axis). Four indices viz. observed ASV richness (i.e., "Richness"), Shannon diversity (i.e., "Shannon"), Pielou evenness (i.e., "Evenness") and Gini dominance (i.e., "Dominance")

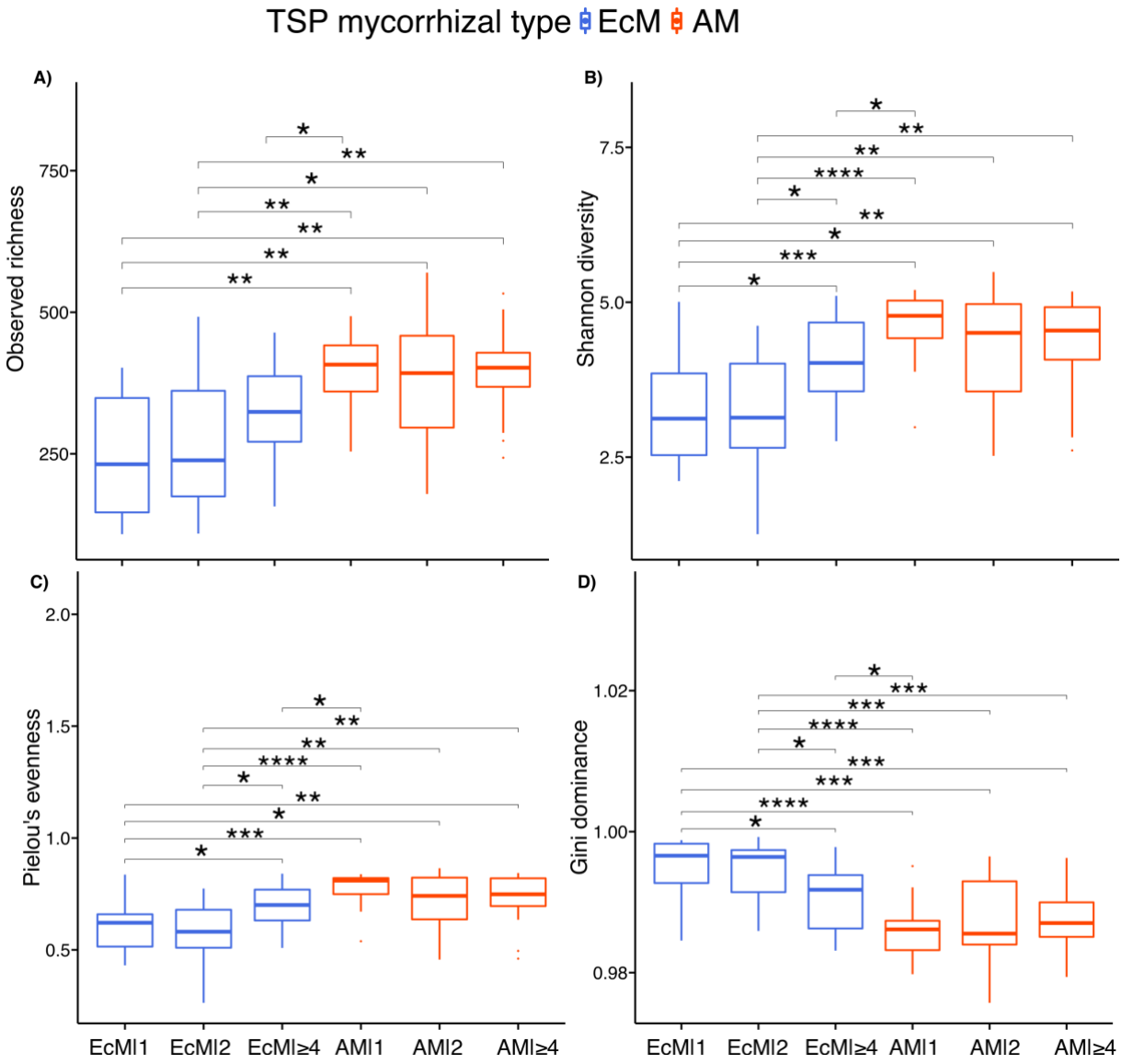


Figure S II.2 | Pair-wise Wilcoxon tests among EcM and AM TSPs soil fungal communities along the tree diversity levels. (A-D) Fungal ASV richness, Shannon diversity, Pielou's evenness and Gini dominance index, respectively. The asterisks above the boxplots show the p-value (for multiple testing correction) significance level; ns.: $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$

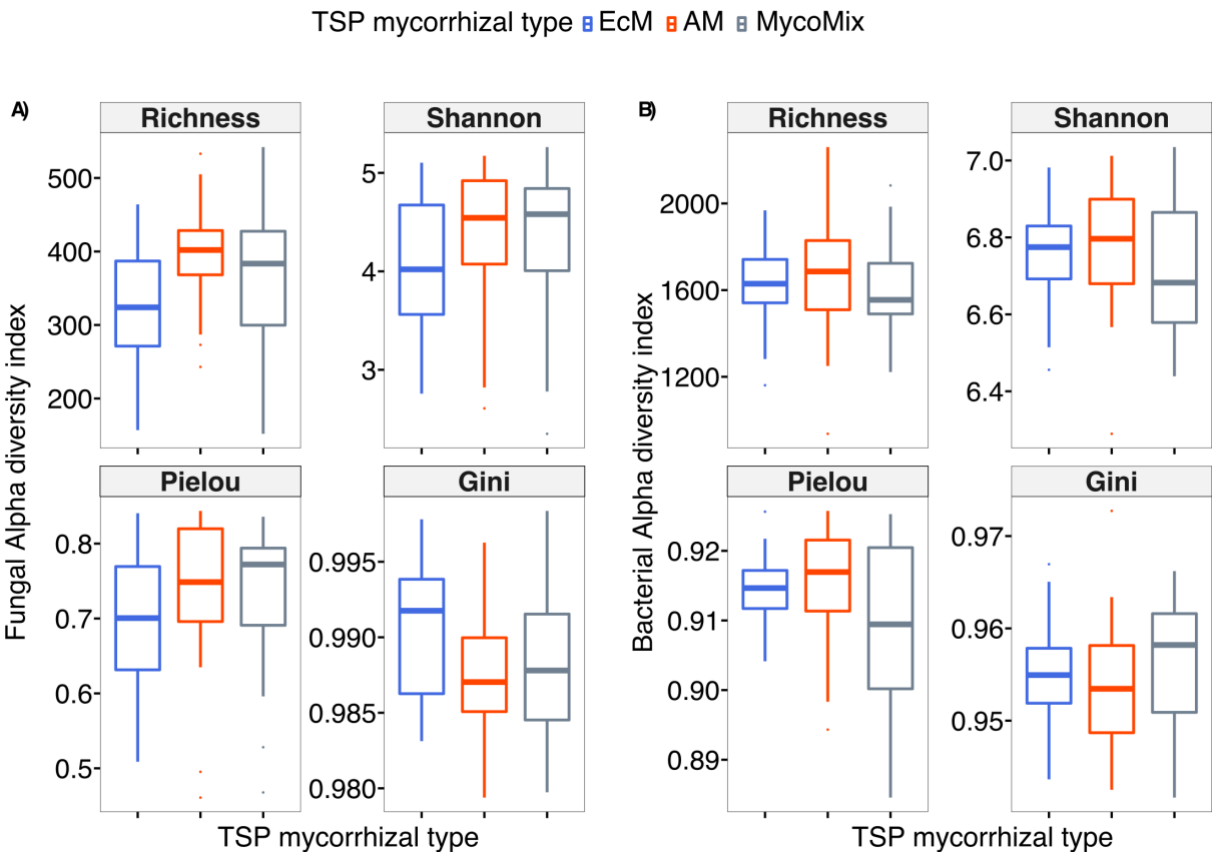


Figure S II.3| Comparison of soil microbial alpha diversity indices of tree species pairs (TSPs) in multi-tree species mixtures. **A**) Fungal communities **B**) Bacterial communities

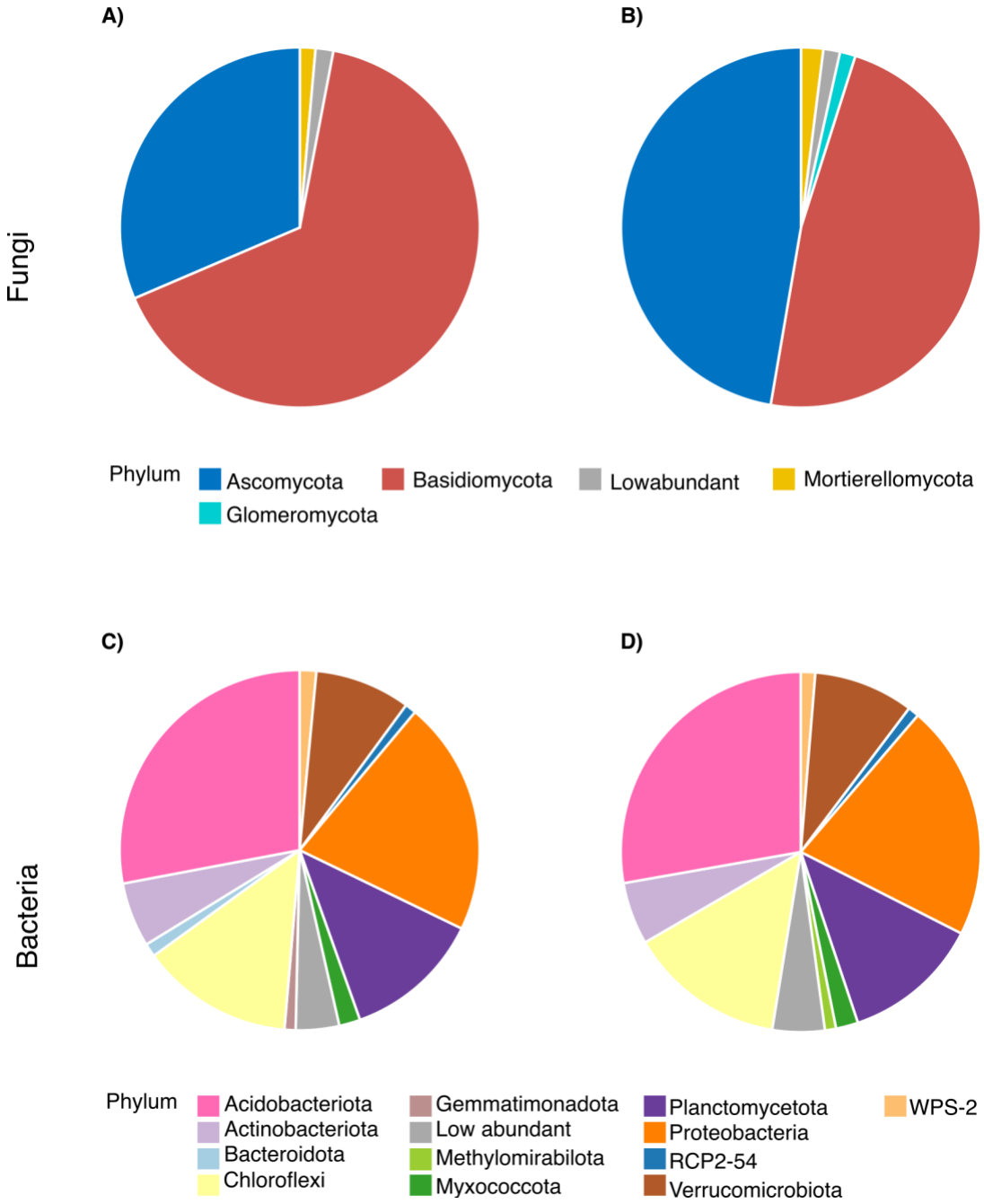


Figure S II.4 | Phylum-level taxonomic composition of TSP soil fungal and bacterial communities. **A)** Fungal composition under EcM TSPs **B)** Fungal composition under AM TSPs **C)** Bacterial composition under EcM TSPs **D)** Bacterial composition under AM TSPs

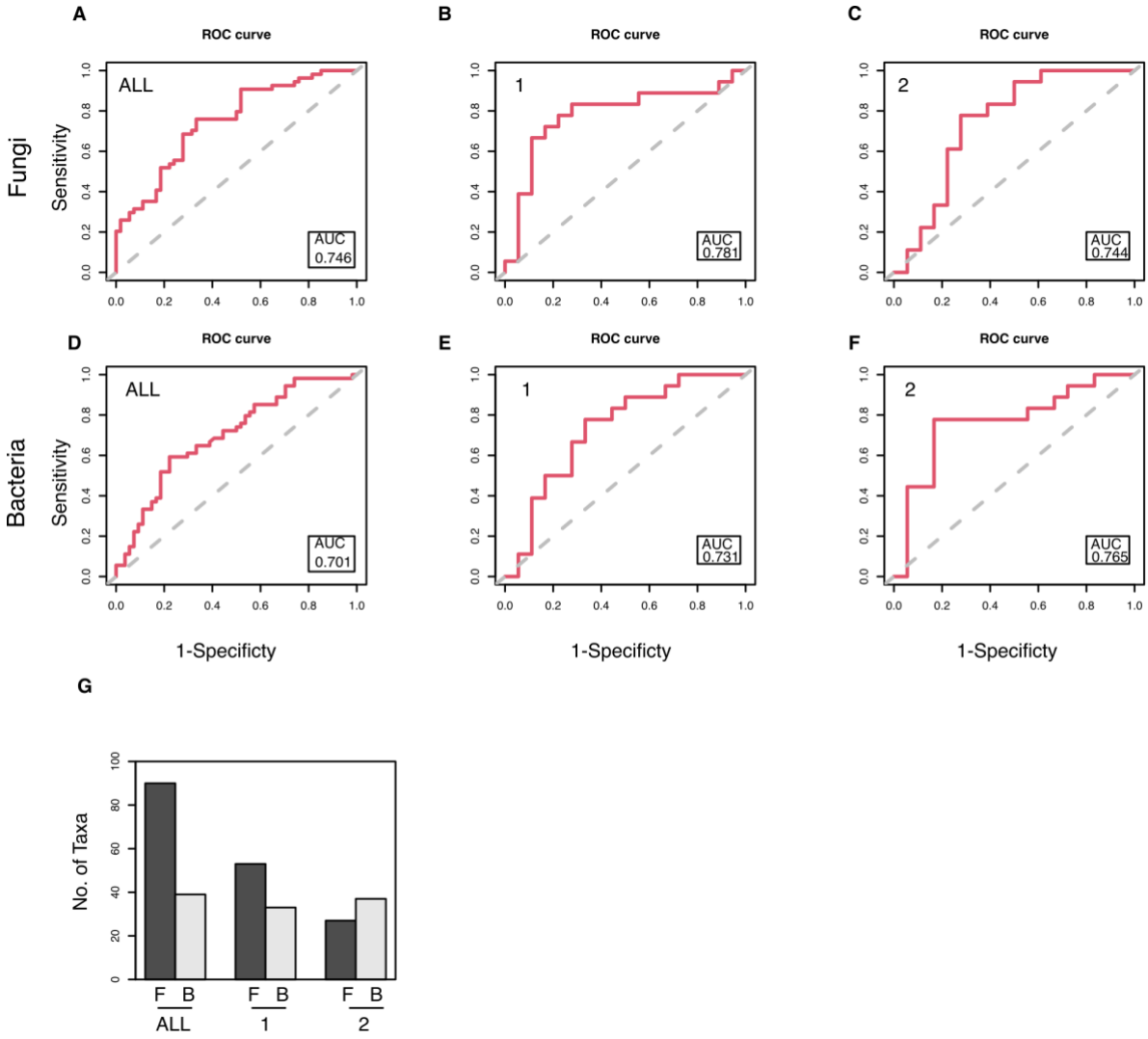


Figure S II.5 | RF model performance metrics. **A-C**) ROC curves of RF models of soil fungal communities **B-F**) ROC curves of RF models of soil bacterial communities **G)** Bar plots showing the number of Significant classifier taxa determined by RF models. F- Fungi; B- Bacteria; All- Combined dataset; 1- monocultures; 2- two-species mixtures. The RF models were not significant in multi-species mixtures.

Table S II.1

Two-way-ANOVA effects of tree mycorrhizal type and tree diversity on alpha diversity metrics of the soil fungal communities

	Mycorrhizal type (M)			Tree diversity level (L)			Interaction (MxL)		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Observed richness	1	35.64	***	2	1.65	n.s.	2	2.10	n.s.
Shannon	1	34.65	***	2	2.60	n.s.	2	3.70	*
Pielou's evenness	1	30.26	***	2	2.54	n.s.	2	3.50	*
Gini dominance	1	43.66	***	2	1.37	n.s.	2	3.53	*

The asterisks represent the p value significance level; n.s.: p > 0.05, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

Table S II.2

EcM and non-EcM fractions of fungal orders Agaricales, Cantharellales, Russulales, Sebacinales & Thelephorales

Taxonomic Order *(fungi)*	**Functional group** *(fungi)*	**EcM TSPs** *(abundance %)*	**AM TSPs** *(abundance %)*
Agaricales	EcM	58.91	11.61
	Non- EcM	41.09	88.39
Cantharellales	EcM	89.20	1.84
	Non- EcM	10.80	98.16
Russulales	EcM	96.52	99.94
	Non- EcM	3.48	0.06
Sebacinales	EcM	97.59	70.32
	Non- EcM	2.41	29.68
Thelephorales	EcM	8.88	13.49
	Non- EcM	91.12	86.51

Table S II.3

Pair-wise PERMANOVA of the EcM and AM TSPs soil microbial communities along the tree diversity levels

Pairwise-comparisons	Fungal communities		Bacterial communities	
	R^2	p.adj	R^2	p.adj
EcM: mono spp ***vs*** EcM: two spp	0.028	0.512	0.048	0.217
EcM: two spp *vs* EcM: multi spp	**0.045**	**0.006****	0.036	0.277
EcM: multi spp *vs* EcM: mono spp	**0.039**	**0.006****	**0.062**	**0.045***
AM: mono spp ***vs*** AM: two spp	0.032	0.184	0.025	0.589
AM: two spp ***vs*** AM: multi spp	0.031	0.249	0.026	0.589
AM: multi spp ***vs*** AM: mono spp	0.036	0.09	0.028	0.488
♣ EcM: mono spp *vs* AM: mono spp	**0.058**	**0.003****	0.039	0.253
EcM: mono spp *vs* AM: two spp	**0.051**	**0.003****	0.048	0.220
EcM: mono spp *vs* AM: multi spp	**0.045**	**0.006****	0.040	0.243
EcM: two spp *vs* AM: mono spp	**0.065**	**0.003****	0.038	0.261

Table S II.3 ***(cont.)***

Pairwise-comparisons	Fungal communities		Bacterial communities	
	R^2	p.adj	R^2	p.adj
♣ EcM: two spp *vs* AM: two spp	**0.058**	**0.003****	0.038	0.253
EcM: two spp *vs* AM: multi spp	**0.051**	**0.003****	0.033	0.321
EcM: multi spp *vs* AM: mono spp	**0.037**	**0.043***	0.043	0.243
EcM: multi spp ***vs*** AM: two spp	0.029	0.337	0.033	0.352
♣ EcM: multi spp ***vs*** AM: multi spp	0.025	0.774	0.023	0.687

*All the p values were fdr corrected for multiple testing and are followed by significance level codes (***p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001**). Significant values were bolded. ♣ symbolled rows indicate the EcM and AM comparison at the same diversity level.*

Table S II.4

Pair-wise analysis of TSP effects on soil microbial communities

Mycorrhizal type	TSP pair	Fungi		Bacteria	
		F.model	P.adj	F.model	P.adj
EcM	CaHe|CaHe vs QuFa|QuFa	1.09	0.336	1.96	0.103
EcM	CaHe|CaHe vs LiGl|LiGl	1.44	0.056	1.28	0.273
EcM	CaHe|CaHe vs QuSe|QuSe	1.11	0.336	1.20	0.353
EcM	CaHe|CaHe vs CaSc|CaSc	1.30	0.075	1.24	0.306
EcM	CaHe|CaHe vs CyGl|CyGl	1.09	0.336	1.90	0.103
EcM	QuFa|QuFa vs LiGl|LiGl	1.52	0.056	1.73	0.103
EcM	QuFa|QuFa vs QuSe|QuSe	1.11	0.336	1.65	0.103
EcM	QuFa|QuFa vs CaSc|CaSc	1.47	0.056	2.49	0.053
EcM	QuFa|QuFa vs CyGl|CyGl	1.00	0.490	0.97	0.541
EcM	LiGl|LiGl vs QuSe|QuSe	1.28	0.165	0.67	0.945
EcM	LiGl|LiGl vs CaSc|CaSc	0.90	0.594	0.99	0.495
EcM	LiGl|LiGl vs CyGl|CyGl	1.39	0.056	1.66	0.103
EcM	QuSe|QuSe vs CaSc|CaSc	1.09	0.340	0.85	0.613
EcM	QuSe|QuSe vs CyGl|CyGl	0.98	0.546	1.46	0.116
EcM	CaSc|CaSc vs CyGl|CyGl	1.25	0.085	2.51	0.053
AM	NySi|NySi vs LiFo|LiFo	1.77	**0.050***	2.23	0.123
AM	NySi|NySi vs SaSe|SaSe	1.20	0.172	1.09	0.444
AM	NySi|NySi vs KoBi|KoBi	1.11	0.270	1.05	0.444
AM	NySi|NySi vs SaMu|SaMu	1.24	0.165	1.46	0.214

Table S II.4 ***(cont.)***

Mycorrhizal type	TSP pair	Fungi		Bacteria	
		F.model	**P.adj**	**F.model**	**P.adj**
AM	NySi|NySi vs ChAx|ChAx	1.66	**0.050***	2.46	0.123
AM	LiFo|LiFo vs SaSe|SaSe	1.41	0.060	1.45	0.205
AM	LiFo|LiFo vs KoBi|KoBi	1.57	**0.050***	1.75	0.123
AM	LiFo|LiFo vs SaMu|SaMu	1.10	0.270	0.93	0.448
AM	LiFo|LiFo vs ChAx|ChAx	1.05	0.387	1.24	0.418
AM	SaSe|SaSe vs KoBi|KoBi	1.10	0.253	0.82	0.732
AM	SaSe|SaSe vs SaMu|SaMu	1.01	0.397	1.01	0.444
AM	SaSe|SaSe vs ChAx|ChAx	1.23	0.165	1.92	0.123
AM	KoBi|KoBi vs SaMu|SaMu	1.09	0.270	1.02	0.444
AM	KoBi|KoBi vs ChAx|ChAx	1.40	0.072	1.99	0.123
AM	SaMu|SaMu vs ChAx|ChAx	1.09	0.273	1.05	0.444

*All the p values were fdr corrected for multiple testing and are followed by significance level codes (***p ≤ 0.05**). Significant p-values were bolded. The abbreviations used for the tree species were as follows, "*CaHe - *Castanea henryi;* CaSc - *Castanopsis sclerophylla;* CyGl - *Cyclobalanopsis glauca;* LiGl - *Lithocarpus glaber*; QuFa - *Quercus fabri;* QuSe - *Quercus serrata;* SaMu - *Sapindus mukorossi;* SaSe - *Sapium sebiferum;* ChAx - *Choerospondias axillaris;* KoBi - *Koelreuteria bipinnata;* LiFo - *Liquidambar formosana;* NySi - *Nyssa sinensis"*

Table S II.5

Significant factors associated with the fungal and bacterial community compositional variation based on dbRDA model selection

		Fungal communities						Bacterial communities					
		All TSPs		**EcM TSPs**		**AM TSPs**		**All TSPs**		**EcM TSPs**		**AM TSPs**	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Soil	Nitrate	2.215	0.001***	1.429	0.008**	1.732	0.001***	3.377	0.001***	2.653	0.004**	3.226	0.001***
	pH	2.189	0.001***	1.393	0.009**	2.410	0.001***	10.047	0.001***	5.175	0.001***	7.423	0.001***
	Ammonia	1.823	0.002**	1.364	0.019*	1.310	0.038*	1.589	0.049*	-	-	-	-
	Soil moisture	1.438	0.007**	-	-	-	-	1.956	0.019*	1.866	0.029*	1.611	0.04*
	Phosphorus	1.329	0.021*	1.311	0.038*	1.317	0.031*	1.646	0.046*	-	-	-	-
	TOC	-	-	-	-	-	-	2.483	0.006**	2.593	0.005**	-	-
Tree	Tree composition	1.661	0.001***	1.575	0.001***	1.596	0.001***	2.138	0.001***	2.234	0.001***	1.934	0.024*
	TSP identity	1.621	0.001***	1.614	0.003**	1.690	0.003**	2.025	0.001***			2.105	0.003**
	EcM-Neighbor abundance	1.650	0.002**	-	-	-	-	-	-	-	-	-	-
	Neighborhood abundance	-	-	-	-	1.393	0.020*	-	-	-	-	-	-
	Tree Simpson	-	-	-	-	-	-	2.173	0.014*	-	-	-	-
Topo	Altitude	2.298	0.001***	1.771	0.001***	2.224	0.001***	5.456	0.001***	3.199	0.001***	4.057	0.001***
	Slope	2.899	0.001***	1.811	0.001***	2.170	0.001***	6.048	0.001***	2.959	0.001***	3.578	0.001***
	Eastness	1.269	0.043*	-	-	1.500	0.012*	2.557	0.004**	2.001	0.011*	2.492	0.009**
	Northness	1.420	0.007**	-	-	1.515	0.008**	-	-	2.000	0.009**	1.914	0.028*
Spatial	Spatial variables	1.550	0.001***	1.395	0.001***	1.573	0.001***	2.339	0.001***	2.136	0.001***	2.439	0.001***

*Significant factors were stepwise selected by ordistep function in R. Only significant factors (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001) in at least one of the given communities were shown. Fields with '-' indicate non-significance of the variable in that particular category. Spatial variables are represented by PCNM vectors, tree community composition and TSP identity are characterized by principal components.*

Table S II.6

Significant factors associated with the fungal community compositional variation across tree diversity levels based on dbRDA

		TSP Mycorrhizal types													
		EcM						AM						Mycomix	
		Mono		Two-spp		Multi-spp		Mono		Two-spp		Multi-spp		Multi-spp	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Soil	Nitrate	-	-	-	-	1.630	0.001***	-	-	-	-	1.767	0.001***	1.69	0.001***
	pH	1.300	0.028*	-	-	-	-	2.215	0.001***	1.547	0.001***	-	-	1.86	0.001***
	Ammonium	-	-	-	-	-	-	1.317	0.046*	-	-	-	-	-	-
	Phosphorus	-	-	1.576	0.009**	-	-	1.893	0.001***	-	-	-	-	-	-
	Nitrogen	-	-	-	-	-	-	-	-	1.317	0.034*	1.421	0.032*	-	-
Tree	Tree composition	-	-	1.563	0.004**	-	-	1.526	0.001***	1.545	0.001***	1.478	0.027*	1.620	0.001***
	TSP identity	1.518	0.004**	1.469	0.039*	-	-	1.579	0.001***	-	-	-	-	1.435	0.017*
	AM-neighbor richness	-	-	-	-	-	-	-	-	1.680	0.002**	-	-	-	-
	Neighborhood abundance	-	-	-	-	-	-	-	-	-	-	1.470	0.019*	-	-
Topo	Altitude	1.431	0.038*	1.431	0.033*	1.283	0.012*	1.502	0.023*	1.604	0.002**	1.433	0.026*	-	-
	Slope	-	-	-	-	1.425	0.002**	1.337	0.050*	1.751	0.001***	1.861	0.003**	2.174	0.001***
	Eastness	-	-	-	-	-	-	1.482	0.021*	-	-	-	-	-	-
	Northness	-	-	-	-	-	-	1.860	0.003**	1.388	0.008**	-	-	-	-
Spatial	Spatial variables	1.224	0.023*	1.820	0.001***	1.332	0.001***	1.686	0.001***	1.659	0.001***	1.549	0.002**	1.882	0.001***

*Significant factors were stepwise selected by ordistep function in R. Only significant factors (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001) in at least one of the given communities were shown. Fields with '-' indicate non-significance of the variable in that particular category. Spatial variables are represented by PCNM vectors and tree community composition is characterized by principal components.*

Table S II.7

Significant factors associated with the bacterial community compositional variation across tree diversity levels based on dbRDA

		TSP Mycorrhizal types													
		EcM						AM						Mycomix	
		Mono		Two-spp		Multi-spp		Mono		Two-spp		Multi-spp		Multi-spp	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Soil	Nitrate	-	-	2.084	0.020*	-	-	-	-	-	-	1.700	0.041*	2.200	0.004**
	pH	2.980	0.001***	4.421	0.001***	4.039	0.001***	4.961	0.001***	4.085	0.001***	3.492	0.001***	4.046	0.001***
	Ammonium	-	-	2.372	0.003**	-	-	-	-	-	-	-	-	-	-
	Soil moisture	-	-	-	-	-	-	-	-	-	-	1.913	0.029*	-	-
	Phosphorus	-	-	-	-	-	-	2.113	0.031*	-	-	-	-	-	-
	Nitrogen	-	-	-	-	-	-	-	-	1.796	0.037*	-	-	-	-
Tree	Tree composition	-	-	2.597	0.002**	1.834	0.037*	-	-	2.179	0.002**	-	-	2.317	0.001***
	TSP identity	-	-	-	-	-	-	2.029	0.013*	-	-	-	-	1.784	0.039*
	EcM neighbor richness	-	-	1.682	0.048*	-	-	-	-	-	-	-	-	-	-
	AM neighbor richness	-	-	-	-	-	-	-	-	2.240	0.008**	-	-	-	-
Topo	Altitude	2.154	0.003**	-	-	2.248	0.015*	-	-	2.573	0.0021***	2.338	0.008**	-	-
	Slope	-	-	1.862	0.033*	3.321	0.001***	-	-	2.956	0.002**	3.183	0.001***	2.950	0.001***
	Eastness	1.613	0.043*	3.030	0.001***	-	-	2.782	0.008**	-	-	-	-	-	-
	Northness	-	-	1.761	0.028*	-	-	4.075	0.001***	-	-	-	-	-	-
Spatial	Spatial variables	1.667	0.018*	2.582	0.001***	2.749	0.001***	2.972	0.001***	3.803	0.001***	2.332	0.001***	2.608	0.001***

*Significant factors were stepwise selected by ordistep function in R. Only significant factors (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001) in at least one of the given communities were shown. Fields with '-' indicate non-significance of the variable in that particular category. Spatial variables are represented by PCNM vectors and tree community composition and TSP identity are characterized by principal components.*

Chapter III: The functional potential of soil microbial communities and their sub-communities varies with tree mycorrhizal type and tree diversity

This chapter is a modified version of the publication. This chapter has been published in the ***Microbiology Spectrum*** journal as

Singavarapu B, Du J, Beugnon R, Cesarz S, Eisenhauer N, Xue K, Wang Y, Bruelheide H & Wubet T (2023). Functional Potential of Soil Microbial Communities and Their Subcommunities Varies with Tree Mycorrhizal Type and Tree Diversity. Microbiology spectrum, e0457822.

https://doi.org/10.1128/spectrum.04578-22

Abstract

Soil microbial communities play crucial roles in the earth's biogeochemical cycles. Yet, their genomic potential for nutrient cycling in association with tree mycorrhizal type and tree-tree interactions remained unclear, especially in diverse tree communities. Here, we studied the genomic potential of soil fungi and bacteria under arbuscular (AM) and ectomycorrhizal (EcM) conspecific tree species pairs (TSPs) at three tree diversity levels in a subtropical tree diversity experiment (BEF China). The soil fungi and bacteria of the TSPs' interaction zone were characterized by amplicon sequencing and their sub-communities were determined using a microbial inter-kingdom co-occurrence network approach. Their potential genomic functions were predicted with regard to the three major nutrients carbon (C), nitrogen (N), and phosphorus (P), and their combinations. We found the microbial sub-communities that were significantly responding to different soil characteristics. The tree mycorrhizal type significantly influenced the functional composition of these co-occurring sub-communities in mono- and two- but not in multi-tree species mixtures. Differentiation of sub-communities was driven by differentially abundant taxa producing different sets of nutrient cycling enzymes across the tree diversity levels, predominantly enzymes of the P (11 and 16) followed by N (9) and C (9) cycles in mono- and two-species mixtures respectively. *Agaricomycetes*, *Sordariomycetes*, *Eurotiomycetes*, *Leotiomycetes*, *Verrucomicrobiae*, *Acidobacteriae*, *Alphaproteobacteria and Actinobacteria* were the major differential contributors (48% to 62%) to the nutrient cycling functional abundances of soil microbial communities across tree diversity levels. Our study demonstrated the versatility and significance of microbial sub-communities in different soil nutrient cycling processes of forest ecosystems.

Importance

Loss of multi-functional microbial communities can negatively affect ecosystem services, especially forest soil nutrient cycling. Therefore, exploring the genomic potential of soil microbial communities particularly their constituting sub-communities and taxa for nutrient cycling is vital to get an in-depth mechanistic understanding for better management of forest soil ecosystems. This study revealed soil microbes with rich nutrient cycling potential, organized in sub-communities that are functionally resilient and abundant. Such microbial communities mainly found in multi-tree species mixtures associated with different mycorrhizal partners can foster soil microbiome stability. A stable and

functionally rich soil microbiome is involved in the cycling of nutrients such as carbon, nitrogen, and phosphorus and their combinations could have positive effects on the ecosystem functioning including increased forest productivity. The new findings could be highly relevant for afforestation and reforestation regimes notably in the face of growing deforestation and global warming scenarios.

Introduction

Microorganisms, especially bacteria and fungi, contribute enormously to terrestrial ecosystem services, for example by playing a vital role in soil nutrient cycling (Van Der Heijden et al., 2008; Bender and van der Heijden, 2015; Kappler and Bryce, 2017; Jansson and Hofmockel, 2020). Particularly, the contribution of plant symbiotic microbes in soil nutrient cycling has been well reported. For example, mycorrhizal fungi form symbiotic associations with around 90% of terrestrial plant species and take part in nutrient cycling by mobilizing nitrogen (N) and phosphorus (P) in soils (Read and Perez-Moreno, 2003; Tedersoo and Bahram, 2019). Similarly, plant-symbiotic bacteria belonging to *Rhizobium* and *Frankia* can fix nitrogen and thus essentially participate in N-cycling (Olivares et al., 2013). Moreover, at the community level, it is also important to consider the extensive contribution of free-living soil bacteria and fungi to soil nutrient cycling as they constitute a major part of soil microbiota (Fierer, 2017). A few examples include carbon-fixing Actinobacteria (Zhao et al., 2018; Jiao et al., 2021a), nitrogen-fixing *Azotobacter* (Zhao et al., 2018; Jiao et al., 2021a) and phosphate-solubilizing Acidobacteria (Chen et al., 2006; Liang et al., 2020). Likewise, *Penicillium*, *Aspergillus*, and *Trichoderma* are free-living fungi and known for being actively involved in the decomposition of soil organic compounds (C-cycle), nitrification (N-cycle), and P-solubilization (P-cycle), respectively (Gaiero et al., 2021; Lebreton et al., 2021; Martikainen, 2022).

Soil stoichiometry of nutrients like C:N:P ratios are known to affect the soil microbial communities depending upon their constituting members' organismal nutrient stoichiometric ratios (Elser et al., 1996; Luo et al., 2020). For example, it was reported that high N and P abundances in soil favor the abundance of fast-growing bacteria (i.e., copiotrophic, r-strategists) like Actinobacteria and Alphaproteobacteria while discriminating slow-growing bacteria (i.e., oligotrophic, K-strategists) like Acidobacteriae (Leff et al., 2015; Wang et al., 2021). Also, previous research suggests that ectomycorrhizal fungi (EMF) preferentially associate with soils of high C:N substrates, whereas

saprotrophic fungi prevail in soils with low C:N ratios (Högberg et al., 2003; Lin et al., 2017; Keller and Phillips, 2019). There is a surge in recent studies showing the link between microbial diversity, community composition, and soil ecosystem multifunctionality (Wagg et al., 2014; Delgado-Baquerizo et al., 2016; Delgado-Baquerizo et al., 2017; Wagg et al., 2019; Jiao et al., 2021b). However, there is still a knowledge gap about how the soil microbial communities vary in the stoichiometry of their nutrient cycling genomic potential which can be the relative combinations of genes coding for different nutrient cycling enzymes. In a study taking a genomic perspective on soil carbon cycling, (Hartman et al., 2017) reported links between microbial community composition, the microbe's C, N, and P substrate utilization potential, and C turnover. This highlights the importance of studying the genomic potential of microbial communities to better understand soil nutrient cycling.

Given the fact that soil C, N, and P cycles are linked it is essential to study the co-occurring bacterial and fungal communities together for their genomic potential in the cycling of different major nutrients and their combinations (viz. C, N, P, CN, CP, NP, and CNP). For instance, the ability to decompose soil organic matter (SOM) with varying nutrient ratios depends on the composition of soil microbial communities (Yarwood, 2018). Subsequently, the decomposed SOM would be available for bacteria and fungi conditioned on their abilities to continue with either N fixation or denitrification (Fan et al., 2014; Almagro et al., 2021) and/or concurrently also be available for P mineralization or solubilization (Wang et al., 2016; Zhang et al., 2016). This linkage between different soil nutrient cycling processes and different microbes involved can be viewed from a 'microbial syntrophy' (microbial metabolic interrelationships) perspective (Morris et al., 2013) which is affected by many factors (for example, available nutrient ratios, etc.,) but essentially depends on the genomic potential of the members of the microbial communities.

The ecological processes and relationships within a microbial community can cumulatively emerge from the constituting microbial groups/clusters (i.e., taxa that are more strongly associated within that group than with other groups), which are also known as sub-communities (Nemergut et al., 2013; Vick-Majors et al., 2014). Based on network theory, studying sub-communities, also known as modules, can provide key insights into the overall functioning of the microbial community, allowing us to assess the metabolic potential based on the single microbes' functional roles, which otherwise remains a black box. In addition, knowledge of sub-communities also sheds light on the ecological

processes that shape and regulate the community structure and organization, such as environmental filtering or niche differentiation (Röttjers and Faust, 2018). For example, recent studies in soil microbial ecology have taken the advantage of sub-community-based analyses to develop a deeper understanding of environment-specific relationships (Delgado-Baquerizo et al., 2018; Ma et al., 2020) and the functional roles of microbial communities (Purahong et al., 2016; Feng et al., 2021; Wang et al., 2022).

One of the key factors influencing the soil microbial communities in forests is the tree mycorrhizal type (Singavarapu et al., 2021), which is also known to impact microbial functional genes (Bahram et al., 2020) and soil nutrient cycling (Cheeke et al., 2017). In addition, tree diversity has also been reported to affect the soil microbial communities (Barberan et al., 2015; Hiiesalu et al., 2017; Gan et al., 2022) and soil nutrient availability (Liu et al., 2021). Despite these efforts, still, there is a great need to understand how the tree mycorrhizal type and tree diversity affect the co-occurring soil bacterial and fungal communities at the sub-community level, and in consequence, their genomic functional potential for nutrient cycling. Insight into these processes would provide a broader understanding of the intrinsic characteristics of soil microbial groups operating in ecological processes and the functional potential emerging at the community level. Such in-depth mechanistic understanding would also be the basis for managing forest soil ecosystems to maintain or increase forest multifunctionality.

To fill this knowledge gap, this study was conducted at the BEF-China experimental research platform (Bruelheide et al., 2014), using tree species of two mycorrhizal types, namely, ectomycorrhizal (EcM) and arbuscular (AM) mycorrhizal types in different tree diversity levels (Singavarapu et al., 2021). We employed the fungal-bacterial inter-kingdom co-occurrence network approach (Tipton et al., 2018) to derive the microbial sub-communities (hereafter, interchangeably used with ‘modules’) and used PICRUSt2 (Douglas et al., 2020) to predict the potential genomic functions with regard to nutrient cycling from the amplicon sequencing data. Our main objective was to understand how the stoichiometry in genomic functional potential of soil microbial communities and their sub-communities with regards to the three major nutrient cycles and their combinations (C, N, P, CN, CP, NP, and CNP) varies under EcM and AM trees at different tree diversity levels. In particular, we asked the following research questions.

1. How do the EcM and AM TSP soil bacterial and fungal community co-occurrence network structures differ across tree diversity levels, and which soil characteristics drive the composition of the sub-communities in these networks?
2. What are the effects of tree diversity and tree mycorrhizal type on the predicted genomic functional potential (in terms of C, N, P cycles and their combinations) of the co-occurring bacterial and fungal communities?
3. How do EcM and AM TSPs soil microbial sub-communities differ in their genomic functional abundances in the three nutrient cycles and their combinations within the tree diversity levels, and which microbial taxa drive these differences?

Material and methods

For detailed descriptions of the study site and design, sampling procedures, laboratory analyses and data generation, please refer to 'Materials and Methods' section of Chapter II, as well as Singavarapu et al., 2021. For this study, the soil bacterial and fungal community datasets of only conspecific TSPs (n=108) were considered with the following six combinations: 'EcM|Mono'(n=18), 'EcM|Two'(n=18), 'EcM|Multi'(n=18), 'AM|Mono'(n=18), 'AM|Two'(n=18), and 'AM|Multi'(n=18).

Bioinformatics analysis

To identify the microbial taxa that are faithfully represented in each of the tree mycorrhizal type and tree diversity combinations (viz, *EcM|Mono, EcM|Two, EcM|Multi, AM|Mono, AM|Two and AM|Multi*), stringent filtering steps were applied to fungal and bacterial datasets prior to further data analyses. First, all taxa with an abundance of >3% mean total sequencing reads were filtered resulting in 798 bacterial and 728 fungal taxa. Next, in each of the tree mycorrhizal type and tree diversity combinations, the taxa were further filtered with a frequency of presence in at least 2/3rd of the samples (≥33%) in their respective datasets. These filtered datasets from each combination were merged into one bacterial and one fungal dataset each and were used as input into PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) software for the prediction of metagenome functional abundances (Douglas et al., 2020).

In PICRUSt2, briefly, first, the AS representative sequences of bacteria and fungi were multiple aligned with the 16S and ITS reference genome database files using hidden Markov models (HMMER tool). For bacteria, we used default settings and for fungi, we

used the minimum alignment option of 0.5 (default 0.8) to include all of the taxa that were classified until genus level in the output. Then these aligned sequences were placed into the reference phylogenetic tree constructed by the Maximum Likelihood Phylogenetic Placement method using EPA-ng (Barbera et al., 2019) and Gappa tools (Czech et al., 2020). Next, gene family content was predicted for both bacterial and fungal ASVs based on EC (Enzyme Commission/Classification) numbers (Boyce and Tipton, 2001) using the castor package (Louca and Doebeli, 2018). Here, we filtered the predicted EC content tables of bacteria and fungi for the carbon, nitrogen and phosphorus nutrient cycling-related EC numbers (enzymes) based on previously available literature (Table S III.3). Finally, these filtered EC content tables were used to determine the gene family abundances per sample with respect to nutrient cycling for both bacterial and fungal datasets. Here, one ASV in each bacterial and fungal dataset was removed as they were above the default NSTI (Nearest-sequenced taxon index) values, the metric which identifies the ASVs that are far from all the reference sequences, thus allowing to exclude of less reliable predictions.

Statistical analysis

All the statistical analyses were done in R (version 4.0.2) software. EcM and AM TSPs soil bacterial and fungal inter-kingdom co-occurrence networks were constructed at each tree diversity level (viz, EcM|Mono, EcM|Two, EcM|Multi, AM|Mono, AM|Two and AM|Multi) using the filtered datasets (*i.e., a. abundance of >3% mean total sequencing reads and b. present in at least 2/3rd of the samples)* mentioned in the bioinformatics analysis. Networks were constructed using the R package SpiecEasi (Kurtz et al., 2015). SpiecEasi controls the spurious co-occurrences by controlling for the lack of independence in normalized count data, which accounts for the high number of edges in the network-based analysis of amplicon datasets. Networks were estimated by the Meinshausen and Bühlmann graph inference method. The minimum lambda ratio was 10^{-3}, and network assessment was done over 100 values of lambda for every 50 cross-validations. Network structural and topological properties including edges, centrality indices, modularity, etc, were calculated using the igraph package (Csardi and Nepusz, 2006). Modules that are considered to be sub-communities in each network were determined based on a hierarchical agglomeration algorithm with modularity optimization using the 'cluster_fast_greedy' function. Differences in the distribution of four network centrality measures (degree, betweenness, closeness and eigen centralities) between EcM and AM TSPs soil microbial

networks were tested by bootstrapping with 10,000 iterations followed by a two-sample Kolmogorov–Smirnov test using the 'ks.test' function in R. Further, these distributions were visualized with sinaplots using the ggforce and ggplot2 packages. Network modules that were significantly associated with soil chemical properties were determined using dbRDA (distance-based redundancy analysis) models based on the Bray–Curtis distance using the 'capscale' function in the vegan package (Oksanen et al., 2019) and for this, modules with size ≥40 were considered. Soil variables (C, N, P, C/N, C/P, N/P, TOC, SOM, NH_4^+, NO_3^-, pH and moisture) were standardized to a mean of zero and standard deviation of one ('decostand' function in vegan). Multi-co-linearity was checked using the 'vifstep' function in usdm package (Naimi et al., 2014). Further, important soil variables were selected using stepwise model selection ('ordistep' function in vegan) and the variables selected were included in the final model for each sub-community. Variables that were significant in the final model were considered the significant soil characteristics and the sub-communities that were associated with at least one of these significant soil variables were treated as soil-responsive sub-communities, in the following called significant modules.

The predicted gene family abundance matrices from PICRUSt2 output were merged per EC number to yield the co-occurring community enzyme /gene family abundance (functional abundance) matrices. These functional compositions were categorized into nutrient cycling combinations (C, N, P, CN, CP, NP and CNP) based on the constituent EC numbers. Shannon diversity of these functional abundance matrices was calculated as a measure for functional diversity and tested for the effects of tree diversity and tree mycorrhizal type using two-way ANOVA with the 'aov' function in R. Furthermore, within each tree diversity level, pairwise comparison of tree mycorrhizal type was done with t-tests followed by Benjamini-Hochberg (BH) multiple testing correction. The effects of the tree diversity and tree mycorrhizal type on the functional compositions were tested with Bray–Curtis distance-based permutational analysis of variance (PERMANOVA) using the vegan package. Moreover, the functional composition of the whole community was compared with those of the soil-responsive modules, and consequently, all the analyses based on sub-communities were rerun using only the soil-responsive sub-communities.

To derive sub-community relative functional abundances, first, mean taxa relative abundances of sub-communities in each network were calculated using the normalized

bacterial and fungal ASVabundances from PICRUSt2 output. Next, matrix multiplication was applied using the mean taxa relative abundances of sub-communities and the predicted EC content (gene family numbers) matrix of the taxa as shown in the exemplary formula (1). In the formula (1), the matrix on the left-hand side is a module (mod1, mod2) by taxa (t1, t2, t3) matrix with the taxa's mean relative abundances in the modules and the one on the right-hand side is a taxa (t1, t2, t3) by enzyme (e1, e2) matrix with the number of enzyme gene families per taxa. The result is a matrix with gene family abundances of enzymes (i.e, functional abundances) in each module (mod1, mod2).

$$\begin{array}{c} \\ mod1 \\ mod2 \end{array}\begin{array}{c} \begin{array}{ccc} t1 & t2 & t3 \end{array} \\ \begin{bmatrix} 0.10 & 0.19 & 0.07 \\ 0.02 & 0.03 & 0.06 \end{bmatrix} \end{array} \times \begin{array}{c} \\ t1 \\ t2 \\ t3 \end{array}\begin{array}{c} \begin{array}{cc} e1 & e2 \end{array} \\ \begin{bmatrix} 1 & 7 \\ 2 & 1 \\ 4 & 3 \end{bmatrix} \end{array} = \begin{array}{c} \\ mod1 \\ mod2 \end{array}\begin{array}{c} \begin{array}{cc} e1 & e2 \end{array} \\ \begin{bmatrix} 0.76 & 1.10 \\ 0.32 & 0.35 \end{bmatrix} \end{array} \quad (1)$$

The obtained sub-community functional abundances across tree diversity levels were visualized by ordination with PCoA, using the ape package (Paradis et al., 2004). Moreover, enzymes related to C, N, and P cycling were fitted to the ordination using 'envfit' function in vegan. Those enzymes with $p<0.01$ were considered significantly associated with the differentiation of modules. Furthermore, pairwise comparisons of sub-community functional abundances at each tree diversity level were done with Wilcoxon signed-rank tests followed by BH multiple testing correction with a significance threshold of $p<0.01$, using the rstatix package and presented as a heatmap using ComplexHeatmap package (Gu et al., 2016). In addition, taxa differential abundance tests were performed for all EcM and AM modules that were significantly different on the overall CNP relative functional abundance of each ASV per sub-community. The latter was obtained by multiplying the relative abundance of that ASV with its predicted EC content. Pairwise Wilcoxon rank sum-tests (BH multiple testing correction with a significance threshold of $p<0.01$) were used to determine the differentially abundant ASVs between sub-community pairs and aggregated these significant ASVs at the Class taxonomic level. The relative functional abundance proportions of the top two of each fungal and bacterial Classes per tree diversity level in sub-communities of each of EcM and AM TSPs soil microbial networks were visualized as Sankey diagrams using the networkD3 package (Allaire et al., 2017).

Results

EcM and AM TSPs soil microbial inter-kingdom network characteristics

The differences in the number of input bacterial taxa used for the construction of networks at each tree diversity level were minuscule between EcM and AM (ranging from 796 -798 ASVs). While the fungal input varied most in two-tree species mixtures with 430 and 503ASVs for EcM and AM networks, respectively (Table S III.1). Consistently we found no contrasting differences in clustering coefficient and modularity, however, there are three more modules in the EcM than AM in each of the mono- and two-species tree diversity levels (Table S III.1). To assess the underlying network community organization and also the importance of the community members, we tested the distribution of four important network centrality indices, namely node degree (used to identify community hub taxa), betweenness (a measure of taxa's influence in the network), closeness (a measure of closeness of a taxon to all other members), and eigenvector centrality (measures taxa's linkage to others accounting for how connected the others are). We found significant differences ($p<0.05$) in the distributions of these four centrality indices between EcM and AM networks at all tree diversity levels (Figure III.1). AM networks had higher median values of these distributions except for betweenness centrality wherein EcM networks had higher values, especially in mono- and two-species tree diversity levels indicating differences in the organization of microbial taxa in their respective communities (Figure III.1).

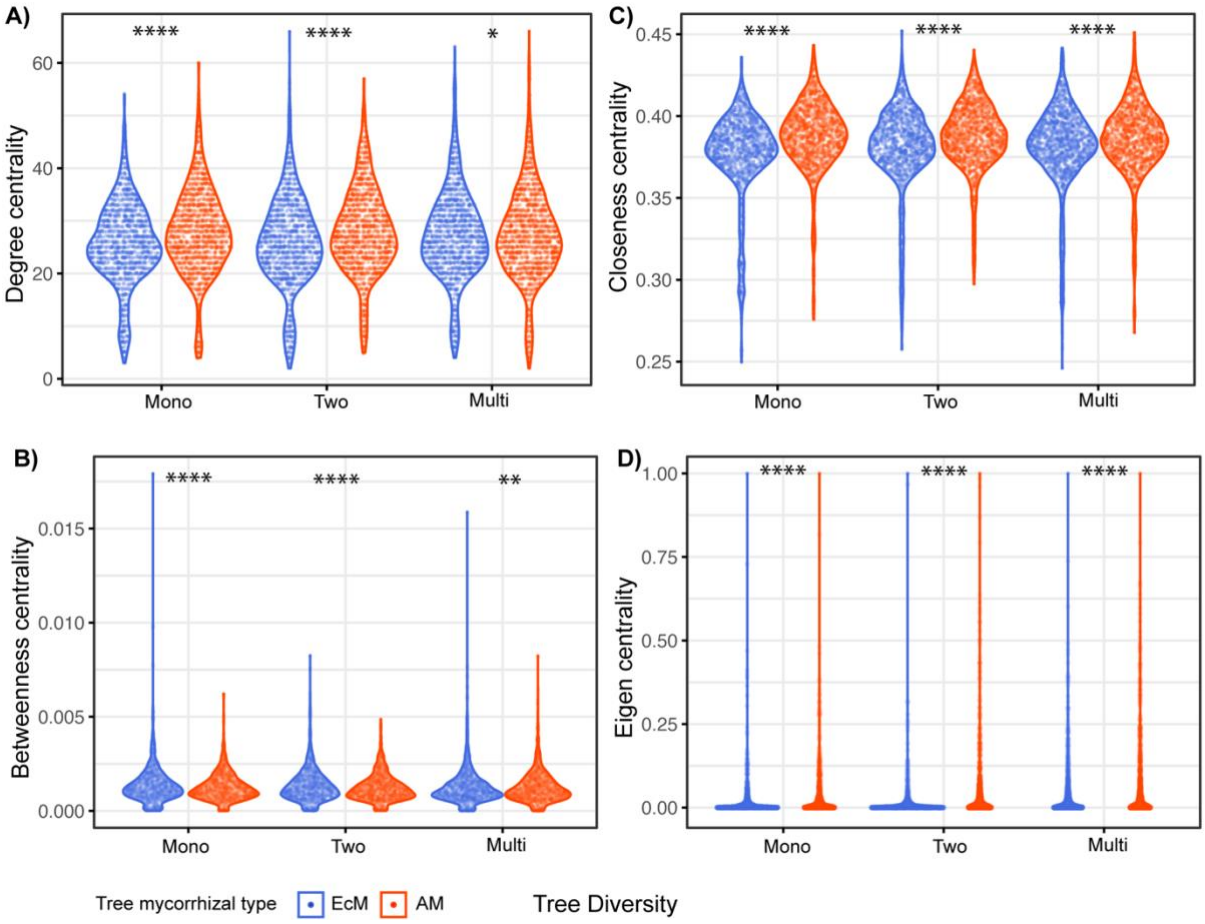


Figure III.1 Comparison of EcM and AM inter-kingdom network centrality indices along the tree diversity levels. On the y-axis centrality indices and on the x-axis, EcM and AM TSPs and the tree diversity levels (Mono for monospecific stands, Two for two-species mixtures and Multi for multi-tree species mixtures) (A) Node Degree centrality (B) Betweenness centrality (C) Closeness centrality (D) Eigen vector centrality. The asterisks show the p-value significance level, *p < 0.05, **p < 0.01, ***p < 0.001, ****p< 0.0001.

Sub-communities significantly responding to the soil environment

We identified the sub-communities of all EcM and AM networks that were significantly associated with the soil variables using the dbRDA models (Table S III.2). Overall, 21 of the 43 identified modules were found to be significantly responsive to the soil environment. For AM 4 (out of 5), 4 (out of 6), 3 (out of 8), and for EcM 3 (out of 8), 4 (out of 9) and 3 (out of 7) significant modules were found in the mono-, two-, and multi-tree species mixtures, respectively. Except for one AM module in two-species mixtures, all of the significant modules (both AM and EcM) were strongly pH-sensitive. We found one AM module in each of the tree diversity levels associated with nitrate, while in EcM communities all modules in two-species mixtures were associated with nitrate in addition to a module in monospecific stands. Although all of the significant AM modules in

monospecific stands were related to P, this was only the case for one of the EcM modules (F=2.09, p=0.04). Furthermore, one module of each EcM (F=1.51, p=0.04) and AM (F=1.56, p=0.03) network in monospecific stands was associated with C. Total N and NH4+ were found to be significantly related to both EcM and AM modules in two-species mixtures. In multi-tree species mixtures, AM modules were significantly related to NO_3^- and moisture in addition to pH, which was the only significant soil variable associated with EcM modules. Collectively, this indicated the differential roles of different sub-communities of AM and EcM networks varying in different tree diversity levels.

Tree mycorrhizal type and tree diversity level effects on the predicted functional potential of co-occurring bacterial and fungal communities

In total, 57 nutrient cycling-related EC numbers known to be part of the C, N, and P cycles, were used to filter the PICRUSt2 predicted gene family content for both bacterial and fungal datasets that were used to construct the co-occurrence networks (Table S III.3). We found a total of 64 (43 for bacteria and 21 for fungi) ECs, where the functional abundance matrix contained 45 unique ECs comprised of 11, 16, and 18 enzymes related to C, N, and P cycling, respectively (Table S III.4). Significant effects of the tree mycorrhizal type were observed on the functional diversity of the co-occurring microbial community in all nutrient cycling combinations, except for C, N, and CN. In contrast, the effects of tree diversity and the interaction with mycorrhizal type were not significant in any of the nutrient cycling combinations (Table S III.5). Moreover, the post-hoc analysis revealed that a tree mycorrhizal type effect was only present in monospecific stands (except for C), but was absent in two- and multi-tree species mixtures (Figure III.2).

Table III. 1
Effects of tree mycorrhizal type and tree diversity level on the nutrient cycling functional compositional differences of co-occurring soil fungal and bacterial communities based on PERMANOVA with 999 permutations

Nutrient Cycle	Factor	df	F	R^2	pval.adj
C	Mycorrhizal_Type (M)	1	6.281	0.055	**0.003****
	Tree_Diversity (L)	2	1.097	0.019	0.488
	Interaction (MxL)	2	1.553	0.027	0.209
N	Mycorrhizal_Type (M)	1	15.663	0.128	**0.003****
	Tree_Diversity (L)	2	0.504	0.008	0.707
	Interaction (MxL)	2	2.067	0.034	0.192
P	Mycorrhizal_Type (M)	1	14.342	0.116	**0.003****
	Tree_Diversity (L)	2	1.05	0.017	0.488
	Interaction (MxL)	2	2.438	0.04	0.092
CN	Mycorrhizal_Type (M)	1	11.902	0.1	**0.003****
	Tree_Diversity (L)	2	0.617	0.01	0.707
	Interaction (MxL)	2	2.184	0.037	0.103
CP	Mycorrhizal_Type (M)	1	14.789	0.117	**0.003****
	Tree_Diversity (L)	2	0.619	0.01	0.707
	Interaction (MxL)	2	3.938	0.063	**0.021***
NP	Mycorrhizal_Type (M)	1	15.158	0.122	**0.003****
	Tree_Diversity (L)	2	0.615	0.01	0.707
	Interaction (MxL)	2	2.679	0.043	0.092
CNP	Mycorrhizal_Type (M)	1	15.022	0.12	**0.003****
	Tree_Diversity (L)	2	0.544	0.009	0.707
	Interaction (MxL)	2	3.353	0.054	**0.042***

*All significant adjusted p values are highlighted in bold followed by significance level codes, *: $p \leq 0.05$, **: $p \leq 0.01$.*

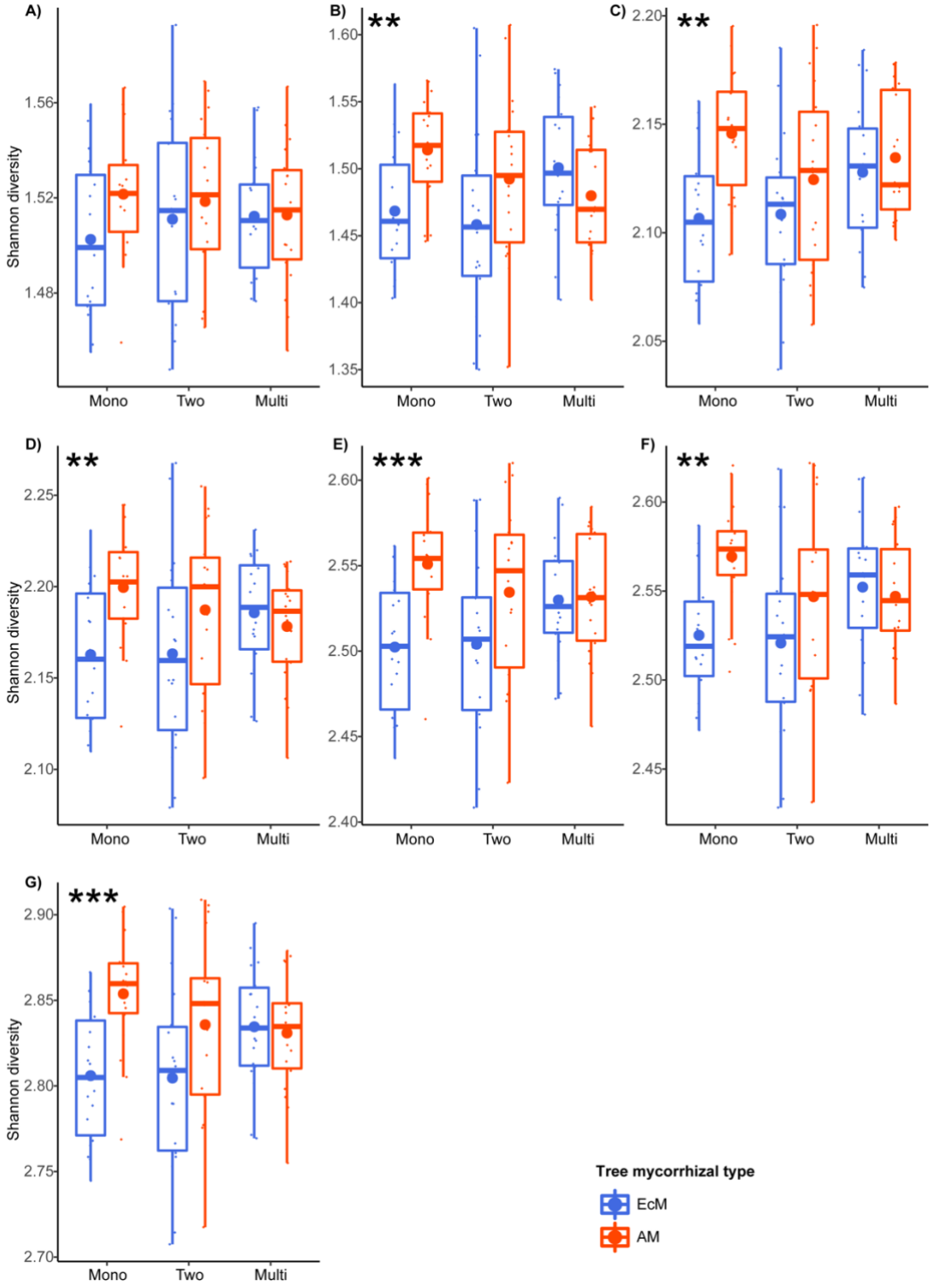


Figure III.2 Comparison of functional diversity of EcM and AM TSPs soil microbial communities along the tree diversity levels. On the y-axis Shannon diversity index and on the x-axis, EcM and AM TSPs and the tree diversity levels (Mono for monospecific stands, Two for two-species mixtures and Multi for multi-tree species mixtures). (A) Carbon (B) Nitrogen (C) Phosphorus (D) Carbon and nitrogen (E) Carbon and phosphorus (F) Nitrogen and phosphorus (G) Carbon, nitrogen and phosphorus. The asterisks show the p-value significance level, *p < 0.05, **p < 0.01, ***p < 0.001, ****p< 0.0001

PERMANOVA tests of the effects of tree mycorrhizal type and tree diversity level on the microbial community genomic functional potential of nutrient cycling combinations

showed a strong effect of tree mycorrhizal type on all combinations of genomic functional compositions (R^2 values range: 5.5 – 12.8%). In addition, significant interaction effects of tree mycorrhizal type and tree diversity were found for CP and CNP combinations (Table III.1). Furthermore, post hoc analysis of the whole community revealed that the tree mycorrhizal type effect was not significant in multi-tree species mixtures (Table S III.6). Comparative analysis of the functional compositions of the whole community with those of the significantly soil-responsive modules showed similar results, except for the additional significance of interaction terms for CN and NP (Table S III.7). Similarly, the tree mycorrhizal type effect was also not significant in multi-tree species mixtures (Table S III.8).

Pairwise comparison of functional abundances of EcM and AM TSPs soil microbial sub-communities

The PCoA ordination based on the relative functional abundances showed that the significant sub-communities of EcM and AM TSPs soil microbial networks became decreasingly distant from monospecific stands to two-species and multi-tree species mixtures (Figure III.3). In addition, envfit analysis ($p < 0.01$) indicated that the differentiation of these sub-communities might be driven by the different sets of nutrient cycling enzymes across the tree diversity levels, predominantly by enzymes of the P cycle (Table S III.9). In monospecific stands, the significantly correlated enzymes were predominantly related to P (11), followed by N (9) cycles, while in two-species mixtures they were related to P (16), followed by C (9) cycles. In contrast, in multi-tree species mixtures, fewer enzymes were correlated with the differentiation of modules, and those were mainly related to the C (6) and P (6) cycles (Table S III.9).

Furthermore, pairwise comparisons across the significant sub-communities of EcM and AM TSP soil microbial networks revealed that 25 module pairs were significantly different in terms of their genomic potential for nutrient cycling. Except for C and N, in all nutrient cycling combinations, we found a higher number of significantly abundant AM modules across the tree diversity levels (Figure III.4). Interestingly, no significant differences were found in N cycling potential in multi-tree species mixtures. Furthermore, for C-related gene families, only EcM modules were significantly abundant in monospecific stands; while for C and CN combinations in multi-tree species mixtures, AM modules were significantly abundant (Figure III.4). In addition, the pairwise comparisons of significant modules within

tree mycorrhizal type (i.e., AM vs AM and EcM vs EcM modules) indicated that the proportion of significant differences was higher in AM sub-communities in all combinations, except for CNP (equal proportion), compared to EcM sub-communities (Figure S III.1).

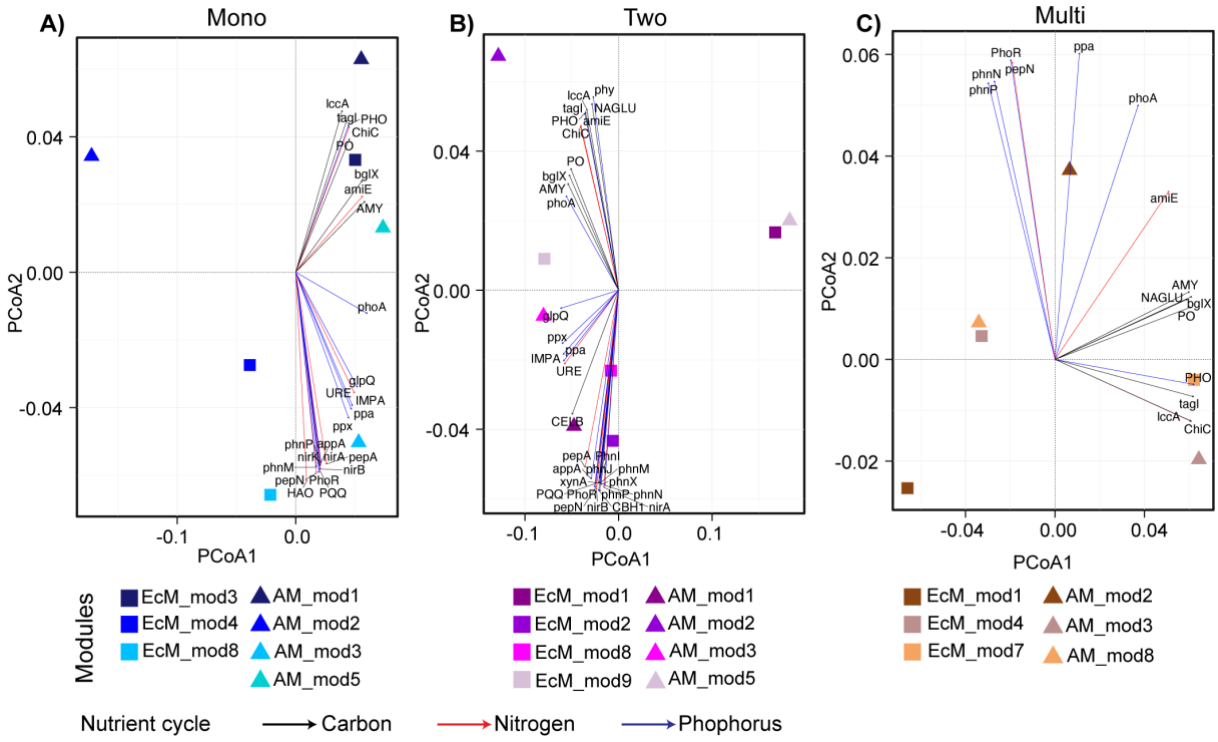


Figure III.3 Significant enzymes in envfit analysis were fitted as arrows onto ordination. The full name for the abbreviations of enzymes are : 'PQQ' - Quinoprotein glucose dehydrogenase; 'nirB' - Nitrite reductase (NADH); 'nirK' - Nitrite reductase (NO-forming); 'nirA' - Ferredoxin-nitrite reductase; 'HAO' - Hydroxylamine reductase; 'PhoR' - Histidine kinase; 'tagl' - Triacylglycerol lipase; 'PhoA' - Alkaline phosphatase; 'PHO' – Acid phosphatase; 'IMPA' - Inositol-phosphate phosphatase; 'appA' - 4-phytase; 'glpQ' - Glycerophosphodiester phosphodiesterase; 'phnP' - Phosphoribosyl 1,2-cyclic phosphate phosphodiesterase; 'AMY' - Alpha-amylase; 'ChiC' – Chitinase; 'bglX' - Beta-glucosidase; 'pepA' - Leucyl aminopeptidase; 'pepN' - Membrane alanyl aminopeptidase; 'amiE' – Amidase; 'URE' – Urease; 'ppa' - Inorganic diphosphatase; 'ppx' – Exopolyphosphatase; 'phnM' - Alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase; 'lccA' – Laccase; 'PO' – Peroxidase; 'phnN' - Ribose 1,5-bisphosphate phosphokinase; 'phnI' - Alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase; 'phy' - 3-phytase; 'phnX' - Phosphonoacetaldehyde hydrolase; 'CELB' – Cellulase; 'NAGLU' - Alpha-N-acetylglucosaminidase; 'xynA' - Endo-1,4-beta-xylanase; 'CBH1' - Cellulose 1,4-beta-cellobiosidase; 'phnJ' - Alpha-D-ribose 1-methylphosphonate 5-phosphate C-P-lyase

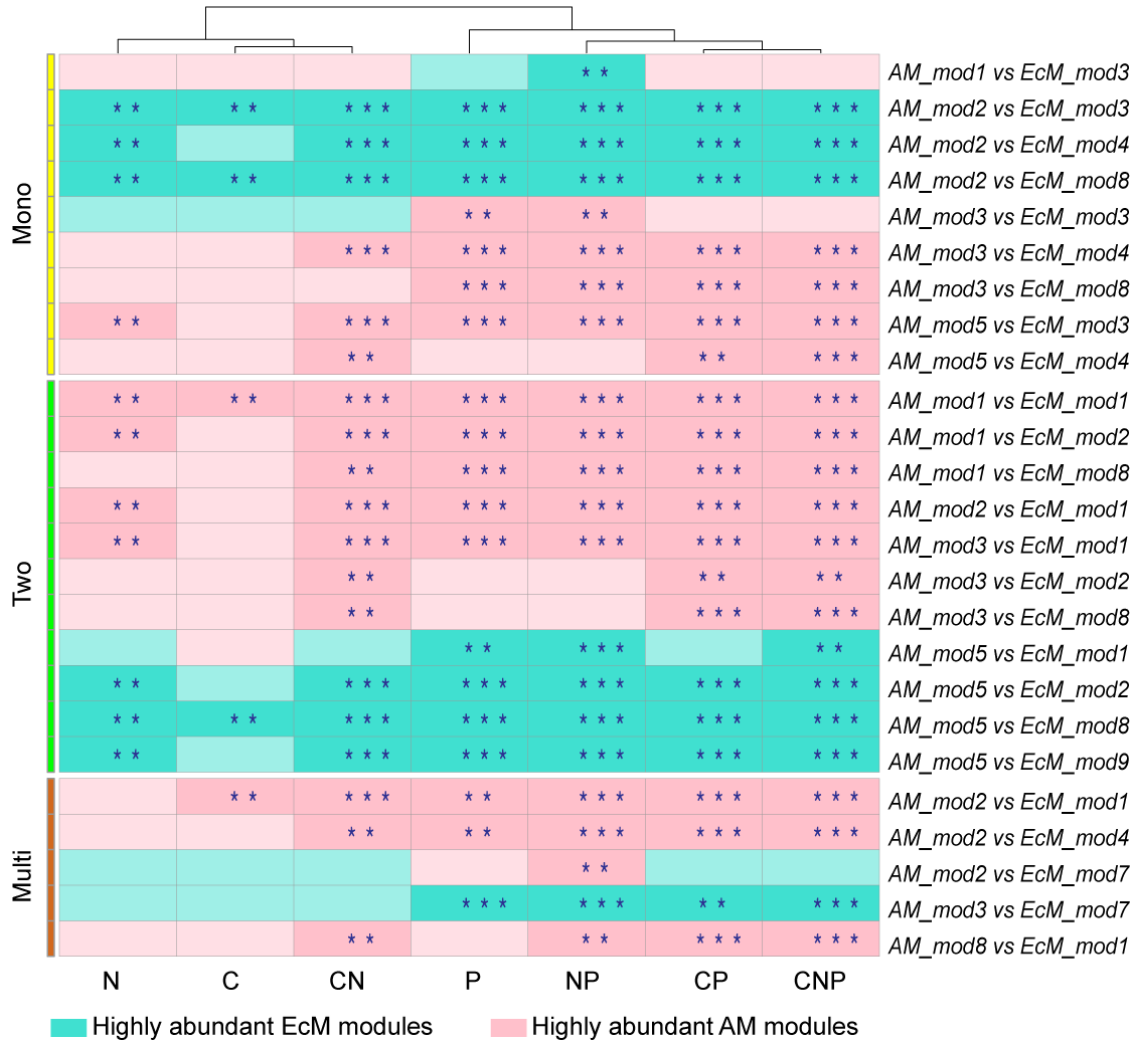


Figure III.4 Heat map of pairwise comparisons of modules EcM and AM modules along the tree diversity levels. The asterisks show the p-value significance level, *p < 0.05, **p < 0.01, ***p < 0.001, ****p< 0.0001

Differentially abundant taxa behind the observed functional abundance differences of EcM and AM TSPs soil microbial sub-communities

We tested the differences in relative functional abundances of taxa between each EcM and AM significantly soil-responding module pairs within each tree diversity level and found a total of 995 unique differentially abundant ASVs. Further, all the ASVs were aggregated at the Class taxonomic level, and we identified the two most differentially abundant classes in both bacteria and fungi that strongly contributed to the functional abundances of EcM and AM TSP soil microbial communities at each tree diversity level for all nutrient cycling combinations (Figure III.5). These contributions ranged from 48% to 62% of the relative functional abundances. In monospecific stands for EcM modules, Agaricomycetes and Sordariomycetes were the predominant fungi contributing to the functional abundances of

all nutrient cycling combinations. In AM modules, Sordariomycetes were the top fungi followed by Leotiomycetes contributing to all nutrient cycling combinations except for P (4.3%) and NP (5.4%) combinations, while Eurotiomycetes were of second most important. In the case of bacteria, Acidobacteriae and Alphaproteobacteria were the predominant contributors in both EcM and AM modules except to the C cycle, not only in mono-but also in two- and multi-tree species mixtures.

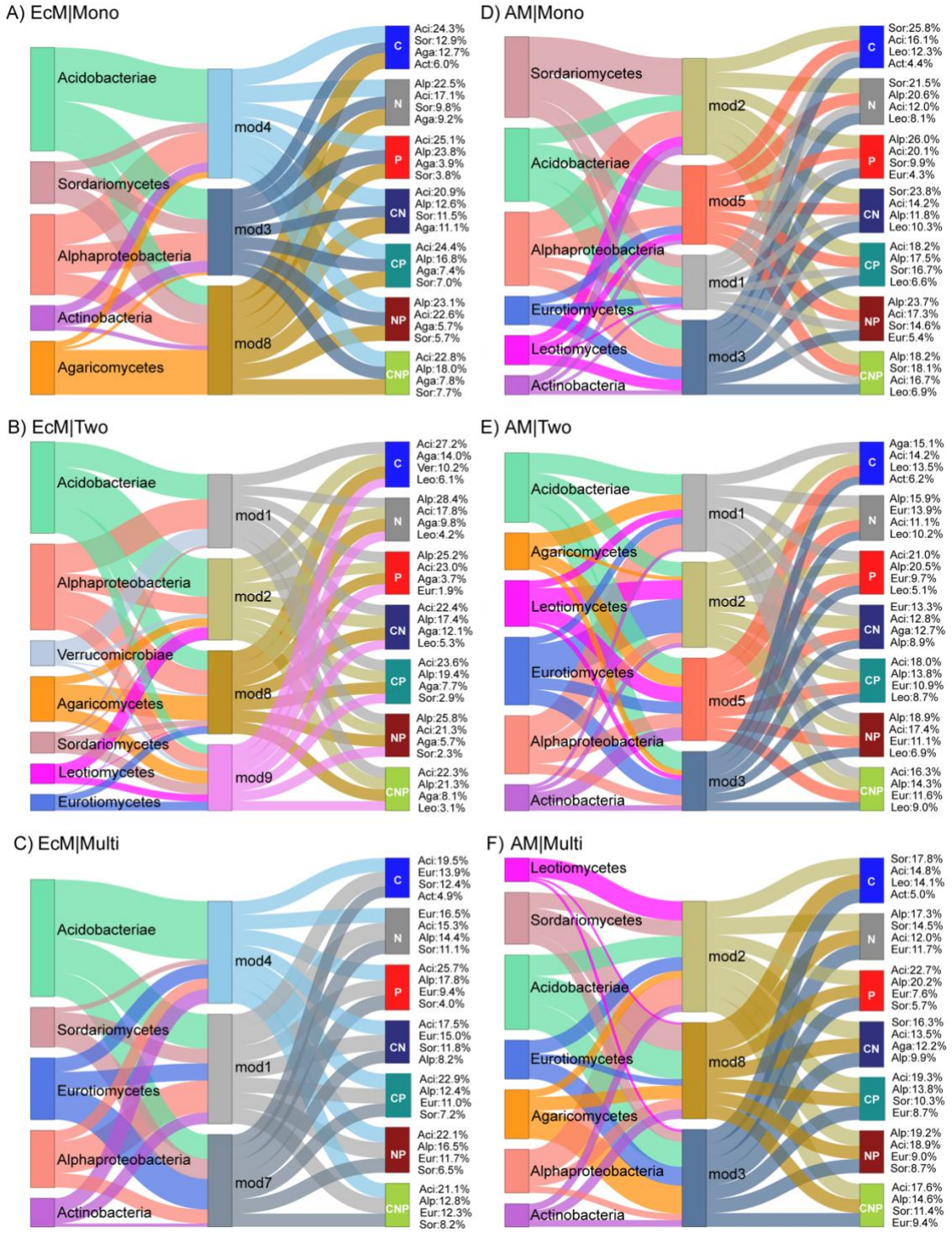


Figure III.5 Sankey plots showing the top differentially abundant taxa from each EcM and AM networks along the tree diversity levels and their proportional contributions to the functional abundances. A- C) EcM networks D- F) AM networks. Connections (edges) represent the proportion of relative functional abundances of each top two bacterial and fungal taxa and their distribution in each soil-responsive sub-communities of EcM and AM networks. The text beside the C, N, P, CN, CP, NP and CNP nodes denotes the top two contributing bacterial and fungal taxa per network to the respective nutrient combinations. "Aci"- *Acidobacteriae*;"Sor"- *Sordariomycetes*;"Ver"- *Verrucomicrobiae*; "Alp"- *Alphaproteobacteria*; "Agr"- *Agaricomycetes*; "Eur"- *Eurotiomycetes*; "Leo"- *Leotiomycetes*; "Act"- *Actinobacteria*

Interestingly, Actinobacteria were the second most contributor to the C cycle across the tree diversity levels, except in EcM modules of two-tree species mixtures where Verrucomicrobiae (10.2%) took that place. In two-tree species mixtures, for EcM modules, Agaricomycetes were the predominant fungal contributor to all nutrient cycling combinations, followed by Leotiomycetes in C, N, CN, and CNP combinations, Sordariomycetes in CP (3%) and NP (2.3%), and by Eurotiomycetes (1.9%) in the P cycle. While for AM modules, Eurotiomycetes followed by Leotiomycetes were the major contributors to most of the nutrient cycling combinations, except in C (15.1%) and CN (12.7%), where Agaricomycetes were predominant. In multi-tree species mixtures, Eurotiomycetes followed by Sordariomycetes were the main fungal contributors to all nutrient cycling combinations in EcM modules. This was also the case for AM modules, except for the C and CN combinations, wherein Leotiomycetes and Agaricomycetes were the second major contributors, respectively. Across the tree diversity levels, in both EcM and AM modules, bacteria outweighed fungi as major differentially abundant contributors to the P-cycle. Furthermore, compared to EcM, higher fungal contribution in AM modules was found in monospecific stands and two-tree species mixtures (Figure III.5).

Discussion

EcM and AM TSPs soil microbial inter-kingdom networks and their sub-communities differ in their ecological properties

The network topological parameters provide key insights into the associations between taxa and the influence of some taxa on particular modules or the whole community. In our study, the observed significant differences between EcM and AM TSP soil microbial co-occurrence networks revealed differences in the taxa assembly and organization in the respective communities. Similarly, in a recent greenhouse experimental study, (Yuan et al.,

2021) reported significant differences in the co-occurrence network topology between arbuscular mycorrhizal fungal (AMF) - bacterial networks and nonmycorrhizal fungal (comprising saprotrophs, pathogens, endophytes, and unclassified) - bacterial networks. Relatively high values of degree centrality and betweenness centrality may indicate stronger relationships among the taxa and a powerful influence of some taxa in bridging or communicating between different parts of the network, respectively (Ma et al., 2016). Our results show that EcM TSPs soil microbial networks had relatively higher betweenness centrality than that of AM networks, especially in mono- and two-tree species mixtures, suggesting that some key taxa might exert control over other taxa members of the network. A relatively higher abundance of ectomycorrhizal fungi (EMF) in EcM TSPs soils which were known to regulate other microbes in the community (Moore et al., 2015; Bahram et al., 2020) might be a possible reason for the higher betweenness centrality. In contrast, the higher degree centrality in AM networks, especially in mono- and two-tree species mixtures could be attributed to the relatively higher abundance of saprotrophs in AM TSP soils (Rudnick et al., 2015).

Microbes belonging to a sub-community/module may share similar ecological processes like nutrient cycling functions or be affected by the same environmental filtering processes (Purahong et al., 2016; Röttjers and Faust, 2018). In our analysis, we identified such modules; for instance, in AM monospecific stands, all of the modules had significant relationships with P which is compliant with the fact that AM trees acquire P through the arbuscular mycorrhizal fungi (AMF), and P is a limiting nutrient for the soil microbes in the sub-tropical systems with AM-dominated stands (Camenzind et al., 2018). Interestingly, the modules (both EcM and AM) in two-tree species mixtures were strongly related to N or its inorganic forms NO_3^- and NH_4^+. It is well known that N is a vital limiting nutrient for both plants and microbes (Bingham and Cotrufo, 2016) and that the EcM and AM tree-dominated systems have contrasting N acquisition and allocation strategies, where organic N is preferred in EcM systems, while this is the case for inorganic N in AM systems (Phillips et al., 2013). One possible reason for the observed association of modules with N or the inorganic N compounds in two-tree species mixtures could be the co-existence of different mycorrhizal type trees in a plot (i.e., AM tree species with EcM trees and *vice versa*). This proportional addition of contrasting N acquisiting tree individuals in one plot would have triggered the mechanisms that may limit the preferred source of N for the associated soil microbial sub-communities. In multi-tree species mixtures, all EcM and AM

modules were significantly associated with pH, which is known to affect both bacterial and fungal communities (Rousk et al., 2010; Glassman et al., 2017) and has a subtle relationship with soil nutrients. For example, low pH was reported to impede N mineralization and nitrification (Read and Perez-Moreno, 2003; Cheng et al., 2013; Tian et al., 2013), while P availability was suggested to be high at near-neutral pH i.e., 6.5–7 (Penn and Camberato, 2019) but see, (Barrow et al., 2020)). Consequently, the microbial sub-communities in multi-tree species mixtures might have dynamic functional roles in nutrient cycling.

Functional potential of EcM and AM TSP soil co-occurring bacterial and fungal communities were strongly impacted by tree mycorrhizal type

As expected, we found a significant tree mycorrhizal type effect on the functional compositions of the co-occurring microbial communities. Our results are in line with a study from boreal and temperate regional sites by Bahram et al. (2020), who reported significant differences in the composition of microbial functional genes between sites dominated by EcM and AM mycorrhizal type plants. Through their specific mycorrhizal partners, trees can select the associated microbial communities with the required functional abilities (Nuccio et al., 2013; Nguyen and Bruns, 2015; Tedersoo et al., 2020a). For example, given the genomic potential to release oxidative and hydrolytic extracellular enzymes to directly break down the soil organic matter (Phillips et al., 2013; Tedersoo and Bahram, 2019), EMF have been reported to outcompete and limit the saprotrophs in microbial communities of EcM tree-dominated systems (Bödeker et al., 2016). In contrast, AMF are known to have very little genomic repertoire for enzymatic degradation of soil organic matter. In consequence, they rely upon and enrich saprotrophic fungi and bacteria in soils under AM trees (Herman et al., 2012; Keller and Phillips, 2019). Furthermore, we found significant interactive effects of tree diversity and tree mycorrhizal type in some nutrient cycling combinations (CP, CNP for whole communities and CN, NP, CP, CNP for significant modules), wherein multi-tree species mixtures neutralize the tree mycorrhizal type effect on the functional compositions of soil microbial communities. More co-occurring tree species and including different mycorrhizal type trees in multi-tree species mixtures could be the potential explanation for the observed absence of significant differences in the functional compositions of soil microbial communities (Singavarapu et al., 2021).

Similar to the functional composition analysis, we found a significant tree mycorrhizal type effect on the functional diversity of soil microbial communities. Nonetheless, this effect was relatively weak and found only in monospecific stands. The results are in line with the significant effect of tree mycorrhizal type on the functional gene ortholog (OG) richness of fungi and bacteria as reported by Bahram et al. (2020). We did not encounter any significant tree diversity effect on the functional diversity of soil microbial co-occurring communities, which was contrary to previous findings of the positive effects of plant diversity on microbial community functions and activities (Zak et al., 2003; Eisenhauer et al., 2013; Lange et al., 2015). Although this effect was not significant, we observed the tendency of increased functional microbial diversity under EcM trees in multi-tree species mixtures. One might expect that the positive effect of tree diversity on the functional diversity of microbial communities might become significant in the long term (Eisenhauer et al., 2010; Chen et al., 2019a).

Moreover, our findings revealed that high tree diversity that includes both AM and EcM mycorrhizal type trees can harbor rich and converging functional genomic potential, which in turn, can have a positive effect on the studied ecosystem. This conforms to the previous findings of our study site of higher stand-level productivity in multi-tree species mixtures compared to monospecific stands (Huang et al., 2018). Hence, our study warrants further research on the detailed mechanisms of how soil microbial communities contribute to the increased above-ground productivity in more species-rich stands.

Insights into the functional abundance differences of EcM and AM TSP soil co-occurring microbial sub-communities

Further, we investigated how EcM and AM TSP soil microbial sub-communities at each tree diversity level differ in their genomic functional abundances. The ordination coupled with the fitting of the significantly contributing enzymes showed for monospecific stands that all the C-cycling and most of the P-cycling enzymes were diverging in opposite directions of the ordination. These C-cycling enzymes along with amidase and chitinase (N-cycling enzymes) might have similar functional roles in the community, which in this case could be the decomposition of complex carbohydrates for microbial utilization (López-Mondéjar et al., 2016; Zang et al., 2018; Canarini et al., 2021). In the other direction, the P-cycling enzymes were broadly involved in inorganic P-solubilization and organic P-mineralization, along with a set of N-cycling enzymes that take part in

nitrification (e.g., hydroxylamine reductase) and nitrate reduction (e.g., Ferredoxin-nitrite reductase). These findings indicate that these sub-communities might have major functional roles in producing plant- and microbe-available forms of N and P (Kuypers et al., 2018; Black et al., 2019; Canarini et al., 2021). This view was corroborated by the response of these modules to the soil chemistry as seen from dbRDA analysis. In contrast, in two-tree species mixtures, a higher number of nutrient cycling enzymes did not show any distinct pattern, and this might indicate that the module differentiation was possibly driven by multiple functional differences. In multi-tree species mixtures, fewer correlated enzymes were found and this might reflect that the module differentiation was driven by fewer functional differences. Expectedly, P-cycle enzymes were predominantly correlated with the module differentiation at all tree diversity levels, and together with their relationship to soil nutrients in monospecific stands, suggests that the soil microbial sub-communities at our study site are shaped by the P-limitation which is in line with previous reports (Huang et al., 2013; Camenzind et al., 2018; Du et al., 2020). Intriguingly, our sub-community level functional analysis pointed out the natural selection of microbes with required functional potential suitable to the habitat at community and sub-community levels.

Furthermore, we encountered differences in functional abundances of nutrient cycling combinations at the module level among the EcM and AM TSP soil microbial communities. Overall, AM modules had a higher number of significantly abundant modules, except for C and N cycles. In particular, significantly abundant EcM modules for the C-cycle were encountered more often in monospecific stands, while not a single significantly abundant EcM module was found in multi-tree species mixtures. The higher abundance pattern in monospecific stands of such modules can be explained by the fact that ectomycorrhizal fungi can efficiently sequester carbon from plants (Soudzilovskaia et al., 2015; Tedersoo and Bahram, 2019), influence the recruitment of co-occurring microbes including bacteria (Johansson et al., 2004; Bonfante and Anca, 2009), and then can allocate the C to them (Sun et al., 1999; Warmink et al., 2009; Churchland and Grayston, 2014). In support of this interpretation, we observed a major contribution of bacteria compared to fungi to the nutrient cycling potential in EcM modules in monospecific stands. In monospecific stands, for the N-cycle, we found three and one significantly abundant EcM and AM modules, respectively. In a recent soil metagenomics-based study from temperate forests, (Mushinski et al., 2021) reported a larger estimated amount of N-cycling genes in AM compared to

EcM tree-dominated soils. In our study, we focused on those sub-communities that fulfil specific functional roles, which would explain the aforementioned observation. Nevertheless, in concord, we found a relatively higher number of significantly abundant AM modules in two-tree species mixtures. It is known that soils under AM trees have more open and faster nutrient cycling rates than EcM systems (Phillips et al., 2013; Tedersoo and Bahram, 2019), which is facilitated by the specifically associated fast-cycling versus slow-cycling microbes (Herzog et al., 2019; Fanin et al., 2020; Su et al., 2020). In agreement with this assumption, we found an overall higher number of significantly abundant AM modules in the remaining nutrient cycling combinations (P, CN, CP, NP, & CNP).

Moreover, the number of modules that differed between EcM and AM was fewer in multi-species compared to monospecific stands and two-tree species mixtures. Taken together, these findings suggest converging functional genomic potential of EcM and AM soil microbiota at the sub-community level with increasing tree species richness. Additionally, pairwise module analysis within tree mycorrhizal type resulted in a higher proportion of significant differences within AM sub-communities than that of EcM sub-communities in all nutrient cycling combinations, except for CNP where equal proportions were observed. This might point to a higher functional equivalence in EcM sub-communities, which is probably facilitated by the slow-cycling members such as ectomycorrhizal fungi as reflected by members of the Agaricomycetes, which were the predominant differentially abundant fungal contributors to the nutrient cycling in mono- and two-tree species mixtures. In contrast, a higher number of specialized functional units in the AM sub-communities might be promoted by fast-cycling microbes such as saprotrophs, which is reflected in their higher functional abundances in most of the nutrient cycling combinations and also by their differentially abundant taxa. Higher functional abundance in their sub-communities might confer resilience to the AM TSPs soil microbial communities. This expected functional resilience in AM and the functional equivalence in EcM TSP soil microbial communities can foster soil microbiome stability, which would be most pronounced in multi-tree species mixtures (Naylor et al., 2020).

Differentially abundant taxa and the top contributors to the functional abundance and nutrient cycling combinations

Finally, differential abundance analysis revealed the taxa behind the differences between each EcM and AM significantly soil-responsive module pairs within each tree diversity

level. Agaricomycetes are a phylogenetically diverse group of fungi containing both biotrophs, such as ectomycorrhizal fungi and saprotrophs (James et al., 2006; Watkinson, 2016) which explains their predominant contributions to the nutrient cycling combinations. Sordariomycetes were one of the major contributors to the nutrient cycling combinations in AM monospecific stands and also for both EcM and AM in multi-tree species mixtures. Sordariomycetes are known to contain decomposers of wood and leaf litter (Spatafora and Blackwell, 1993; Lutzoni et al., 2004). A recent study identified some Sordariomycetes taxa to function as connector hubs in soil microbial networks and were positively correlated to the abundance of functional genes involved in C, N, and P cycling (Shi et al., 2020). Eurotiomycetes and Leotiomycetes, which contributed to various nutrient cycling combinations in our study were also shown to have a significant link to the production of C-cycling enzymes (Trivedi et al., 2016). In addition, Eurotiomycetes were also found to be involved in denitrification (Mothapo et al., 2015). Acidobacteriae and Alphaproteobacteria were the predominant contributors in all nutrient cycling combinations. Together with the Actinobacteria, which showed the second highest association with C in our study, all these groups are known from the literature to be involved in the C cycle (Fierer et al., 2007; Trivedi et al., 2016), N (Mushinski et al., 2021) and P cycle (Liang et al., 2020; Gaiero et al., 2021). We have also shown the functional potential of these groups for other nutrient combinations including CN, CP, NP, and CNP. This information can be helpful in future studies on the relationship between microbial taxa and nutrient cycling. Although these top differentially abundant classes were common in both EcM and AM modules, it is worth noting that they differ in their role at the lower taxa levels such as ASVs. Moreover, the top two contributing fungal and bacterial classes differed between EcM and AM modules in the different tree diversity levels, especially in two-tree species mixtures. This indicates that the sub-communities recruit groups of different taxa depending on their functional roles and niche requirements.

Conclusions

Taken together, our study highlights the importance of inter-kingdom soil microbial co-occurrence networks and their sub-communities to understand the factors that shape their community composition and functional roles. We comprehensively characterized the predicted genomic functional potential of co-occurring EcM and AM TSPs soil microbial sub-communities. Our analysis indicated that the nutrient cycling potential of the soil microbiota at the community level was a cumulative effect of their sub-communities. More

importantly, functional potential differences, driven by differentially enriched taxa, were revealed among sub-communities that were not obvious at the community level. Our results highlight the key role of the tree mycorrhizal type in the recruitment and organization of these networks. Furthermore, higher tree diversity levels of co-existing AM and EcM mycorrhizal trees were found to foster microbial communities with rich and converging functional genomic potential, thereby promoting stable and better functioning of the forest soil ecosystem. These findings underlined the versatility and significance of microbial sub-communities in different soil nutrient cycling processes, which contribute to maintaining multi-functionality and modulating tree-tree interactions in diverse forest ecosystems.

Appendix S III

Table S III.1

Network properties of EcM and AM TSPs soil co-occurring microbial communities across the tree diversity levels

	Input Bacteria	Input Fungi	Nodes	Edges negative	Edges positive	Average path length	Clustering coefficient	Modularity	Modules	Diameter
AM | Mono	798	473	1271	8180	9486	2.585	0.085	0.208	5	5
AM | Two	798	503	1301	8048	10124	2.587	0.087	0.214	6	5
AM | Multi	797	491	1288	7848	9670	2.602	0.083	0.211	8	5
EcM | Mono	796	448	1244	7042	8867	2.671	0.094	0.236	8	6
EcM | Two	798	430	1228	7260	8538	2.638	0.083	0.210	9	5
EcM | Multi	798	514	1312	8114	9703	2.634	0.091	0.222	7	5

Table S III.2

Significant soil variables associated with EcM and AM TSPs soil microbial network sub-communities

Network	***Module***	***Size***	***pH***		***NO3***		***Moisture***		***TOC***		***P***		***N***		***NH4***	
			F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
AM | Mono	1	355	1.91	0.004	-	-	-	-	1.56	0.031	2.13	0.003	-	-	-	-
	2	88	4.38	0.001	2.11	0.026	-	-	-	-	2.20	0.014	-	-	-	-
	3	418	8.53	0.001	-	-	-	-	-	-	1.78	0.012	-	-	-	-
	5	403	2.79	0.003	-	-	-	-	-	-	2.15	0.014	-	-	-	-
AM | Two	1	363	5.90	0.001	-	-	-	-	-	-	-	-	-	-	-	-
	2	419	2.09	0.004	1.51	0.04	-	-	-	-	-	-	-	-	-	-
	3	450	2.68	0.001	-	-	-	-	-	-	-	-	2.55	0.002	-	-
	5	40	-	-	-	-	-	-	-	-	-	-	-	-	2.34	0.02
AM | Multi	2	434	3.17	0.006	-	-	2.27	0.027	-	-	-	-	-	-	-	-
	3	403	1.73	0.009	1.51	0.01	-	-	-	-	-	-	-	-	-	-
	8	372	4.75	0.001	-	-	2.12	0.041	-	-	-	-	-	-	-	-
EcM | Mono	3	414	1.40	0.083	-	-	-	-	1.51	0.042	-	-	-	-	-	-
	4	351	1.62	0.037	1.57	0.035	-	-	-	-	-	-	-	-	-	-
	8	388	3.19	0.003	-	-	-	-	-	-	2.09	0.042	-	-	-	-
EcM | Two	1	82	5.61	0.001	2.42	0.03	-	-	-	-	-	-	-	-	3.13	0.008
	2	373	4.02	0.001	2.88	0.003	-	-	-	-	-	-	-	-	2.03	0.023
	8	303	3.60	0.001	2.21	0.01	-	-	-	-	-	-	-	-	2.77	0.004
	9	411	2.74	0.002	1.93	0.011	-	-	-	-	-	-	-	-	-	-
EcM | Multi	1	340	6.81	0.001	-	-	-	-	-	-	-	-	-	-	-	-
	4	433	1.98	0.028	-	-	-	-	-	-	-	-	-	-	-	-
	7	437	2.39	0.002	-	-	-	-	-	-	-	-	-	-	-	-

Significant soil parameters were selected based on dbRDA models for each network. Variables that were significant only ($p < 0.05$) in the final model were shown here. Fields with '-' indicate the non-significance of the variable in that particular category.

Table S III.3

EC (Enzyme Commission/Classification) numbers of the enzymes known to participate in C, N and P cycles based on the literature

S.No	Name	EC	Description	Nutrient cycle	Reference
1	Beta-glucosidase	EC:3.2.1.21	Cleaving of cellobiose to free glucose molecules by hydrolysis of β-glucosidic linkages	Carbon	Zang et al. (2018); López-Mondéjar et al. (2016); Canarini et al. (2021)
2	Exoglucanase	EC:3.2.1.91	*CBH1; CBH2 [K19668];* cellulose 1,4-beta-cellobiosidase; exo-cellobiohydrolase;	Carbon	Canarini et al. (2021)
3	Endoglucanase	EC:3.2.1.4	Also known as Cellulase [K19357]; *bcsZ [*K20542*]*	Carbon	Das, S. K., & Varma, A. (2010); Zang et al. (2018);López-Mondéjar et al. (2016)
4	xylan 1,4-betaxylosidase	EC:3.2.1.37	Degradation of polysaccharide xylan into xylose. Catalysing the hydrolysis of the glycosidic linkage (β-1,4) of xylosides	Carbon	Zang et al. (2018);López-Mondéjar et al. (2016); Canarini et al. (2021)
5	Endo-1,4-betaxylanase	EC:3.2.1.8	Degradation of polysaccharide xylan into xylose. Catalysing the hydrolysis of the glycosidic linkage (β-1,4) of xylosides	Carbon	Zang et al. (2018);López-Mondéjar et al. (2016)
6	Laccase	EC:1.10.3.2	lccA; Other KO is K05909. Also known as benzenediol:oxygen oxidoreductase. A group of multi-copper proteins of low specificity acting on both o- and p-quinols, and often acting also on aminophenols and phenylenediamine.	Carbon	Das, S. K., & Varma, A. (2010);Zang et al. (2018);López-Mondéjar et al. (2016)
7	Pectin lyase	EC:4.2.2.10	PL; Other KO is K05909. Also known as pectolyase and polymethylgalacturonic transeliminase.	Carbon	Das, S. K., & Varma, A. (2010)
8	Peroxidase	EC:1.11.1.7	Oxidoreductases; Acting on a peroxide as acceptor. Other KO is K19511.	Carbon	Das, S. K., & Varma, A. (2010);Zang et al. (2018);López-Mondéjar et al. (2016)

9	Alpha-N-acetylglucosaminidase	EC:3.2.1.50	NAGLU; Hydrolysis of terminal non-reducing N-acetyl-D-glucosamine residues in N-acetyl-alpha-D-glucosaminides	Carbon	Das, S. K., & Varma, A. (2010); López-Mondéjar et al. (2016)
10	Alpha-amylase	EC:3.2.1.1	AMY, amyA, malS; Endohydrolysis of (1->4)-alpha-D-glucosidic linkages in polysaccharides containing three or more (1->4)-alpha-linked D-glucose units	Carbon	Das, S. K., & Varma, A. (2010); Zang et al. (2018)
11	Triacylglycerol lipase	EC:3.1.1.3	triacylglycerol acylhydrolase; Glycerolipid metabolism	Carbon	Canarini et al. (2021)
12	Nitrogenase	EC:1.18.6.1	*anfG [K00531]; nifD[K02586]; nifK* [K02591]; ;Nitrogenase, the enzyme complex catalysing N2 fixation. Reduced ferredoxin:dinitrogen oxidoreductase (ATPhydrolysing)	Nitrogen	Pajares and Bohannan (2016); Kuypers et al. (2018); Black et al. (2018)
13	Ammonia monooxygenase subunit A	EC:1.14.99.39	*AMO [K10944-K10946]*; Conversion of N into usable forms by oxidation. The enzyme catalyses the first reaction in the pathway of ammonia oxidation to nitrite	Nitrogen	Pajares and Bohannan (2016); Kuypers et al. (2018); Isobe et al. (2020); Black et al. (2018)
14	Urease	EC:3.5.1.5	URE; Also known as urea amidohydrolase. *ureA*, *ureB*, *ureC* (urease subunit gamma, beta & alpha) [K01428 - K01430, K14048]	Nitrogen	Kuypers et al. (2018); Isobe et al. (2020); Black et al. (2018)
15	Hydroxylamine reductase	EC:1.7.99.1	This enzyme participates in nitrogen metabolism acting on other nitrogenous compounds as donors with a cytochrome as an acceptor	Nitrogen	Pajares and Bohannan (2016); Kuypers et al. (2018)
16	Nitrite reductase / Hydroxylamine reductase	EC:1.7.2.1	*nirK [K00368] ;nirS [K15864]*; Also known as nitric-oxide:ferricytochrome-c oxidoreductase.	Nitrogen	Pajares and Bohannan (2016); Kuypers et al. (2018) ; Black et al. (2018)
17	Hydrazine synthase	EC:1.7.2.7	*HZS [K20932-K20934]*; anaerobic ammonium oxidation	Nitrogen	Black et al. (2018)
18	Hydrazine dehydrogenase	EC:1.7.2.8	*hdh*; anaerobic ammonium oxidation	Nitrogen	Black et al. (2018)
19	Nitrous-oxide reductase	EC:1.7.2.4	*nosZ*; N2O reductase;	Nitrogen	Black et al. (2018)
20	Nitric oxide reductase	EC:1.7.2.5	*norB*; nitric oxide reductase subunit B. nitric oxide reductase (cytochrome c)	Nitrogen	Black et al. (2018)
21	Ferredoxin-nitrite reductase	EC:1.7.7.1	*nirA*; ferredoxin-nitrite reductase. Also known as ammonia:ferredoxin oxidoreductase	Nitrogen	Black et al. (2018)

22	Ferredoxin-nitrate reductase	EC:1.7.7.2	*narB*; assimilatory ferredoxin-nitrate reductase	Nitrogen	Black et al. (2018)
23	Nitrate reductase	EC:1.7.5.1	*narG, narZ, nxrA; narV; NarGHI; [K00370, K00371, K00374]* Dissimilatory nitrate reductase	Nitrogen	Black et al. (2018)
24	Periplasmic nitrate reductase	EC:1.9.6.1	*napA; napB[K02568];*respiratory nitrate reductase; nitrate reductase (cytochrome);	Nitrogen	Black et al. (2018)
25	Hydroxylamine dehydrogenase	EC:1.7.2.6	*hao;* The enzyme converts hydroxylamine to nitrite	Nitrogen	Black et al. (2018)
26	Nitrite reductase (NADH)	EC:1.7.1.15	*nirB;* NADH large subunit. *nirD [K00363];* NADH small subunit	Nitrogen	Black et al. (2018)
27	Nitrite reductase (cytochrome c-552)	EC:1.7.2.2	*nrfA;*	Nitrogen	Black et al. (2018)
28	Chitinase	EC:3.2.1.14	ChiC; The enzyme binds to chitin and randomly cleaves glycosidic linkages in chitin and chitodextrins in a non-processive mode, generating chitooligosaccharides and free ends on which exo-chitinases and exo-chitodextrinases can act.	Nitrogen	Canarini et al. (2021)
29	Endo-chitodextinase	EC:3.2.1.202	endo_I; Also known as chitodextrinase.	Nitrogen	Canarini et al. (2021)
30	Exo-chitinase (reducing end)	EC:3.2.1.201	ChiA; The enzyme hydrolyses the second glycosidic (1->4) linkage from reducing ends of chitin and chitodextrin molecules, liberating N,N'-diacetylchitobiose disaccharides	Nitrogen	Canarini et al. (2021)
31	Exo-chitinase (non-reducing end)	EC:3.2.1.200	ChiB; The enzyme hydrolyses the second glycosidic (1->4) linkage from reducing ends of chitin and chitodextrin molecules, liberating N,N'-diacetylchitobiose disaccharides	Nitrogen	Canarini et al. (2021)
32	Endo-beta-N-acetylglucosaminidase	EC:3.2.1.96	ENGASE; Glycosidases, i.e. enzymes that hydrolyse O- and S-glycosyl compounds.Endohydrolysis of the N,N'-diacetylchitobiosyl unit in high-mannose glycopeptides and glycoproteins	Nitrogen	Canarini et al. (2021); Isobe et al. (2020)
33	Leucyl aminopeptidase	EC:3.4.11.1	CARP, pepA; Also known as leucine aminopeptidase and peptidase S. Release of an N-terminal amino acid. Amino acid amides and methyl esters are also readily hydrolysed, but rates on arylamides are exceedingly low	Nitrogen	Canarini et al. (2021)

34	Aminopeptidase N	EC:3.4.11.2	pepN; Also known as aminopeptidase M and alanine aminopeptidase. Release of an N-terminal amino acid, Xaa!Yaa- from a peptide, amide or arylamide.	Nitrogen	Canarini et al. (2021)
35	Amidase	EC:3.5.1.4	amiE; Also known as acylamidase and acylamide amidohydrolase. Acting on carbon-nitrogen bonds, other than peptide bonds; In linear amides	Nitrogen	Das, S. K., & Varma, A. (2010)
36	Acid phosphatase	EC:3.1.3.2	*olpA;* PHO;Transformation of P from soil organic matter into available forms. Hydrolytic enzymes that cleave the ester bond between the phosphate group and the organic residue of the organic phosphates	Phosphorus	Eivazi and Tabatabai (1977); Margalef et al. (2017)
37	Inorganic Pyrophosphatase	EC:3.6.1.1	*ppa*; Also known as diphosphate phosphohydrolase	Phosphorus	Eivazi and Tabatabai (1977); Margalef et al. (2017)
38	Exopolyphosphatase	EC:3.6.1.11	*ppx;* Also known as guanosine-5'-triphosphate,3'-diphosphate pyrophosphatase	Phosphorus	Dai et al. (2020)
39	phosphoribosyl 1,2-cyclic phosphate phosphodiesterase	EC:3.1.4.55	*phnP*; C-P lyase sub-unit	Phosphorus	Gaiero et al. (2021)
40	Alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase	EC:3.6.1.63	*phnM*; C-P lyase sub-unit	Phosphorus	Dai et al. (2020)
41	Alpha-D-ribose 1-methylphosphonate 5-phosphate C-P lyase	EC:4.7.1.1	*phnJ*; C-P lyase sub-unit. Participates in processing of phophonates into usable phosphate	Phosphorus	Gaiero et al. (2021)
42	Alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase	EC:2.7.8.37	*phnI*; C-P lyase sub-unit. ; *phnH ; phnG ; phnL*	Phosphorus	Dai et al. (2020)
43	Ribose 1,5-bisphosphokinase	EC:2.7.4.23	*phnN*; C-P lyase sub-unit.	Phosphorus	Dai et al. (2020)
44	Aminoalkylphosphonate N-acetyltransferase	EC:2.3.1.280	phnO;	Phosphorus	Dai et al. (2020)
45	Quinoprotein glucose dehydrogenase	EC:1.1.5.2	*gcd; PQQ*;	Phosphorus	Dai et al. (2020)
46	Phosphonoacetaldehyde hydrolase	EC:3.11.1.1	*phnX ;* Also known as phosphonatase	Phosphorus	Gaiero et al. (2021)

47	2-aminoethylphosphonate-pyruvate transaminase	EC:2.6.1.37	*phnW;*	Phosphorus	Dai et al. (2020)
48	Phosphonoacetate hydrolase	EC:3.11.1.2	*phnA*; A zinc-dependent enzyme. Belongs to the alkaline phosphatase superfamily of zinc-dependent hydrolases.	Phosphorus	Gaiero et al. (2021)
49	Inositol-phosphate phosphatase	EC:3.1.3.25	IMPA; suhB; Also known as myo-inositol-1(or 4)-monophosphatase. Acts on five of the six isomers of myo-inositol phosphate, all except myo-inositol 2-phosphate,	Phosphorus	Gaiero et al. (2021)
50	Alkaline phosphatase	EC:3.1.3.1	*phoA, phoB;* Also known as phosphate-monoester phosphohydrolase. Wide specificity. Also catalyses transphosphorylations. ; *phoD*	Phosphorus	Gaiero et al. (2021)
51	3-phytase	EC:3.1.3.8	*phy;* Also known as myo-inositol-hexakisphosphate 3-phosphohydrolase	Phosphorus	Liang et al. (2020)
52	4-phytase	EC:3.1.3.26	*appA*; Also known as myo-inositol-hexakisphosphate 4-phosphohydrolase.Inositol phosphate metabolism	Phosphorus	Gaiero et al. (2021)
53	Glycerophosphoryl diester phosphodiesterase	EC:3.1.4.46	*glpQ, ugpQ;* Phosphoric-diester hydrolases. Glycerophospholipid metabolism.Broad specificity for glycerophosphodiesters	Phosphorus	Gaiero et al. (2021)
54	Phosphate regulon sensor histidine kinase	EC:2.7.13.3	*PhoR* ; Also known as histidine kinase. Two-component system. Transferring phosphorus-containing groups	Phosphorus	Gaiero et al. (2021)
55	Glycerol 3-phosphate transport system ATP-binding protein	EC:7.6.2.10	*ugpC*; Also known as ABC-type glycerol 3-phosphate transporter and ATP phosphohydrolase.Linked to the hydrolysis of a nucleoside triphosphate.	Phosphorus	Gaiero et al. (2021)
56	Phosphonate transport system ATP-binding protein	EC:7.3.2.2	*phnC*; ABC-type phosphonate transporter. Linked to the hydrolysis of a nucleoside triphosphate. Also known as phosphonate-transporting ATPase	Phosphorus	Gaiero et al. (2021)
57	Phosphate transport system ATP-binding protein	EC:7.3.2.1	*pstB*; ABC-type phosphate transporter. Also known as phosphate-transporting ATPase	Phosphorus	Gaiero et al. (2021)

Table S III.4
List of PICRUSt2 predicted unique gene families/Enzymes in the EcM and AM TSPs soil co-occurring microbial communities

EC number	Name	Symbol	Nutrient Cycle
EC:3.1.1.3	*Triacylglycerol lipase*	*tagl*	*Carbon*
EC:3.2.1.1	*Alpha-amylase*	*AMY*	*Carbon*
EC:3.2.1.21	*Beta-glucosidase*	*bglX*	*Carbon*
EC:3.2.1.37	*Xylan 1.4-beta-xylosidase*	*xynB*	*Carbon*
EC:3.2.1.4	*Cellulase*	*CELB*	*Carbon*
EC:3.2.1.50	*Alpha-N-acetylglucosaminidase*	*NAGLU*	*Carbon*
EC:3.2.1.8	*Endo-1.4-beta-xylanase*	*xynA*	*Carbon*
EC:3.2.1.91	*Cellulose 1. 4-beta-cellobiosidase (non-reducing end)*	*CBH1*	*Carbon*
EC:4.2.2.10	*Pectin lyase*	*PL*	*Carbon*
EC:1.10.3.2	*Laccase*	*lccA*	*Carbon*
EC:1.11.1.7	*Peroxidase*	*PO*	*Carbon*
EC:1.14.99.39	*Ammonia monooxygenase*	*AMO*	*Nitrogen*
EC:1.18.6.1	*Nitrogenase*	*anfG*	*Nitrogen*
EC:1.7.1.15	*Nitrite reductase (NADH)*	*nirB*	*Nitrogen*
EC:1.7.2.1	*Nitrite reductase (NO-forming)*	*nirK*	*Nitrogen*
EC:1.7.2.2	*Nitrite reductase (cytochrome; ammonia-forming)*	*nrfA*	*Nitrogen*
EC:1.7.2.4	*Nitrous-oxide reductase*	*nosZ*	*Nitrogen*
EC:1.7.2.5	*Nitric-oxide reductase (cytochrome c)*	*norB*	*Nitrogen*
EC:1.7.2.6	*Hydroxylamine dehydrogenase*	*haoA*	*Nitrogen*
EC:1.7.7.1	*Ferredoxin--nitrite reductase*	*nirA*	*Nitrogen*
EC:1.7.7.2	*Ferredoxin--nitrate reductase*	*narB*	*Nitrogen*
EC:1.7.99.1	*Hydroxylamine reductase*	*HAO*	*Nitrogen*
EC:3.2.1.14	*Chitinase*	*ChiC*	*Nitrogen*
EC:3.4.11.1	*Leucyl aminopeptidase*	*pepA*	*Nitrogen*
EC:3.4.11.2	*Membrane alanyl aminopeptidase*	*pepN*	*Nitrogen*
EC:3.5.1.4	*Amidase*	*amiE*	*Nitrogen*
EC:3.5.1.5	*Urease*	*URE*	*Nitrogen*
EC:1.1.5.2	*Quinoprotein glucose dehydrogenase*	*PQQ*	*Phosphorus*
EC:2.6.1.37	*2-aminoethylphosphonate--pyruvate transaminase*	*phnW*	*Phosphorus*
EC:2.7.13.3	*Histidine kinase*	*PhoR*	*Phosphorus*
EC:2.7.4.23	*Ribose 1.5-bisphosphate phosphokinase*	*phnN*	*Phosphorus*
EC:2.7.8.37	*Alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase*	*PhnI*	*Phosphorus*
EC:3.1.3.1	*Alkaline phosphatase*	*phoA*	*Phosphorus*
EC:3.1.3.2	*Acid phosphatase*	*PHO*	*Phosphorus*
EC:3.1.3.25	*Inositol-phosphate phosphatase*	*IMPA*	*Phosphorus*
EC:3.1.3.26	*4-phytase*	*appA*	*Phosphorus*
EC:3.1.3.8	*3-phytase*	*phy*	*Phosphorus*
EC:3.1.4.46	*Glycerophosphodiester phosphodiesterase*	*glpQ*	*Phosphorus*
EC:3.1.4.55	*Phosphoribosyl 1.2-cyclic phosphate phosphodiesterase*	*phnP*	*Phosphorus*
EC:3.11.1.1	*Phosphonoacetaldehyde hydrolase*	*phnX*	*Phosphorus*
EC:3.11.1.2	*Phosphonoacetate hydrolase*	*phnA*	*Phosphorus*
EC:3.6.1.1	*Inorganic diphosphatase*	*ppa*	*Phosphorus*

EC:3.6.1.11	*Exopolyphosphatase*	*ppx*	*Phosphorus*
EC:3.6.1.63	*Alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase*	*phnM*	*Phosphorus*
EC:4.7.1.1	*Alpha-D-ribose 1-methylphosphonate 5-phosphate C-P-lyase*	*phnJ*	*Phosphorus*

Table S III.5
Two-way-ANOVA effects of tree mycorrhizal type and tree diversity on nutrient cycling functional diversity of the soil co-occurring fungal and bacterial communities

Nutrient Cycle	Factor	df	F	pval.adj
C	Mycorrhizal_Type (M)	1	2.121	0.24
	Tree_Diversity (L)	2	0.073	0.93
	Interaction (MxL)	2	0.758	0.55
N	Mycorrhizal_Type (M)	1	3.627	0.124
	Tree_Diversity (L)	2	1.011	0.482
	Interaction (MxL)	2	3.991	0.077
P	Mycorrhizal_Type (M)	1	9.826	**0.022***
	Tree_Diversity (L)	2	1.735	0.272
	Interaction (MxL)	2	2.136	0.216
CN	Mycorrhizal_Type (M)	1	5.42	0.077
	Tree_Diversity (L)	2	0.307	0.773
	Interaction (MxL)	2	2.974	0.124
CP	Mycorrhizal_Type (M)	1	11.056	**0.022***
	Tree_Diversity (L)	2	0.698	0.552
	Interaction (MxL)	2	2.808	0.124
NP	Mycorrhizal_Type (M)	1	7.4	**0.04***
	Tree_Diversity (L)	2	1.522	0.312
	Interaction (MxL)	2	3.273	0.11
CNP	Mycorrhizal_Type (M)	1	9.165	**0.022***
	Tree_Diversity (L)	2	0.839	0.538
	Interaction (MxL)	2	3.341	0.11

*All significant adjusted p values are highlighted in bold followed by significance level codes. *: $p \leq 0.05$. **: $p \leq 0.01$.*

Table S III.6
Post-hoc analysis for effects of tree mycorrhizal type at each tree diversity level on the nutrient cycling functional compositional differences of soil co-occurring fungal and bacterial (whole) communities based on PERMANOVA with 999 permutations

Nutrient Cycle	Tree Diversity	df	F	R^2	pval.adj
C	Mono	1	3.407	0.091	**0.023***
	Two	1	3.657	0.097	**0.034***
	Multi	1	1.876	0.052	0.155
N	Mono	1	18.422	0.351	**0.004****
	Two	1	4.293	0.112	0.062
	Multi	1	1.778	0.05	0.222
P	Mono	1	13.226	0.28	**0.004****
	Two	1	5.012	0.128	**0.023***
	Multi	1	1.31	0.037	0.271
CN	Mono	1	11.049	0.245	**0.004****
	Two	1	4.324	0.113	**0.032***
	Multi	1	1.527	0.043	0.222
CP	Mono	1	13.771	0.288	**0.004****
	Two	1	6.981	0.17	**0.015***
	Multi	1	1.036	0.03	0.337
NP	Mono	1	16.469	0.326	**0.004****
	Two	1	4.994	0.128	**0.034***
	Multi	1	1.511	0.043	0.229
CNP	Mono	1	15.159	0.308	**0.004****
	Two	1	6.041	0.151	**0.018***
	Multi	1	1.286	0.036	0.271

*All significant adjusted p values are highlighted in bold followed by significance level codes. *: $p \leq 0.05$. **: $p \leq 0.01$.*

Table S III.7
Effects of tree mycorrhizal type and tree diversity level on the nutrient cycling functional compositional differences of the significantly soil-responsive modules of soil microbial networks based on PERMANOVA with 999 permutations

Nutrient Cycle	Factor	df	F	R^2	pval.adj
C	Mycorrhizal_Type (M)	1	3.852	0.034	**0.034***
	Tree_Diversity (L)	2	1.439	0.025	0.278
	Interaction (MxL)	2	2.252	0.04	0.061
N	Mycorrhizal_Type (M)	1	10.309	0.085	**0.014***
	Tree_Diversity (L)	2	1.439	0.024	0.288
	Interaction (MxL)	2	3.041	0.05	0.064
P	Mycorrhizal_Type (M)	1	6.692	0.058	**0.024***
	Tree_Diversity (L)	2	1.006	0.017	0.4
	Interaction (MxL)	2	2.831	0.049	0.06
CN	Mycorrhizal_Type (M)	1	7.384	0.062	**0.014***
	Tree_Diversity (L)	2	1.398	0.024	0.278
	Interaction (MxL)	2	2.993	0.051	**0.034***
CP	Mycorrhizal_Type (M)	1	5.58	0.047	**0.032***
	Tree_Diversity (L)	2	1.047	0.017	0.375
	Interaction (MxL)	2	4.993	0.083	**0.024***
NP	Mycorrhizal_Type (M)	1	8.103	0.068	**0.014***
	Tree_Diversity (L)	2	0.851	0.014	0.463
	Interaction (MxL)	2	3.614	0.061	**0.034***
CNP	Mycorrhizal_Type (M)	1	6.86	0.057	**0.027***
	Tree_Diversity (L)	2	1.087	0.018	0.375
	Interaction (MxL)	2	4.482	0.075	**0.024***

*All significant adjusted p values are highlighted in bold followed by significance level codes. *: $p \leq 0.05$. **: $p \leq 0.01$.*

Table S III.8
Post-hoc analysis for effects of tree mycorrhizal type at each tree diversity level on the nutrient cycling functional compositional differences of the significantly soil-responsive modules of soil microbial networks based on PERMANOVA with 999 permutations

Nutrient Cycle	Tree Diversity	df	F	R^2	pval.adj
C	Mono	1	2.393	0.066	0.074
	Two	1	3.489	0.093	**0.04***
	Multi	1	2.474	0.068	0.099
N	Mono	1	11.979	0.261	**0.008****
	Two	1	5.161	0.132	**0.032***
	Multi	1	2.049	0.057	0.134
P	Mono	1	6.712	0.165	**0.014***
	Two	1	4.735	0.122	**0.031***
	Multi	1	1.623	0.046	0.186
CN	Mono	1	7.380	0.178	**0.007****
	Two	1	4.727	0.122	**0.03***
	Multi	1	2.152	0.06	0.112
CP	Mono	1	6.516	0.161	**0.008****
	Two	1	6.846	0.168	**0.018***
	Multi	1	2.177	0.06	0.134
NP	Mono	1	9.481	0.218	**0.007****
	Two	1	5.275	0.134	**0.031***
	Multi	1	2.202	0.061	0.121
CNP	Mono	1	8.098	0.192	**0.007****
	Two	1	6.144	0.153	**0.026***
	Multi	1	2.321	0.064	0.112

*All significant adjusted p values are highlighted in bold followed by significance level codes. *: $p \leq 0.05$. **: $p \leq 0.01$.*

Table S III.9
***envfit* analysis showing the significant gene families/Enzymes correlated to the ordination of significant modules of soil microbial networks**

Axis.1	Axis.2	Symbol	Name	Nutrient Cycle	Role	R^2	Tree diversity
0.324	-0.935	PQQ	Quinoprotein glucose dehydrogenase (PQQ. quinone)	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.978	Mono
0.333	-0.931	nirB	Nitrite reductase (NADH)	Nitrogen	DNRA (Denitrification and Dissimilatory Nitrate Reduction to Ammonia)	0.977	Mono
0.329	-0.916	nirK	Nitrite reductase (NO-forming)	Nitrogen	Denitrification / AnAmmOx	0.947	Mono
0.339	-0.905	nirA	Ferredoxin--nitrite reductase	Nitrogen	Assimilatory nitrate reduction	0.934	Mono
0.148	-0.988	HAO	Hydroxylamine reductase	Nitrogen	Nitrification / AnAmmOx	0.998	Mono
0.315	-0.947	PhoR	Histidine kinase	Phosphorus	P-starvation response regulation	0.996	Mono
0.689	0.718	tagl	Triacylglycerol lipase	Carbon	Glycerolipid metabolism	0.991	Mono
0.969	-0.195	phoA	Alkaline phosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.977	Mono
0.716	0.696	PHO	Acid phosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.998	Mono
0.767	-0.631	IMPA	Inositol-phosphate phosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.986	Mono
0.342	-0.898	appA	4-phytase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.924	Mono
0.834	-0.544	glpQ	Glycerophosphodiester phosphodiesterase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.991	Mono
0.332	-0.915	phnP	Phosphoribosyl 1.2-cyclic phosphate phosphodiesterase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.947	Mono
0.936	0.335	AMY	Alpha-amylase	Carbon	Carbohydrate hydrolysis	0.989	Mono
0.724	0.688	ChiC	Chitinase	Nitrogen	Nitrogen metabolism	0.997	Mono
0.897	0.432	bglX	Beta-glucosidase	Carbon	cellulose hydrolysis	0.992	Mono
0.416	-0.907	pepA	Leucyl aminopeptidase	Nitrogen	Protein degradation	0.995	Mono
0.281	-0.949	pepN	Membrane alanyl aminopeptidase	Nitrogen	Glutathione metabolism	0.980	Mono
0.906	0.361	amiE	Amidase	Nitrogen	Degradation of aromatic and Nitrogen containing compounds	0.951	Mono
0.794	-0.571	URE	Urease	Nitrogen	Urea cycle	0.957	Mono

0.754	-0.649	ppa	Inorganic diphosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.989	Mono
0.717	-0.690	ppx	Exopolyphosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.991	Mono
0.279	-0.921	phnM	Alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.927	Mono
0.628	0.765	lccA	Laccase	Carbon	Phenols and similar aromatic compounds Oxidation	0.980	Mono
0.728	0.631	PO	Peroxidase	Carbon	peroxidation of phenolic and non-phenolic substrates	0.928	Mono
-0.395	-0.890	PQQ	Quinoprotein glucose dehydrogenase (PQQ. quinone)	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.948	Two
-0.379	-0.919	nirB	Nitrite reductase (NADH)	Nitrogen	DNRA (Denitrification and Dissimilatory Nitrate Reduction to Ammonia)	0.988	Two
-0.252	-0.907	nirA	Ferredoxin--nitrite reductase	Nitrogen	Assimilatory nitrate reduction	0.887	Two
-0.409	-0.906	PhoR	Histidine kinase	Phosphorus	P-starvation response regulation	0.988	Two
-0.236	-0.898	phnN	Ribose 1.5-bisphosphate phosphokinase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.862	Two
-0.327	-0.865	PhnI	Alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.855	Two
-0.567	0.821	tagl	Triacylglycerol lipase	Carbon	Glycerolipid metabolism	0.997	Two
-0.900	0.435	phoA	Alkaline phosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.999	Two
-0.576	0.815	PHO	Acid phosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.996	Two
-0.944	-0.326	IMPA	Inositol-phosphate phosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.997	Two
-0.468	-0.869	appA	4-phytase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.974	Two
-0.433	0.893	phy	3-phytase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.984	Two
-0.995	-0.083	glpQ	Glycerophosphodiester phosphodiesterase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.998	Two

-0.339	-0.924	phnP	Phosphoribosyl 1.2-cyclic phosphate phosphodiesterase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.968	Two
-0.297	-0.895	phnX	Phosphonoacetaldehyde hydrolase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.888	Two
-0.868	0.492	AMY	Alpha-amylase	Carbon	Carbohydrate hydrolysis	0.995	Two
-0.647	0.759	ChiC	Chitinase	Nitrogen	Nitrogen metabolism	0.995	Two
-0.845	0.531	bglX	Beta-glucosidase	Carbon	cellulose hydrolysis	0.996	Two
-0.795	-0.572	CELB	Cellulase	Carbon	cellulose degradation	0.958	Two
-0.455	0.860	NAGLU	Alpha-N-acetylglucosaminidase	Carbon	Glucosamines degradation	0.947	Two
-0.341	-0.889	xynA	Endo-1.4-beta-xylanase	Carbon	Xylan degradation	0.907	Two
-0.322	-0.927	CBH1	Cellulose 1.4-beta-cellobiosidase (non-reducing end)	Carbon	cellulose degradation	0.962	Two
-0.573	-0.816	pepA	Leucyl aminopeptidase	Nitrogen	Protein degradation	0.993	Two
-0.390	-0.908	pepN	Membrane alanyl aminopeptidase	Nitrogen	Glutathione metabolism	0.976	Two
-0.652	0.753	amiE	Amidase	Nitrogen	Degradation of aromatic and Nitrogen containing compounds	0.993	Two
-0.926	-0.340	URE	Urease	Nitrogen	Urea cycle	0.974	Two
-0.952	-0.296	ppa	Inorganic diphosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.993	Two
-0.968	-0.246	ppx	Exopolyphosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.998	Two
-0.319	-0.888	phnM	Alpha-D-ribose 1-methylphosphonate 5-triphosphate diphosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.890	Two
-0.327	-0.865	phnJ	Alpha-D-ribose 1-methylphosphonate 5-phosphate C-P-lyase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.855	Two
-0.544	0.834	lccA	Laccase	Carbon	Phenols and similar aromatic compounds Oxidation	0.992	Two
-0.818	0.561	PO	Peroxidase	Carbon	peroxidation of phenolic and non-phenolic substrates	0.984	Two
-0.317	0.944	PhoR	Histidine kinase	Phosphorus	P-starvation response regulation	0.991	Multi
-0.435	0.877	phnN	Ribose 1.5-bisphosphate phosphokinase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.958	Multi
0.991	-0.117	tagl	Triacylglycerol lipase	Carbon	Glycerolipid metabolism	0.996	Multi
0.596	0.801	phoA	Alkaline phosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.997	Multi

0.995	-0.079	PHO	Acid phosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.997	Multi
-0.478	0.871	phnP	Phosphoribosyl 1.2-cyclic phosphate phosphodiesterase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.988	Multi
0.961	0.213	AMY	Alpha-amylase	Carbon	Carbohydrate hydrolysis	0.970	Multi
0.977	-0.195	ChiC	Chitinase	Nitrogen	Nitrogen metabolism	0.993	Multi
0.978	0.198	bglX	Beta-glucosidase	Carbon	cellulose hydrolysis	0.996	Multi
0.946	0.190	NAGLU	Alpha-N-acetylglucosaminidase	Carbon	Glucosamines degradation	0.931	Multi
-0.306	0.937	pepN	Membrane alanyl aminopeptidase	Nitrogen	Glutathione metabolism	0.971	Multi
0.815	0.530	amiE	Amidase	Nitrogen	Degradation of aromatic and Nitrogen containing compounds	0.944	Multi
0.174	0.965	ppa	Inorganic diphosphatase	Phosphorus	Inorganic P-solubilization and organic P-mineralization	0.961	Multi
0.973	-0.194	lccA	Laccase	Carbon	Phenols and similar aromatic compounds Oxidation	0.985	Multi
0.971	0.165	PO	Peroxidase	Carbon	peroxidation of phenolic and non-phenolic substrates	0.970	Multi

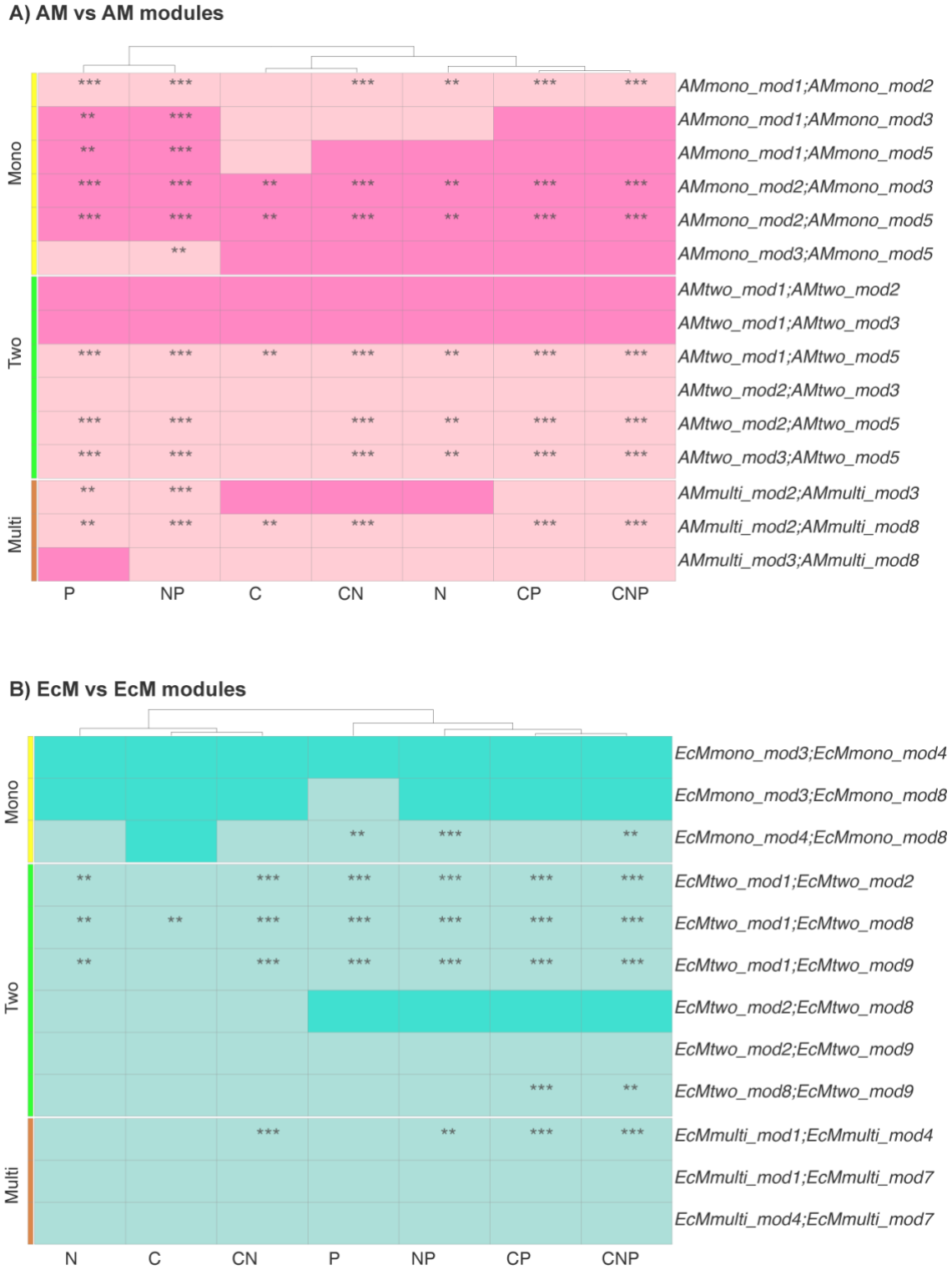


Figure S III.1: Heat map of pairwise comparisons of significantly soil-responding modules within the tree mycorrhizal type along the tree diversity levels. (A) Modules of AM tree mycorrhizal type. (B) EcM tree mycorrhizal type. The asterisks show the p-value significance level, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p≤ 0.0001.

Chapter IV: Phylogeny- and taxon abundance-based comparative analysis provides insights into the root-associated fungal communities of Arbuscular and Ectomycorrhizal tree species across forest tree diversity levels

A modified version of this chapter has been published in the New Phytologist journal as

Singavarapu B, Haq Ul H, Darnstaedt F, Nawaz A, Beugnon R, Cesarz S, Eisenhauer N, Du J, Xue K, Wang Y, Bruelheide H & Wubet T (2024). Influence of tree mycorrhizal type, tree species identity, and diversity on forest root-associated mycobiomes. New Phytologist, 242, 1691–1703.

https://doi.org/10.1111/nph.19722

Abstract

Both ecological and evolutionary forces are of paramount importance in shaping the plant root-fungal associations, and yet little is known about the effects of tree species identity, tree mycorrhizal type and tree diversity on the root fungal communities including their phylogenetic relationships. To address this, we studied different arbuscular (AM) and ectomycorrhizal (EcM) conspecific tree species pairs (TSPs) in a subtropical tree diversity experiment across three tree diversity levels comprising monospecific stands, two- and multi-tree species mixtures. Root-associated fungal communities were determined by paired-end Illumina sequencing of the fungal internal transcribed spacer (ITS2) region, followed by taxon relative abundance and phylogeny-based comparative analyses on fungal communities. We found a significant effect of the tree mycorrhizal type on the observed alpha and phylogenetic diversities of root-associated fungi in monospecific stands but not in the multi-species mixtures. In addition, there was a significant main effect of tree species identity and interactive effects of tree species identity and tree diversity levels. Overall, the root fungal communities were shaped by tree species identity, tree mycorrhizal type, tree diversity, the interactions between tree mycorrhizal type and tree diversity as well as between tree diversity and tree species identity. Moreover, these factors explained more variation in fungal community composition based on phylogeny based (37%) compared to that based on taxon abundance (27%). In multi-species mixtures, both taxon abundance and phylogeny-based compositional differences in root fungal communities between AM and EcM tree species disappeared. Variation partitioning analysis showed that tree-related variables explained the highest amount of variation in fungal phylogenetic community composition compared to abiotic variables, whereas that of abundance-based fungal community composition was firstly explained by spatial distance. Collectively, our results indicate that tree species identity, tree mycorrhizal type and tree diversity are important factors determining the diversity and composition of plant root-associated fungal communities in forests. In particular, accounting for phylogenetic relationships of root fungi provide deeper insights into the fungal community assembly.

Introduction

Root-associated fungal communities play versatile roles such as promoting the growth and survival of the plants, influencing the plant community functioning by modulating the

plant–plant belowground interactions and contributing to the biogeochemical cycling by mediating plant-soil feedbacks (Clemmensen et al., 2013; Chen et al., 2018; Almario et al., 2022). It is known that the diversity and composition of the root-associated fungal communities are influenced by many biotic and abiotic factors. One of the important biotic components is the identity of the host plant species. A plant can selectively recruit root-inhabiting microbes, for example, through specific rhizodeposition (Eisenhauer et al., 2017). Host-fungi specificity is brought about by shared co-evolutionary histories (Hoeksema et al., 2018). Nearly 80% of vascular plant species are estimated to have symbiotic associations with particular root fungi, known as, arbuscular (abbreviated as 'AMF') and ectomycorrhizal fungi (abbreviated as 'EMF') through which plant carbon is exchanged for nutrients such as phosphorus and nitrogen provided by mycorrhizal fungi (Brundrett and Tedersoo, 2018). Previous studies reported positive relationships between the abundance of plants with a given mycorrhizal type and that of their symbiotic mycorrhizal communities, i.e., ectomycorrhizal associated plants (abbreviated as 'EcM plants') being associated with EMF and arbuscular mycorrhizal associated plants (abbreviated as 'AM plants') with AMF (Gao et al., 2013; Neuenkamp et al., 2018). Furthermore, plant mycorrhizal partners can significantly influence the diversity and composition of the opposing root-associated fungal communities (Toju et al., 2014; Ferlian et al., 2021; Heklau et al., 2021). However, research shows that plant species diversity effects on root-associated fungal communities are not unanimous. For instance, some studies reported the absence of any significant effects of plant richness on root fungal communities (Navratilova et al., 2019; Otsing et al., 2021). Nevertheless, the prevailing pattern shows significant positive effects of plant richness on fungal richness (Gao et al., 2013; Ferlian et al., 2021; Mony et al., 2021). In a study highlighting the differences between soil microbial communities under AM and EcM tree species, Singavarapu et al., 2021 reported significantly higher fungal diversity under the AM than that of EcM tree species in monocultures. Furthermore, it was shown that with increasing plot tree diversity the differences in microbial diversity and composition between AM and EcM tree species became non-significant. In the case of forests, trees might only grow in conspecific stands, which should favor symbiotic associations with their corresponding mycorrhiza type, or in mixtures of varying diversity, which also includes mixtures of tree species with opposing mycorrhiza type. We can only get a comprehensive understanding of how the root-inhabiting fungi are structured in these different host species compositions when they were analyzed in parallel rather than individually. However, such available comparative studies

are very limited and there is a necessity to study the effects of tree mycorrhizal type, and tree species identity in differing tree diversity levels on the root-associated fungi to get better insights into their community assembly and consequently their active role in ecosystem functioning.

One of the salient features driving the host–microbe interactions are their evolutionary relationships which can be studied by phylogenetic analysis for example, (Hoeksema et al., 2018) in their meta-analysis reported that evolutionary history explained a larger amount of variation than ecological factors in plant responses to mycorrhizal fungi. Moreover, the study identified different outcomes of EMF versus AMF symbioses that were differentially influenced by evolutionary history. Phylogenetically related plants tend to have similar characteristics, such as morphological and physiological traits, and thus, might recruit similar root-associated fungi (Koyama et al., 2019). Previous research showed that host plant phylogeny explained a major proportion of the variation in their root-inhabiting fungal communities (Tedersoo et al., 2013; Wehner et al., 2014; Wang et al., 2019). Nonetheless, research has also shown that the role of abiotic factors is at least equally important in shaping root-inhabiting fungal communities. For example, the spatial component in the environment was shown to be the major determinant than plant community in structuring AMF assemblages (Horn et al., 2017). Similarly, in one study, the slope aspect of the site was reported to induce differential phylogenetic clustering of AMF communities via modifying the microclimate (Chai et al., 2018), and in another study, strong abiotic environmental filtering provided by the soil environment was reported to shape the root endophytic fungal communities (David et al., 2016). Furthermore, in a recent study, (Zhu et al., 2022) used plant-fungal association networks and reported that fungal affiliation to the network modules was determined by fungal phylogeny indicating the importance of fungal phylogenetic information in regulating the assembly of root fungal communities. These examples show that including phylogenetic information in ecological analyses might greatly improve our understanding of microbial community assembly. However, studying the effects of tree species identity, tree mycorrhizal type and tree diversity in combination with the root-associated fungal community phylogenetic structure has not been attempted yet.

To fill this research gap, we utilized the BEF-China experimental research platform which is the largest tree diversity experiment worldwide (Bruelheide et al., 2014). We assumed

phylogeny-based analyses provide more information since they consider the evolutionary plant-fungal and fungal-fungal relationships along with ecological links. High tree diversity involves complex plant and microbe high-order interactions and can promote species co-existence for instance by nutrient partitioning (Luo et al., 2018) and also diverse neighborhoods to the focal plant can share diverse belowground fungal species pool (Mony et al., 2021). Based on this, we further assumed diluted effects of tree mycorrhizal type and tree species identity at higher tree diversity levels. More specifically, we have hypothesized that

(H1) AM and EcM tree species differ in their observed alpha- and phylogenetic diversity indices with higher diversity in AM than that of EcM tree species. Further, we expected that the effects of tree mycorrhizal type and tree species identity on the alpha and phylogenetic diversity measures decreases with increasing tree diversity.

(H2) Root-associated fungal community composition differs between AM and EcM tree species with stronger effects of tree mycorrhizal type, tree species identity and tree diversity on the fungal community composition based on phylogeny than that of the abundance of taxa. We also expected that the fungal communities between the AM and EcM tree communities and tree species pairs tend to be phylogenetically and taxonomically similar with increasing tree diversity levels.

(H3) The effects of soil, spatial, topographic and tree community-related variables on shaping the root-associated fungal community composition differ between AM and EcM tree species. Moreover, we expected to encounter larger differences in analyses based on phylogenetic distance than in those based on taxon abundance.

Material and methods

Study site, experimental design and sampling

For detailed descriptions of the study site, experimental design and soil characteristic analyses, please refer to ‘Materials and Methods’ section of Chapter II, as well as Singavarapu et al., 2021. For this study, we focused on the conspecific TSPs including six EcM and six AM type tree species pairs (Figure S IV.1) From both adjacent target trees of each TSP (n=108), root samples were collected resulting in a total of 216 samples with the following six combinations: ‘EcM|Mono’(n=36), ‘EcM|Two’(n=36), ‘EcM|Multi’(n=36),

‘AM|Mono’(n=36), ‘AM|Two’(n=36) and ‘AM|Multi’(n=36). Fine root samples were taken from each of the two trees in the TSP in the location between those two trees, called as the tree-tree interaction zone (i.e., the horizontal axis between the two TSP partners). Rhizosphere soil attached to the roots was gently removed by shaking and squeezing and further the fine root samples were washed for 3 min in 400 ml sterile water that contained 0.01 % Tween™ 80 (Merck KGaA, Germany) to efficiently remove remaining soil particles and externally bound epiphytic microbes. Subsequently, two more washing steps with 400 ml of sterile water were performed to remove the remaining traces of the detergent. Finally, the washed roots were surface sterilized in 70 % ethanol for 2 min and transferred into 5 ml tubes filled with 70 % ethanol and stored at -20 °C until DNA extraction.

DNA extraction, amplicon library preparation, and sequencing

Before extraction, 25 - 50 mg fine root material was dried at 40 °C for 20 h in the drying oven. The dried roots were crushed using metal beads from Retsch with a diameter of 5 mm and the vibration mill “MM 300” (Retsch® GmbH, Germany) with run times of 2 - 3 minutes at 30.000 Hz. Genomic DNA was extracted from the crushed root material using the DNeasy® Plant Mini Kit (QIAGEN, Netherlands) following the manufacturer's protocol. DNA concentrations were measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Dreieich, Germany), and the extracts were adjusted to 10 ng/µl template concentration. The fungal amplicon libraries were prepared as previously described in Singavarapu et al. (2021). Briefly, semi-nested PCR was employed to amplify the ITS2 rDNA region using the initial primer combination of ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) followed by the primer pair fITS7 (Ihrmark et al., 2012) and ITS4 containing the Illumina adapter sequences. The fungal amplicon libraries were purified with Agencourt AMPure XP beads (Beckman Coulter, Krefeld, Germany). Illumina Nextera XT Indices were added to both ends of the bacterial and fungal fragments in the indexing PCR. The indexed products were purified again with AMPure beads and then quantified by PicoGreen assay. The amplicon libraries were pooled equimolarly to a final concentration of 4 nM and paired-end sequencing of 2x300 bp was performed on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, United States) using MiSeq Reagent kit v3 at the Department of Environmental Microbiology, UFZ, Leipzig, Germany.

Bioinformatics analysis

High-quality reads were extracted from raw reads generated by the Illumina MiSeq Sequencing platform as described in (Singavarapu et al., 2021)using the Quantitative Insights into Microbial Ecology – QIIME 2 version 2022.2 software (Bolyen et al., 2019). Following demultiplexing of forward and reverse reads based on index combinations, primer sequences were trimmed, followed by sequence denoising and grouping into Amplicon Sequence Variants (ASVs) using cut-adapt (Martin, 2011) (q2-cutadapt) and DADA2 (Callahan et al., 2016) via (q2-dada2) respectively. The q2-ITSxpress Qiime2 plugin (Rivers et al., 2018) was used to analyze the fungal ITS dataset, with the ITS2 fungal sequences being detected and trimmed, then denoised and grouped into ASVs using the DADA2 plugin. The q2-feature-classifier (Bokulich et al., 2018) was used to classify fungal ITS ASVs using the classify-sklearn naive Bayes taxonomy classifier against the unite-ver8-99-classifier-04.02.2020. The fungal phylogenetic tree was constructed using the best fit model GTR+F+R10 selected based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) using the align-to-tree-mafft-iqtree pipeline. The respective metadata, ASV matrices, taxonomic tables, representative sequences and phylogenetic tree were imported into R using the phyloseq package (McMurdie and Holmes, 2013) for further statistical analysis.

Statistical analysis

All statistical analyses and data visualizations were performed using R version 4.1.1 (R Core Team, 2020). The datasets were controlled for non-fungal ASVs and were rarefied to 7130 reads per sample. The fungal alpha diversity measures were calculated using the microbiome package (Lahti and Shetty, 2017). Faith's phylogenetic diversity was calculated using the btools package (Battaglia, 2018). The relationships between alpha- and phylogenetic diversity measures were tested with Pearson's correlations and visualized using the ggpubr package (Kassambara and Kassambara, 2020). Wilcoxon rank sum-tests were used to test for the tree mycorrhizal type effect on the observed richness, Shannon and phylogenetic diversity indices. A two-way ANOVA was used to test the main and interactive effects of tree species identity and tree diversity on the alpha and phylogenetic diversity indices. For this purpose, the data were checked for normality and homogeneity of variance using the Shapiro-Wilk test and Levene’s test, respectively and further Box-Cox transformed to meet the normality and homogeneity of variance assumptions using the

car package (Fox and Weisberg, 2018). Furthermore, the significant differences among the TSPs within each tree diversity level were first verified with Kruskal Wallis tests followed by the pairwise t-tests with Benjamini-Hochberg correction for multiple comparisons using the rstatix package (Kassambara, 2021). To test the main and interactive effects of mycorrhizal types, tree species identity and diversity levels on the root-associated fungal community compositional variation, non-parametric permutational ANOVA (PERMANOVA) analysis was performed with Bray-Curtis distance and weighted UniFrac phylogenetic distances using the 'adonis2' function of the vegan package (Oksanen et al., 2019), followed by a pairwise multilevel comparison using the 'pairwise.adonis' function of the pairwiseAdonis package with Benjamini-Hochberg correction for multiple comparisons (Martinez Arbizu, 2017). The pairwise distances were presented as a heatmap using the 'pheatmap' function of the ComplexHeatmap package (Gu et al., 2016). Distance-based redundancy analysis (dbRDA) ordination analysis was used to visualize the fungal community composition using the phyloseq package. The ASVs were agglomerated at the phylum level using the phyloseq package to visualize the relative abundance of the top 10 phyla across tree diversity levels of the two mycorrhizal types. The fungal functional groups were defined using FUNGuild (Nguyen et al., 2016a) and FungalTraits (Põlme et al., 2020) databases where we were able to assign about 47.9 % of ASVs identified in this study, excluding the NAs and guilds with a confidence ranking of " possible" as suggested by Nguyen et al., 2016. The relative abundances of the fungal functional guilds were presented at the tree diversity and tree species levels of the two mycorrhizal types using barplots. We used variation partitioning analysis to measure the contribution of soil, tree, topography and spatial characteristics on both the taxon abundance- and phylogeny-based fungal community compositional variation. The tree community variables as a biotic component included tree community composition, tree species identity, tree and shrub species richness, tree Shannon and Simpson diversity indices, abundance and richness of tree neighborhood, abundance and richness of neighbor AM and EcM TSPs. Soil characteristics (C, N, P, C/N, C/P, N/P, TOC, SOM, NH4+, NO3-, pH and moisture) and topographical variables (altitude, slope, northness, and eastness) were considered as abiotic components. Vectors from principal coordinates of neighborhood matrices (PCNM) computed from the sampling locations (latitude and longitude) were used to represent the spatial component (Dray et al., 2006). Prior to variation partitioning, significant variables from each of the four components were selected using distance-based redundancy analysis (dbRDA) models ('capscale' function in vegan) with stepwise model selection ('ordistep'

function in vegan). All graphs were created with the ggplot2 package (Wickham et al., 2016) or base R functions unless otherwise mentioned.

Results

The raw sequencing data output for the 216 samples included 3,559,881 reads in total. After quality filtering via denoising, merging, chimera and non-target taxa removal, 2,989,877 reads (84 %) remained and were then clustered to 6629 amplicon sequence variants (ASVs). All 216 samples were rarefied at 7130 reads per sample resulting in 6394 ASVs in the final dataset.

Observed alpha and phylogenetic diversity of root-associated fungi

The observed ASV richness and Shannon diversity were found to be significantly correlated ($R = 0.80$, $p < 0.001$). The fungal Faith's phylogenetic diversity index showed a stronger correlation with the fungal ASV richness ($R = 0.91$, $p < 0.001$) than with Shannon diversity ($R = 0.60$, $p < 0.001$) (Figure S IV.2). Analysis of the effect of the host tree's mycorrhizal type indicated a significant effect on the observed ASV richness and Shannon diversity but not on phylogenetic diversity (Figure IV.1A, C, E). However, the effect of mycorrhizal type was significant for all three indices in monospecific stands and ASV richness and Shannon diversity indices at two species mixtures. In contrast, any significant effect of mycorrhizal type was absent in the multi-species plots (Figure IV.1B, D, F). The responses varied between species as was revealed by subsequent two-way analysis of variance (ANOVA), showing a significant main effect of tree species and interactive effects of tree species and tree diversity level on fungal ASV richness, Shannon and phylogenetic diversity indices (Table IV.1). Further analysis of the role of tree species identity on the alpha and phylogenetic diversity measures within the tree diversity levels revealed that the number of significantly different tree species pairs declined with increasing tree diversity, according to Kruskal Wallis tests ($p < 0.05$) (Figure IV.2, Figure S IV.3). Although there were some significant differences within mycorrhizal types, the majority of the significant pairwise differences were encountered between AM and EcM tree species in both mono and two-species mixtures.

Table IV.1:

Two-way-ANOVA effects of tree species identity and tree diversity level on the fungal ASV richness, Shannon diversity and Faith's phylogenetic diversity indices

		Richness		Shannon		Faith's PD	
	Df	F	p value	F	p value	F	p value
Tree Diversity (D)	2	0.648	0.525	0.027	0.973	1.699	0.186
Tree species (I)	11	4.420	7.01E-06***	5.951	3.07E-08***	3.703	9.08E-05***
Interaction (D x I)	22	2.938	4.13E-05***	2.511	0.000461***	2.402	0.00084***

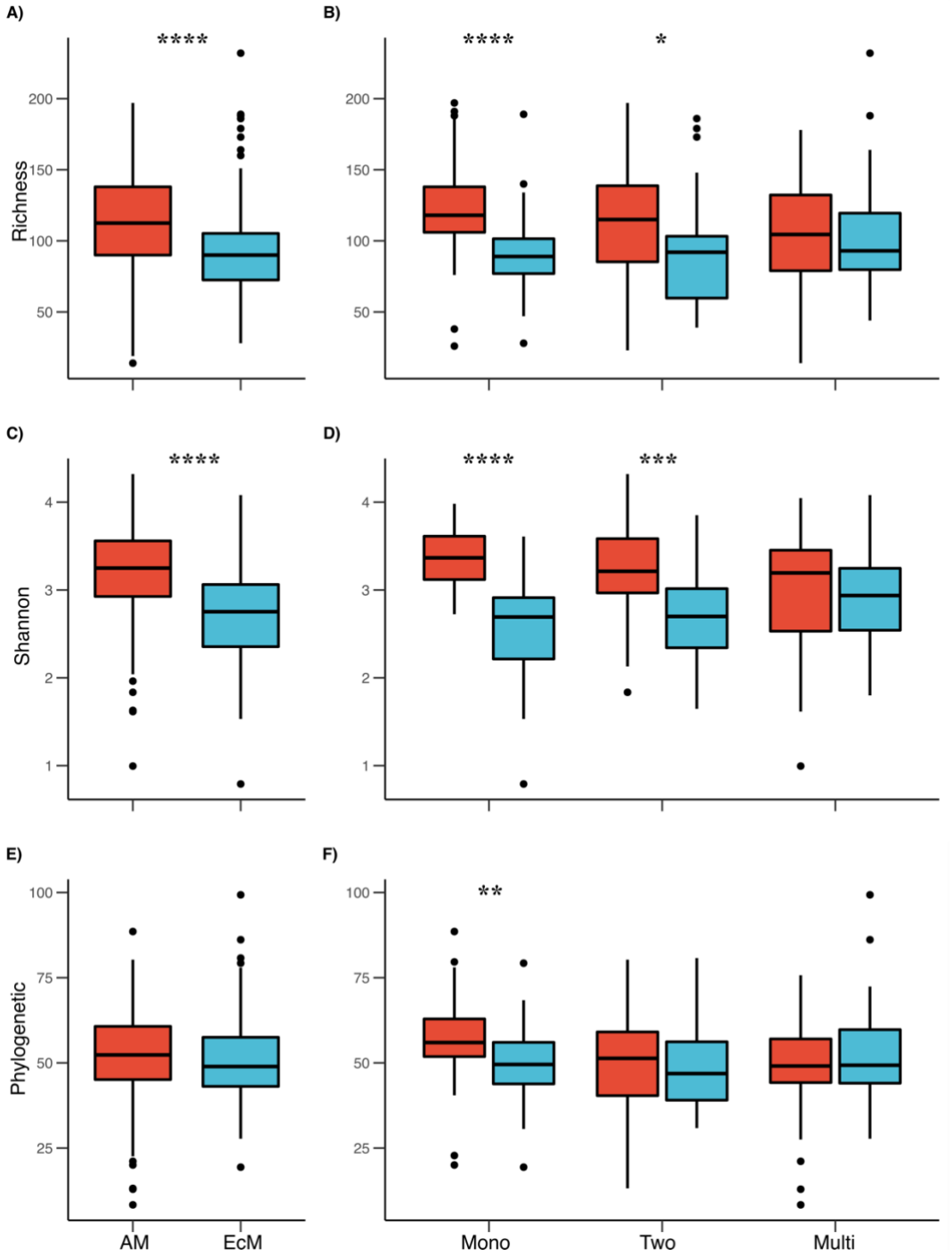


Figure IV.1 Alpha diversity indices of root-associated fungal communities. A), C), E): Comparison of fungal observed richness, Shannon and Faith's phylogenetic diversities, respectively between all EcM and AM TSPs. B), D), F): Within the tree diversity level differences between EcM and AM TSPs for the respective fungal alpha diversity measures. Abbreviations: Mono = monospecific stands, Two = Two species mixtures, Multi = Multi species mixtures. P values: '****' $p<0.0001$; '***' $p< 0.001$; '**' $p< 0.01$; '*' $p< 0.05$

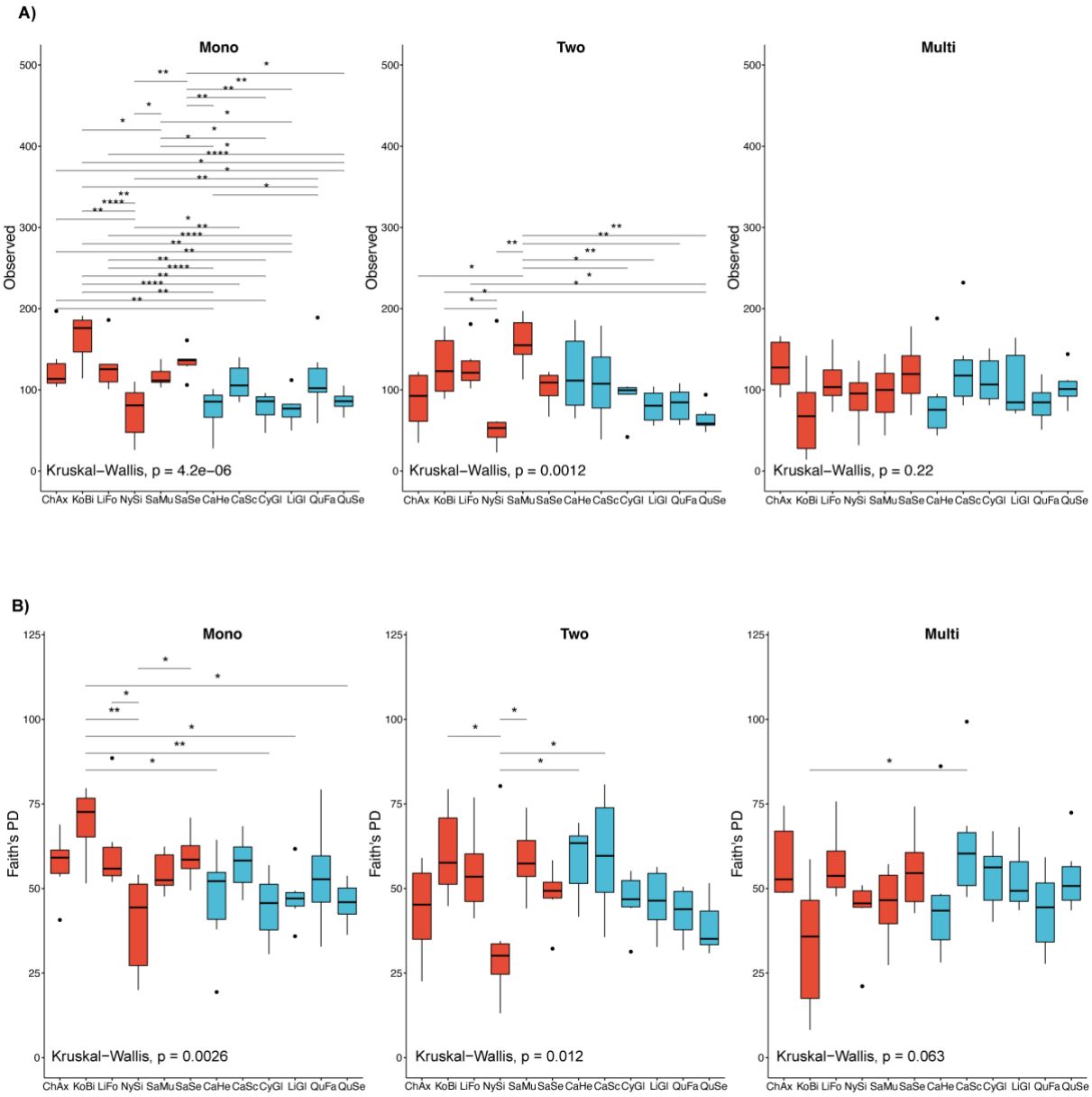


Figure IV.2 : Observed fungal ASV richness (A) and Faith's phylogenetic diversity (B) across the three tree diversity levels. Significant Pairwise t-test results and the respective Kruskal-Wallis-test p values were presented. Abbreviations: Mono = monospecific stands, Two = Two species mixtures, Multi = Multi species mixtures; ChAx = Choerospondias axillaris, KoBi = Koelreuteria bipinnata, LiFo = Liquidambar formosana, NySi = Nyssa sinensis, SaMu = Sapindus mukorossi, SaSe = Sapium sebiferum, CaHe = Castanea henryi, CaSc = Castanopsis sclerophylla, CyGl = Cyclobalanopsis glauca, LiGl = Lithocarpus glaber, QuFa = Quercus fabri, QuSe = Quercus serrata. BH adjusted P values: '****' p<0.0001; '***' p< 0.001; '**'p< 0.01; '*' p< 0.05; '.' p< 0.1.

Tree mycorrhizal type, tree species identity and tree diversity effects on root-associated fungal community composition

Analysis of the main and interactive effects of tree mycorrhizal type, tree diversity level and tree species identity on the root-associated fungal community composition revealed a significant effect of all three factors, and in addition, the interactions between tree mycorrhizal type with tree diversity and of tree diversity with tree species identity (Table IV.2). These effects were significant for the two distance measures used, that is both for those based on taxon relative abundance and phylogeny. In total, across all factors, the phylogeny-based analysis explained much more variance (37.33%) compared to that based on taxon abundance (26.57%). The main difference between these two measures was that the mycorrhiza type explained 10.6% in the analysis on distances based on phylogeny compared to 1.8% in that based on taxon abundance (Table IV.2). Consistent with the analysis on fungal diversity, the distance-based redundancy analysis (dbRDA) showed clearly separated fungal communities of AM and EcM tree mycorrhizal types in monospecific stands and two-species mixtures (Figure IV.3A and B), but not in multi-species mixture level. Here, fungal communities of both tree mycorrhizal types clustered closely together. This result was obtained for both the ordination analyses based on phylogeny and taxon abundance except that the first axis of the former explained more variance (58%) than that of the latter (45%) (Figure IV.3).

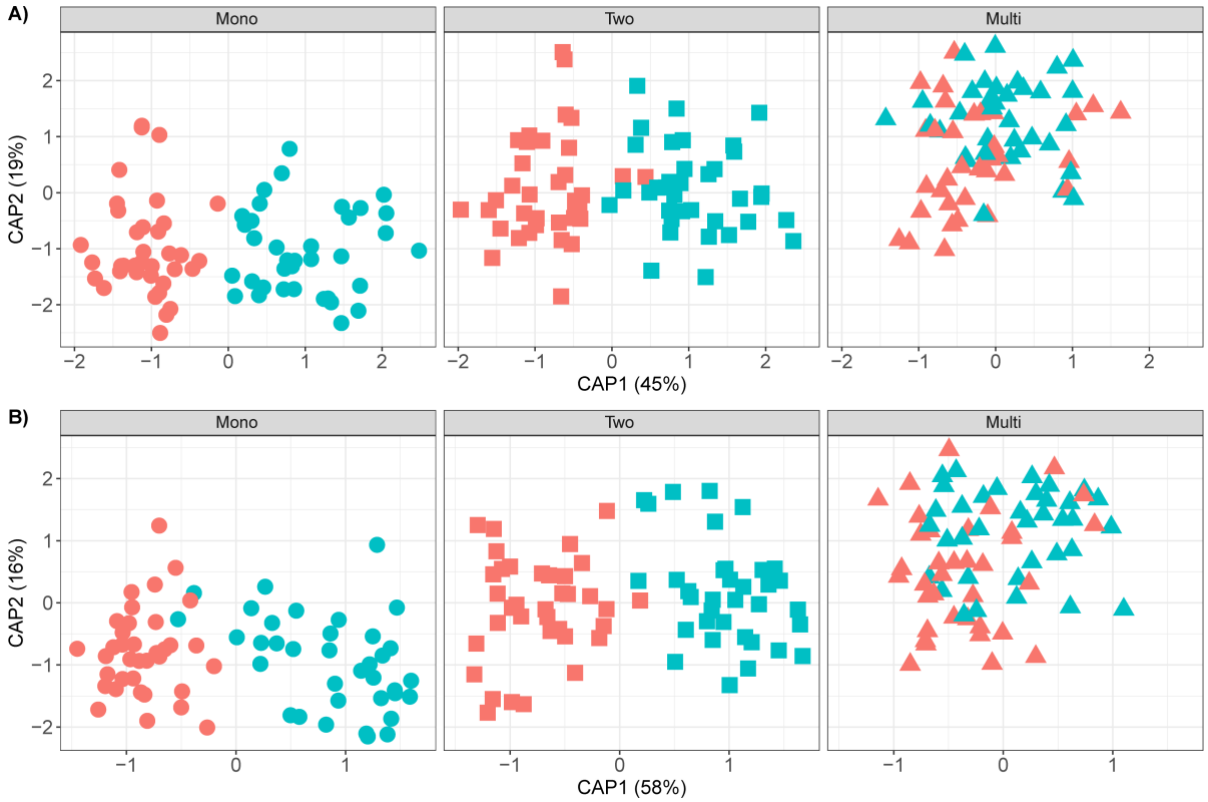


Figure IV.3 Ordination plots of Distance-based redundancy analysis (dbRDA) constrained by mycorrhizal type and tree diversity level using A) Taxon abundance-based Bray-Curtis distance and B) weighted UniFrac based phylogenetic distance matrices. Abbreviations: Mono = monospecific stands, Two = Two species mixtures, Multi = Multi species mixtures. Blue = ectomycorrhiza (EcM) samples, Red = arbuscular mycorrhiza (AM) samples.

Table IV.2:

Permutational multivariate analysis of variance (PERMANOVA) on root-associated fungal communities using Bray-Curtis distance for the taxon abundance-based and weighted UniFrac distance for the phylogeny based compositions. Abbreviations: Mycorrhizal Type (M), Tree Diversity (D), and Tree species identity(I).

		Taxon abundance			Phylogeny		
	Df	R^2	F	p	R^2	F	p
Mycorrhizal Type (M)	1	0.018	4.298	0.001***	0.106	30.31	0.001***
Tree Diversity (D)	2	0.014	1.776	0.001***	0.014	2.040	0.008**
Tree species (I)	10	0.089	2.193	0.001***	0.118	3.380	0.001***
(M x D) Interaction	2	0.015	1.791	0.001***	0.022	3.145	0.002**
(I x D) Interaction	20	0.130	1.588	0.001***	0.114	1.636	0.001***
Residual	180	0.734			0.627		

Tree species identity effects across the tree diversity levels

The strong tree species identity and tree diversity level interaction were further investigated using permanova in each of the tree diversity levels. Tree species identity explained a relatively low percentage of variance using the relative abundance data (30.8%, 26.3% and 19.4%) as compared to the phylogenetic distance-based analysis (44.6%, 38.6%, 25.6%) in mono, two and multi-species mixtures, respectively. Similarly, the contribution of tree mycorrhizal type to the tree species identity effect was also higher in the phylogenetic distance (17%, 16.7% and 4.8%) than the relative abundance-based analysis (4%, 3.8% and 1.9%) in mono, two and multi-species mixtures respectively. Furthermore, the pairwise permanova analysis resulted in 66 potential tree species pairs including 15 AM-AM, 15 EcM-EcM, and 36 AM-EcM pairs (Figure IV.4A-C). The analysis based on relative abundance indicated that all the 66 tested potential tree pairs significantly differed from each other in their fungal community composition in monospecific stands but not in the multi-species mixtures (Figure IV.4A-C). In the two species mixtures, 60 out of the 66 tree species pairs (including all the 36 AM-EcM, 14 AM-AM and 10 EcM-EcM tree species pairs) showed significantly different root fungal community composition (Figure IV.4A-C). The analysis based on phylogenetic distance showed the overall same patterns but differed in important details. In particular, in the EcM-EcM tree species pairs only 7 out of 15 pairs were found to host significantly different fungal communities in monospecific stands (Figure IV.4B). All the 15 EcM-EcM tree species pairs were found to share phylogenetically closely related fungal communities in both two and multi-species mixtures (Figure IV.4B).

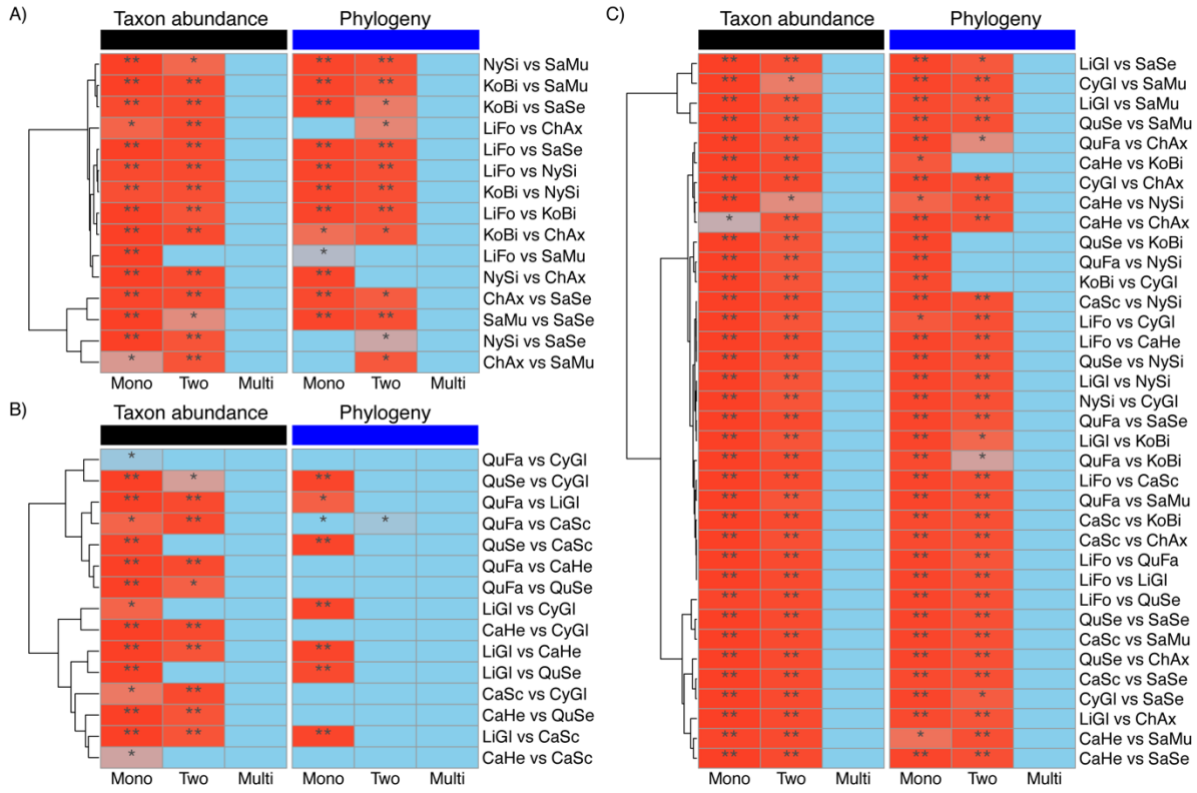


Figure IV.4 Heatmap based on pairwise permutational multivariate analysis of variance (PERMANOVA) to test effects of tree diversity on tree species root-associated fungal community composition based on taxon abundance (black) and phylogenetic distance matrices (blue) across the three tree diversity levels. AM tree species pairs (A), EcM tree species pairs (B) and AM-EcM tree species pairs (C). Abbreviations: Mono = monospecific stands, Two = Two species mixtures, Multi = Multi species mixtures; ChAx = Choerospondias axillaris, KoBi = Koelreuteria bipinnata, LiFo = Liquidambar formosana, NySi = Nyssa sinensis, SaMu = Sapindus mukorossi, SaSe = Sapium sebiferum, CaHe = Castanea henryi, CaSc = Castanopsis sclerophylla, CyGl = Cyclobalanopsis glauca, LiGl = Lithocarpus glaber, QuFa = Quercus fabri, QuSe = Quercus serrata. BH adjusted P values: '****' p<0.0001; '***' p< 0.001; '**'p< 0.01; '*' p< 0.05; ' ' p>=0.05.

Root-associated fungal community taxonomic composition

The root-associated fungal communities were composed of nine fungal phyla, wherein three phyla namely, Ascomycota, Basidiomycota and Glomeromycota covered 99.6 % and 98.89 % proportions in AM and EcM tree species, respectively (Figure IV.5A). The AM trees were predominantly colonized by members of the phyla Ascomycota followed by Basidiomycota and then Glomeromycota, whereas the EcM trees were nearly proportionally colonized by members of the phyla Ascomycota and Basidiomycota. In general, AM tree species had 20.62% more Ascomycota and 7.1% more Glomeromycota fungal ASVs than the EcM tree species. However, the proportion of Basidiomycotan ASVs was 27.02 % lower in AM compared to EcM trees. For AM tree species, there was a slight

increase of Basidiomycota with increasing tree diversity, while Ascomycota showed slightly decreasing proportions in multi-species mixtures compared to monospecific stands and two-species mixtures. Glomeromycota covered the highest share in monospecific stands and decreased consistently from monospecific stands to two-species mixtures and multi-species mixtures. For EcM tree species, the proportions of Ascomycota and Basidiomycota were almost not affected by tree diversity (Figure IV.5A).

Root-associated fungal community functional composition

A total of 3063 ASVs accounting for 47.9 % of the 6394 ASVs identified in this study were assigned to seven trophic modes. Out of those, 1642 ASVs (53.6%) were exclusively assigned to the three major trophic groups of symbiotroph (770 ASVs), saprotroph (747 ASVs) and pathotroph (125 ASVs) lifestyles, accounting for 25.1%, 24.4% and 4.1%, respectively, of the assigned ASVs (Figure IV.5B). The remaining 1421 ASVs (46.4%) were assigned to more than one trophic mode. In the AM tree species, fungi with saprotrophy either as their main trophic mode or as one of their lifestyles were the predominant functional groups with a total of around 79% relative abundance. On contrary, EcM trees harbored Symbiotrophs (31.7%) and Saprotroph-Symbiotrophs (28.8%) as the major functional groups in their roots (Figure IV.5B). The relative importance of these functional groups also changed across the tree diversity levels within the tree mycorrhizal types. For instance, in AM tree species, the combined relative abundance of Symbiotrophs and Saprotroph-Symbiotrophs increased from monospecific plots to multi-species mixtures after a decline in the two species mixtures whereas the opposite is the case for EcM trees (Figure IV.5B). A closer look into the symbiotrophs indicated that both the AM and EcM tree species were mainly colonized by their respective mycorrhizal fungal partners in the monospecific plots. An exception was *Sapindus mukorossi* which, as AM tree species, hosted also ectomycorrhizal fungi (Figure IV.5C). In the two species mixtures, dual colonization was observed in four of the AM and one of the EcM tree species, whereas dual mycorrhization occurred in all species when they grew in the multi-species mixtures. However, the degree to which AM or EcM tree species were associated with their contrasting mycorrhizal partner varied from species to species, with the AM tree species *Liquidambar formosana* and *Koelreutheria bipinnata* hosting even more ectomycorrhizal than arbuscular mycorrhizal fungi (Figure IV.5C).

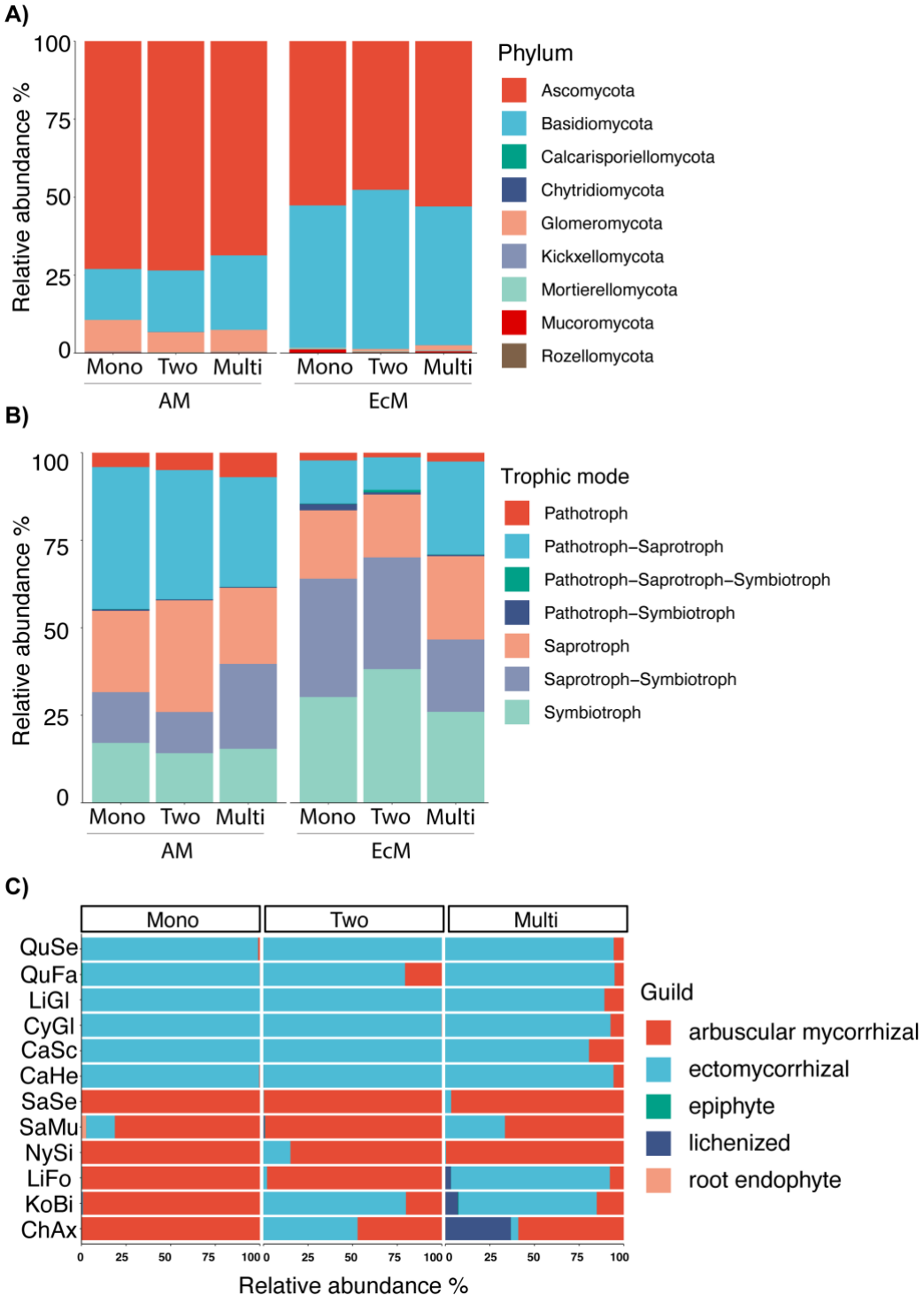


Figure IV.5 Taxonomic and functional composition of root associated fungal communities across tree diversity levels. (A) Phylum level taxonomic composition of AM and EcM tree species, (B) trophic mode level functional composition of AM and EcM tree species, (C) functional guild level composition of symbiotrophs of all tree species. First six EcM tree species followed by six AM tree species.

Biotic and abiotic components shaping the root-associated fungal communities

Variation partitioning analysis unravelled the contribution of soil, tree community, topography and spatial components on the root-associated mycobiome composition. Overall, more variation was explained by phylogenetic distance-based analysis compared to relative abundance-based analysis (Figure IV.6A and D). However, the opposite pattern was found in the EcM dataset (Figure IV.6C and F). Across all tree mycorrhiza types, the relative abundance-based analysis showed that the fungal community composition was mainly influenced by the tree (10.0%), spatial (9.6%) and soil (2.2%) related variables, while their shared contribution was always <1% (Figure IV.6A). In EcM tree species, soil characteristics were more important (5.3%) than in AM tree species (2%). The pattern based on phylogenetic distance revealed similar patterns (Figure IV.6D-F), with, however, a stronger contribution of tree community-related variables.

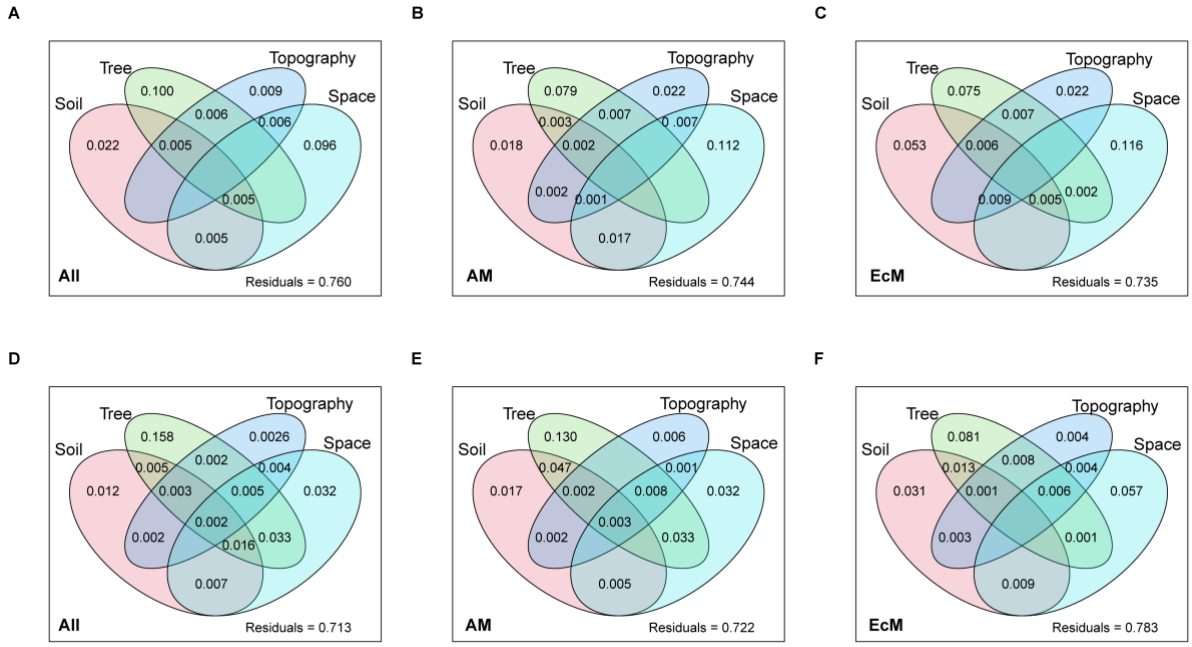


Figure IV.6 Variance partitioning analysis depicting the contribution of soil, tree community variables, topography and spatial parameters on the overall (A and D), AM tree species (B and E) and EcM tree species (C and F) root associated fungal community based on taxon relative abundance-based Bray-Curtis's distance (A, B, and C) and weighted-UniFrac phylogenetic distance (D, E, and F) based analysis

Discussion

High tree diversity dilutes tree mycorrhizal type and tree species identity effects on the observed alpha and phylogenetic diversities of root-associated mycobiota

As expected, (H1) we found significant effects of the tree mycorrhizal type on the alpha diversity (richness and Shannon diversity) of root fungi and a higher diversity in AM than that of EcM tree species. In line with our findings, Heklau et al. (2021) reported higher fungal richness in AM tree species roots compared to that of EcM in a temperate forest experiment. From the same experiment, Ferlian et al. (2021) reported higher AMF in AM and higher EMF in EcM tree species roots, pointing out the importance of the tree mycorrhizal type on root fungal diversity. In addition, Ferlian et al. (2021) also reported the effects of tree species identity and the neighbor tree mycorrhizal types on the AMF and EMF phylogenetic diversity, which fully complies with our results. In our study, the observed high fungal alpha diversity in AM tree species roots can be attributed to the highly diverse fungal species reservoir available in the soils of AM trees (Singavarapu et al., 2021). This might be because, in AM tree-dominated habitats, saprophytic fungi were known to facilitate decomposition and faster nutrient cycling which in turn could support the high fungal diversity compared to the slower decomposition rates associated with habitats dominated by EcM fungi (Midgley et al., 2015; Jacobs et al., 2018; Tedersoo and Bahram, 2019). Moreover, it was shown that phylogenetically more distant trees can harbor more diverse fungal communities (Tedersoo et al., 2013; Cheng and Yu, 2020). In our study, the AM tree species belonged to five different families which are phylogenetically distant, which might explain the high number of significant pairwise comparisons of tree species identity effects on the fungal alpha diversity within AM trees. Additionally, we found that the effects of both tree mycorrhizal type and tree species identity were dependent on the tree diversity level, as all effects that were significant in mono-specific stands or two-species mixtures disappeared in multi-tree species mixtures, confirming the second part of our hypothesis (H1). In general, higher plant diversity facilitates diverse microhabitats, providing divergent niche opportunities and different substrate resources for numerous soil microbes (Hooper et al., 2000; Waldrop et al., 2006). This view is further supported by the higher diversity of fungal taxonomic and functional groups in multi-tree species mixtures in our data and also explains the decreasing importance of tree

mycorrhizal type and tree species identity effects on the observed alpha and phylogenetic diversity. The positive tree diversity effect is also seen in the significant interaction of tree species identity with tree diversity on root fungal diversity of EcM tree species. Both tree mycorrhizal type and tree species identity had generally smaller effects on phylogenetic diversity than on observed alpha diversity. It is known that not only plant root symbionts but also other root-associated fungal communities have co-evolved with plants (Heilmann-Clausen et al., 2016; Zhu et al., 2022). This highlights the importance of considering the phylogeny for plant-associated microbiota studies to better understand the eco-evolutionary dynamics with regard to plant-microbe diversity relationships. This might have further functional implications, which cannot be captured simply by richness and diversity estimates. As demonstrated by Maherali and Klironomos et al. (2007), the increased fungal phylogenetic diversity (here, in EcM tree species) in our multi-tree species mixtures could have a positive effect on ecosystem functioning. In support, a previous experiment of suppressing fungi associated with both AM and EcM tree species, (Yang et al., 2022) showed the key role of fungi in mediating the observed positive tree diversity–plant productivity relationship at our study site.

Phylogenetic relationships explain more variation in the root fungal community composition than taxon abundance

Confirming our hypothesis (H2), root fungal community composition differed between AM and EcM tree species, which was revealed by both analyses based on relative taxon abundance and phylogeny. Overall, the analyses carried on with phylogenetic distances showed stronger effects of tree mycorrhizal type and tree species identity on the root fungal community composition than those based on relative taxon abundance. These findings corroborate the reports from tree species-associated root fungal communities (Kuang et al., 2021; Otsing et al., 2021), tree mycorrhizal type effects on root fungi (Toju et al., 2014; Heklau et al., 2021) and relationships between root fungal community composition and tree species richness (Gao et al., 2013; Heklau et al., 2021) (but see, Otsing et al., 2021). Our results also confirm those of other studies that considered fungal phylogeny on plant identity effects (Wubet et al., 2009; Zhu et al., 2022), and plant functional traits (e.g., photosynthetic traits and mycorrhizal statuses) effects on mycorrhizal fungal communities (Davison et al., 2020; Zhu et al., 2022). A key new finding of our comparative analysis between analyses based on relative taxon abundance and phylogeny was that the amount

of explained variation in fungal community composition was higher when being phylogeny-based. This points to a pivotal role of the evolutionary relationships of root-associated fungal taxa with their tree hosts. This was also evident both in lower phylogenetic diversity in EcM compared to AM trees and in a smaller number of significant differences found between EcM TSPs compared to those between AM TSPs in the pairwise analysis of TSPs' root fungal community composition in monospecific stands and two-tree species mixtures. Ectomycorrhizal symbiosis originated multiple times (ca. ≥80 times) around 190-200 million years ago across multiple lineages of Mucoromycota, Ascomycota and mainly Basidiomycota (Miyauchi et al., 2020). Despite their repeated convergent evolution which results in genetically diverse and functionally similar associations, EMF share broad genetic similarities and are also phylogenetically very close to their saprotrophic ancestors (Miyauchi et al., 2020; Strassert and Monaghan, 2022). Predominantly high abundances of EMF together with the fungal taxa with saprotrophic lifestyle in the root fungal communities of EcM TSPs explains the lower number of significant differences found between EcM TSPs. Furthermore, we assumed that EMF affected the recruitment of other non-mycorrhizal fungal taxa, particularly the saprotrophs through competitive interactions and preferential selection (Churchland and Grayston, 2014; Fernandez and Kennedy, 2016). In contrast, the fungal phylogeny places the AMF, which belongs to the phylum Glomeromycota, in a clade distant to Dikarya (i.e., both Ascomycota and Basidiomycota) and between the Dikarya and Mucoromycota (Strassert and Monaghan, 2022). Probably owing to their limited genomic potential in breaking down organic compounds, AMF were reported to have cooperative interactions with saprotrophs (Verbruggen et al., 2017; Miyauchi et al., 2020). Therefore, they might positively influence the recruitment of various fungal taxa with the saprotrophic lifestyle which was apparent in the root fungal community functional guild composition of AM TSPs. Altogether, these phylogenetically distant taxa comprising Ascomycota, Basidiomycota and Glomeromycota explain the higher number of significant differences found within AM TSPs and between AM and EcM TSPs in monospecific stands and two-tree species mixtures. Furthermore, confirming the second part of hypothesis H2, the fungal communities between the AM and EcM trees converged in multi-tree species mixtures as shown by the ordination and pairwise permanova analyses. This convergence is facilitated by the cooccurrence of different tree species with opposing mycorrhizal types (Singavarapu et al., 2021). This also explains the absence of tree mycorrhizal type and tree species identity effects in multi-tree species mixtures. Besides, it was reported that a phylogenetically and taxonomically

diverse neighborhood can provide a larger fungal species pool for any of the co-occurring tree species (Cheng and Yu, 2020; Mony et al., 2021).

Analyzing the same tree species' root fungal communities at different tree diversity levels provided very intriguing findings. First, the observed niche conservatism in monospecific stands (i.e., co-occurrence of closely related species) observed in the root fungal communities of tree species of both tree mycorrhizal types (but prominently in EcM tree species) was replaced with phylogenetic dispersion in multi-tree species mixtures. While this might seem a contradiction to the phylogenetic niche conservatism (i.e., the tendency of species to maintain their ancestral traits) of root-associated fungi, it might be explained by the new niche opportunities provided by the different tree species (also, of different mycorrhizal partners) co-occurring in multi-tree species mixtures (Wiens and Graham, 2005).

A second key finding was the dual mycorrhization in the roots of both EcM and AM tree species in multi-tree species mixtures, whereas, as expected, they hosted only their respective mycorrhizal partners (i.e., EMF on EcM plants & AMF on AM plants) in monospecific stands. However, despite the careful preparation of our root samples, there is still the possibility of contamination by e.g., spores from the opposing mycorrhiza type. To exclude this, we would have had to carry out morphological identification (Heklau et al., 2021). However, our sequencing-based identification and its taxonomic resolution clearly demonstrate that dual mycorrhization was almost totally absent in monospecific stands. While dual mycorrhization in both EcM and AM plants has been reported in the literature (Teste et al., 2020; Heklau et al., 2021), causal mechanisms are still poorly understood. Our results underline the importance of ecological interactions that might be precursory to evolutionary changes in plant traits (here, plant mycorrhizal status). Using populations of the same native plant species grown in prairies and post-agricultural grasslands, (Delavaux and Bever, 2022) provided evidence for the evolution of differential growth responses to different AMF taxa and shift in their co-evolutionary trajectories. Furthermore, the capacity for facultative biotrophy was reported for saprotrophs when they developed EcM-like structures such as mantle and Hartig net while colonizing the conifer seedling roots in vitro (Smith et al., 2017). These observations suggest that changes in ecological interactions as brought about by mixing host trees can induce rapid shifts in the co-evolutionary relationships between plants and their associated microbes.

Differential contribution of biotic and abiotic factors in driving the assembly of root-associated fungal communities

We had hypothesized (H3) that the contribution of soil, spatial, topographic and tree community variables differ in explaining the variation in root-associated fungal community composition and furthermore, that the phylogenetic distance-based analysis provides better resolution than that based on relative taxon abundance. We found confirmation for this second part of the hypothesis across all host species and for AM trees but not for EcM trees, for which relative taxon abundance explained more variation than phylogeny. A probable explanation for the higher explanatory power of phylogenetic rather than taxonomic community composition might be the co-evolutionary relationships between fungi and their hosts which we discussed above. This might also explain why the trees' mycorrhiza type was such an important predictor for fungal community composition in all analyses of both AM and EcM tree species. Concordant with our results, the importance of plant-related variables such as functional traits, phylogeny and neighborhood in shaping the root-associated fungal communities has been emphasized before (Wang et al., 2019; Cheng and Yu, 2020; Zhu et al., 2022). An even more important predictor for structuring the root-inhabiting fungal communities was spatial distance, which confirms findings from other studies (Toju et al., 2014; Horn et al., 2017; Koyama et al., 2019). However, there was only a very small amount of jointly explained variance with tree mycorrhizal type, which shows the strengths of our experimental design that made sure that these factors were independent of each other. The high importance of spatial distance might be brought about by the fungal spore dispersal ability. Dispersal limitation had stronger effects in the analyses based on taxon abundance than in those based on phylogeny. Another explanation for the importance of space might be unmeasured variables in our study that were captured by space (Horn et al., 2014). Furthermore, soil variables explained more variation in root-associated fungal communities of EcM than that of AM tree species. This observation is also in agreement with previous research demonstrating the importance of soil chemistry structure for EcM assemblages (Nguyen et al., 2020). All in all, our analyses highlighted the differential contribution of biotic and abiotic factors in driving the assembly of root-associated fungal communities of EcM and AM tree species suggesting the importance of understanding the underlying ecological and evolutionary relationships.

Conclusions

Using a comparative approach, we obtained unprecedented insights into the structure of root-associated fungal communities of EcM and AM tree species across tree diversity levels. Our study affirmed the significance of evolutionary relationships among fungal taxa and co-evolutionary relationships with their hosts in the root-associated fungal community assembly. Furthermore, observation of dual mycorrhizal communities in multi-tree species mixtures highlights the importance of ecological interactions in promoting the evolutionary shifts in plant–fungal associations. Borrowing evolutionary biologist Theodosius Dobzhansky's words "Nothing in biology makes sense except in the light of evolution" (Dobzhansky, 1973) to our study's context, we recommend the need for considering phylogenetic information to better understand the plant–microbe relationships and their functional consequences. Moreover, our study warrants further studies in exploring the mechanisms of fungal phylogenetic diversity and plant-productivity relationships and also studying the heritability of observed dual mycorrhizal traits for multiple generations.

Appendix S IV

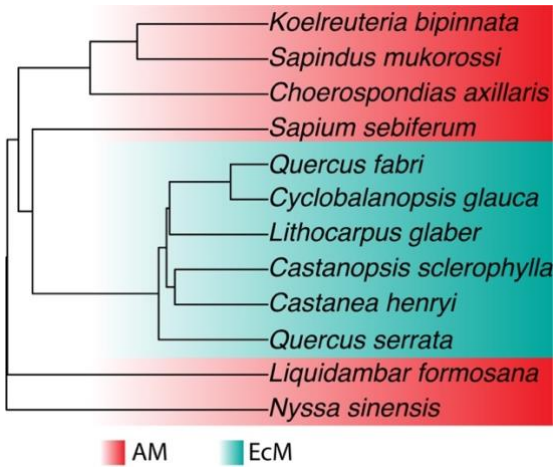


Figure S IV.1: Phylogenetic tree of the 6 AM and 6 EcM tree species used in the study. This is a pruned tree derived from the original phylogenetic tree from (Purschke et al., 2017).

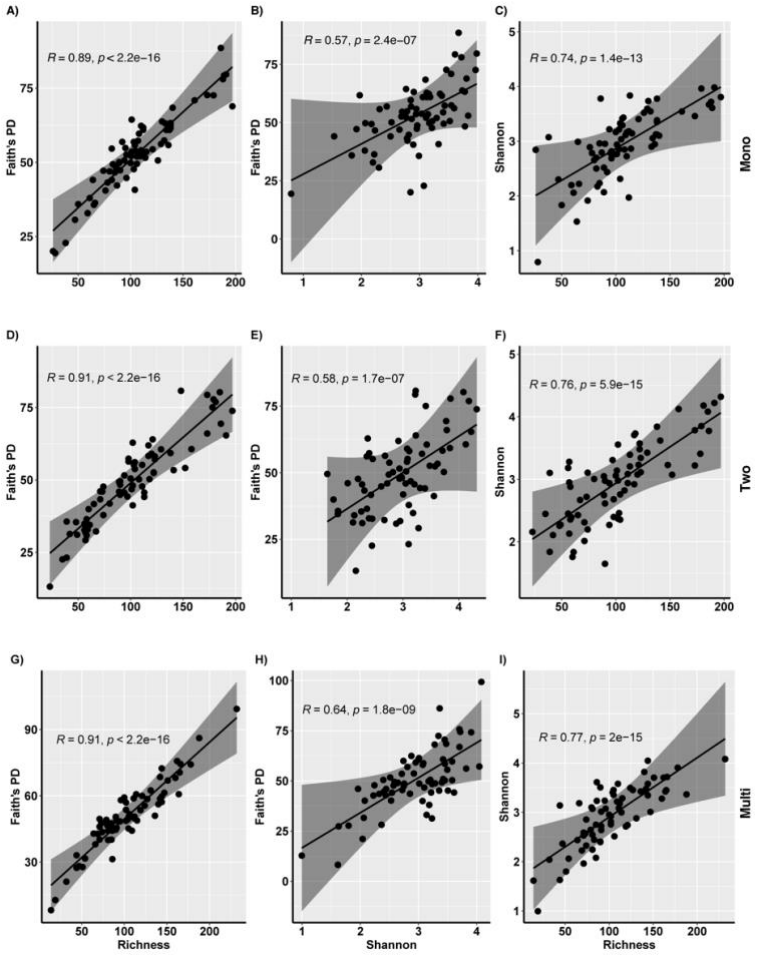


Figure S IV.2: Relationships of the fungal alpha and phylogenetic diversity indices across the tree diversity levels, illustrating strong and significant correlations. Monospecific stands (A – C), two-species mixtures (D – F), multi-species mixtures (G – I).

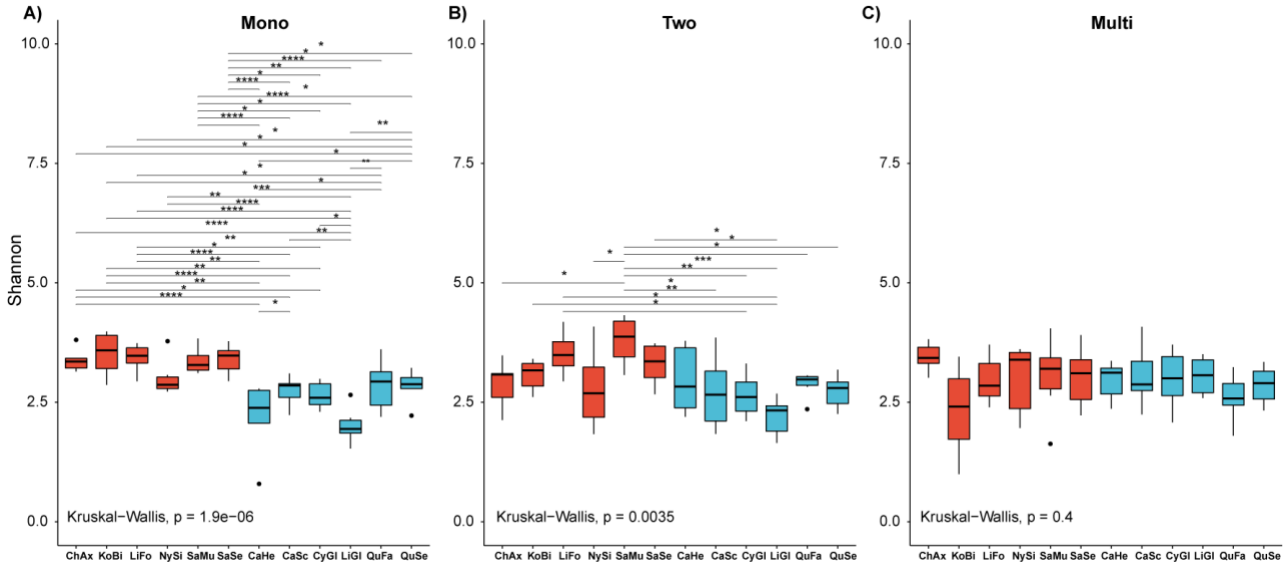


Figure S IV.3: Shannon diversity of fungal communities across the three tree diversity levels. Significant Pairwise t-test results and the respective Kruskal-Wallis-test p values were presented. Abbreviations: Mono = mono species mixture, Two = Two species mixture, Multi = Multi species mixture. BH adjusted P values: '****' p<0.0001; '***' p< 0.001; '**'p< 0.01; '*' p< 0.05; '.' p< 0.1.

Chapter V: General Discussion

It is largely unknown how the mixing of different mycorrhizal type tree species in varying diversity levels affects the belowground fungal and bacterial community structure, including their inter-kingdom network sub-communities and functional potential. In this thesis, I studied the effects of tree mycorrhizal type and tree diversity in association with site-specific environmental factors on the structure and functional potential of forest belowground bacterial and fungal communities. In this last chapter, I summarize the results, discuss the key findings and provide implications. Furthermore, I discuss the study's limitations and perspectives for future research.

Summary of Results

In **chapter II**, I proposed that the diversity and composition of soil microorganisms are affected by tree mycorrhizal type and tree species diversity. Specifically, I hypothesized that EcM Tree Species Pairs (TSPs) exhibit lower diversity than AM TSPs, and that diversity increases with increasing tree species diversity in both AM and EcM TSPs. Additionally, I hypothesized that the tree mycorrhizal type, tree diversity and site-specific environmental conditions affect the composition of the soil microbial community. As hypothesized, my findings revealed significant differences in fungal communities between tree mycorrhizal types, with lower diversity observed in EcM TSPs. A significant interaction between tree mycorrhizal type and tree diversity was detected, with diversity increasing only in EcM TSPs at higher tree diversity levels. These differences were present in monoculture and two-species mixtures, but not in multi-tree species mixtures. Furthermore, I found a significant main effect of tree mycorrhizal type and interactive effects of tree mycorrhizal type and diversity levels on fungal community composition. Additionally, a significant main effect of tree diversity was observed on bacterial communities. With increasing tree species diversity, differences in the fungal and bacterial communities between EcM and AM TSPs decreased, with the most influential taxa being no longer significant between tree mycorrhizal types in multi-tree species mixtures. In addition to tree mycorrhizal type and diversity, environmental factors including soil, spatial, topographic and tree community-related variables also showed significant and varying impacts on shaping the microbial communities.

In **chapter III**, I investigated the differences in EcM and AM TSP soil bacterial and fungal community co-occurrence network structures between tree diversity levels, focusing on their sub-communities and genomic functional potential for nutrient cycles (of C, N, P cycles and their combinations). I found that the distribution of centrality indices between EcM and AM networks differed at all tree diversity levels. In total, 21 out of 43 identified network sub-communities responded significantly to soil characteristics. Tree mycorrhizal type had a significant effect on functional diversity in all nutrient cycling combinations except for C, N, and the CN combination. There was a strong main effect of tree mycorrhizal type on genomic functional composition, with significant interaction effects with tree diversity for CP and CNP combinations. Notably, no significant effects of tree mycorrhizal type were detected in multi-tree species mixtures on the genomic functional potential. My findings indicated that variations in sub-communities were mirrored by their composition of different nutrient-cycling enzymes, particularly those of the P cycle. AM sub-communities with higher functional abundances were prevalent across tree diversity levels, except for enzymes associated with the C and N cycle. Within tree mycorrhizal type, AM sub-communities had a higher proportion of significant differences among them in their functional abundances compared to EcM. Finally, I identified the two most differentially abundant classes of bacteria and fungi that significantly contributed to the functional abundances of EcM and AM TSP soil microbial communities at each tree diversity level.

In **chapter IV**, I hypothesized that AM and EcM tree species differ in their alpha and phylogenetic diversity indices, with AM tree species displaying higher diversity. I proposed that AM and EcM tree species have distinct root-associated fungal communities, with stronger effects on community composition based on phylogeny than taxon abundance. With increasing tree diversity, fungal communities become more similar between AM and EcM tree species. I also expected the contribution of variables related to soil, space, topography and tree community to differ between AM and EcM tree species, with greater differences in analyses based on phylogenetic distance than on taxon abundance. I found that AM TSPs had higher alpha diversity, with significant effects of tree mycorrhizal type on ASV richness and Shannon diversity, but not phylogenetic diversity. In monospecific stands, the effect of tree mycorrhizal type was significant for all three indices, but not in multi-species plots. In addition, the number of significant differences among tree species decreased with increasing tree diversity. Further, I found significant effects of all three

factors on root-associated fungal community composition, with phylogeny explaining more variance than taxon abundance. Tree mycorrhizal type and species identity had stronger effects on phylogenetic distance compared to taxon abundance. The composition of fungal communities of AM and EcM tree mycorrhizal types became similar in multi-species mixtures. Lastly, I found that environmental factors explained more variation in phylogenetic distance-based analysis than that in relative abundance-based analysis, except in the EcM dataset. Fungal community composition of both tree mycorrhiza types was mainly influenced by tree species composition and spatial-related variables. Phylogenetic analysis revealed a strong effect of tree community-related factors in both AM and EcM tree species.

Discussion

The ecosystem concept, as articulated by Arthur Tansley (1935), "*the whole system including not only the organism-complex, but also the whole complex of physical factors forming what we call the environment of the biome – the habitat factors in the widest sense*" highlights the complexity of multiple biotic and abiotic interactions in shaping an ecosystem. In order to gain a comprehensive understanding of ecosystem functioning, it is essential to study as many factors as possible. The results of my thesis clearly demonstrated the importance of considering tree mycorrhizal type, tree diversity, and site-specific environmental factors together at the local scale to enhance our understanding of the structure and functional potential of forest belowground microbial communities. For instance, the significant positive tree diversity effect observed only on the EcM but not AM TSP soil and root fungal communities (chapter II & IV) emphasizes the importance of considering tree mycorrhizal type in studies investigating the relationship between tree diversity and soil microbial diversity. Previous reports indicated absence (Navratilova et al., 2019; Rivest et al., 2019; Otsing et al., 2021) and presence of significant relationships between tree diversity and belowground microbial communities (Gao et al., 2013; Weissbecker et al., 2019; Ferlian et al., 2021). For example, Rivest et al. (2019) in their analyses did not consider the tree mycorrhizal type and reported no tree diversity effect on the fungal diversity. My thesis findings suggest that accounting for the interactive effects of tree mycorrhizal type and diversity could resolve some of the inconsistencies and provide insights into tree diversity effects on the belowground microbial communities in future studies.

Moreover, the study findings in chapter II revealed that site-specific environmental factors, such as topography, have a differential impact on belowground microbial community composition in addition to tree mycorrhizal type and diversity. Specifically, it was observed that soil fungal communities under AM tree species, but not EcM tree species, had a preferential association with eastern and northern aspects of the slope in mono- and two-species mixtures. This correlation can be attributed to the influence of aspect on the amount and distribution of solar radiation received by the slope, which in turn affects microclimate factors such as soil temperature and humidity (Carter and Ciolkosz, 1991; Davies et al., 2006). In line with my findings, previous studies have also reported the impact of aspect on AM fungi and saprotrophs (Chai et al., 2018; Geml, 2019). Furthermore, the effects of environmental factors varied across the tree diversity levels.

Interestingly, in multi-tree species mixtures, the number of environmental factors, including edaphic, floristic, and topographic variables, that were significantly associated with soil fungal and bacterial communities under both AM and EcM trees, decreased in comparison to stands with lower tree diversity. This suggests that high plant diversity influences the local edaphic and microclimatic environment, potentially optimizing the microclimatic conditions such as temperature and humidity that can favor microbial co-existence and buffering the impact of abiotic factors (Bruelheide et al., 2014; Beugnon et al., 2022). My thesis findings (chapters II-IV) provided evidence for increasing microbial co-existence with increasing tree diversity, which indicate biotic facilitation, brought about by tree diversity through the amelioration of abiotic conditions such as microclimate.

Moreover, findings from chapter IV underlined the importance of co-evolutionary relationships among the microbes and also the host (plant) – microbe relationships in structuring the root-associated fungal communities. My results further corroborated the evidence found in the studies on plant-fungal co-evolutionary relationships and host phylogeny effects on root-associated fungal communities (Tedersoo et al., 2013; Hoeksema et al., 2018). One of the noteworthy outcomes was the identification of evolutionary associations of tree mycorrhizal partners that played a key role in the assembly of fungal communities as revealed by the phylogeny-based analyses which were not obvious from taxon-abundance-based analyses. Interestingly, unlike for soil microbial communities, topography was not an important factor in determining the assembly of root-associated fungal communities, indicating clear differences between these two belowground compartments. Soil characteristics were more important for EcM than AM root fungal

communities, which probably reflects their dependency on host mycorrhizal partner for nutrient acquisition from soil organic matter (Frey, 2019). Further, dispersal limitation, as assessed by spatial distance, was found to be an important factor in the assembly of belowground communities, with, as expected, stronger effects in the analyses based on taxon abundance than in those based on phylogeny. Dispersal can be active (for example, by hyphal growth) or passive (for example, by wind) depending on many factors such as microbial traits and environment. Taken together, my results suggest the predominant role of deterministic processes, such as environmental and biotic filtering, in the assembly of belowground microbial communities.

Tree mycorrhizal type is a crucial factor in determining the composition of belowground microbial communities as evident from the PERMANOVA and db-RDA analyses on soil and root microbiota (chapters II & IV), and co-occurrence network analysis of soil bacterial and fungal communities (chapter III). Previous research on the plant mycorrhizal type effect had mainly focused either exclusively on the belowground mycorrhizal communities (Gao et al., 2013; Neuenkamp et al., 2018) or the whole fungal communities (Toju et al., 2014; Weissbecker et al., 2019; Heklau et al., 2021). The plant mycorrhizal type effect on bacterial communities has been rarely studied so far. For example, in their study from boreal and temperate regional sites, Bahram et al. (2020) reported no significant effect of the plant mycorrhizal type on bacterial communities. Although PERMANOVA analyses in our case also did not reveal a significant effect, our random forest models were able to clearly point out the influence of the tree mycorrhizal partner on bacterial community assembly, especially in mono- and two-species mixtures (chapter II, Singavarapu et al. (2021). Additionally, bacterial classifier taxa were identified for the different mycorrhizal types, which, along with fungal classifier taxa, could serve as proxy indicators for determining the dominant mycorrhizal types in unexplored vegetation mixtures. Furthermore, network centrality, ordination and pairwise analyses of co-occurring bacterial and fungal sub-communities also highlighted the impact of tree mycorrhizal partners in the assembly of bacteria along with fungal communities.

One of the novel aspects of this thesis is the identification of the co-occurring fungal and bacterial sub-communities and their genomic functional potential with regard to the nutrient cycling of major elements (chapter III). Furthermore, my results clearly highlighted the significant contribution of microbial interkingdom interactions in performing those processes. For instance, the significant association of AM sub-communities with P and the

predominant correlation of P-cycling enzymes with the differentiation of all (AM & EcM) sub-communities reflects the P-limitation in sub/tropical soils (Huang et al., 2013). In addition, results from the module analyses mirrored the functional differences between AM vs EcM systems, with faster vs. slower nutrient cycling rates, respectively (Phillips et al., 2013). Collectively, these findings indicate the selection forces operating on microbes and their sub-communities, based on the niche requirements and shared functional roles of their members. Moreover, the presence of a large number of differences among subcommunities in their functional abundances within AM networks, which are characterized by a significant abundance of enzymes involved in the cycling of different nutrients and their combinations, has the potential to confer functional resilience. Conversely, the presence of greater similarities in their nutrient cycling enzyme abundances within EcM subcommunities may indicate functional redundancy or equivalence among subcommunities, which could help buffer external stressors and maintain ecosystem process rates. (Naylor et al., 2020). Furthermore, the microbial taxa that were found to differ in their functional abundance between mycorrhizal types across varying levels of tree diversity, if present in high abundance, can serve as indicators of soil systems at unexplored sites and provide insight into their potential for cycling various combinations of nutrients (C, N, P, CN, CP, NP, and CNP).

I encountered intriguing and surprising findings in multi-tree species mixtures. Firstly, the absence of a significant impact of tree diversity on the belowground fungal diversity of AM tree species contrasts with the findings for EcM tree species (chapters II & IV). The positive impact of EcM tree species on the fungal diversity could be due to complementarity effects from new niche opportunities provided by co-occurring tree species and their mycorrhizal partners (Trogisch et al., 2021). However, the absence of an impact of AM tree species requires further investigation. My analysis focused on alpha and phylogenetic diversities at the ASV level. However, the positive effect on richness may occur at a different taxonomic level, such as that of families or classes, which I did not consider yet. Additionally, the converging functional potential in multi-tree species mixtures and the synergy between functional resilience and redundancy in AM and EcM microbial sub-communities, respectively, may contribute to stable microbiome functioning for both AM and EcM trees in multi-species mixtures and enhance the maintenance of multifunctionality (Delgado-Baquerizo et al., 2017).

Secondly, although I expected diluted effects of tree species identity at higher tree diversity levels, the complete absence of significant effects on both diversity and composition of root-associated fungal communities was surprising owing to their strong co-evolutionary relationships and host specificity (Hoeksema et al., 2018). Even more surprising was the occurrence of dual mycorrhization that was absent in mono-specific stands. Based on my findings resulting from the interplay of environment, tree mycorrhizal type and diversity one can assume that dispersal and selection, two fundamental ecological processes operating closely at the top level, are probably the causal forces of these observations. When multiple tree species with different mycorrhizal partners were mixed in a plot, the spatial proximities facilitate a greater probability of both active and passive dispersal, and thus, make the microbial inoculum available to all tree species. Over time, the trees select the microbes through biotic and abiotic filtering, for example through their tree species-specific rhizodeposits (Jones et al., 2019). In addition, my findings suggest that the plant mycorrhizal partners select the co-occurring microbes through biotic interactions (Johansson et al., 2004). Alternatively, spillover effects (i.e., transfer of microbes from one nearby habitat to another) from the tree neighbors could also be a major factor, which would explain my findings of the significant impact of neighborhood on the belowground communities. Root networks (overlapping root systems) and also fungal hyphal networks could mediate this microbial spillover which, over time, may lead to successful colonization.

My thesis findings and the mechanisms operating here at the local scale can be generalized across different forest biomes as similar patterns were observed in studies from other biomes. For example, lower diversity of belowground fungal communities in EcM tree species and EcM-dominated sites were reported in temperate (Heklau et al., 2021), boreal (Bahram et al., 2020) and tropical forests (Tedersoo and Nara, 2010). The negative correlation of saprotrophs with the abundance of EcM plants and the positive correlation with that of AM plants were also reported in temperate and boreal site studies (Bahram et al., 2020; Eagar et al., 2022). In my thesis, for the first time, I have shown that taxonomically, phylogenetically and functionally converging microbial communities with high diversity were found in multi-tree species mixtures and that this high diversity potentially enhances ecosystem functioning (chapters II-IV). Previous research at our study site demonstrated that high tree diversity promotes forest productivity (Huang et al., 2018). Microbial diversity might be one of the underlying mechanisms for this relationship.

Furthermore, a key role of fungi in mediating the observed positive tree diversity–productivity relationship was also shown at our study site (Yang et al., 2022). Taken together, my findings suggest that mixing tree species of different mycorrhizal types in high diversities at the local level can foster diverse belowground microbial communities with converging genomic functional potential, which in turn may strengthen the maintenance of forest ecosystem services.

From the perspective of growing awareness of the essential role of forests in mitigating climate change, a key recommendation from my thesis is to plant different mycorrhizal type tree species in high diversities for afforestation and reforestation regimes. Similarly, for commercial purposes like timber production, planting mixtures would be more productive in the long run as the functionally rich and resilient nutrient-cycling microbial taxa could accelerate tree growth. Besides, phylogenetically diverse soil and root-associated microbial communities may promote the health of the tree communities which is vital for forest ecosystem services including timber production.

Study limitations

First, the universal primers (ITS2 region of 18S rRNA) used in the study effectively captured the taxonomic and phylogenetic diversity of the fungal samples, as evidenced by the clear fungal taxa patterns in my experimental treatments. However, a higher resolution of arbuscular mycorrhizal fungal diversity could have been achieved by using AM fungal-specific primers designed based on the 18S rRNA large and/or small subunit (LSU, SSU). This would have provided more information on the AM fungal communities in the study. Second, the db-RDA and variation partition analyses provided valuable insights into the complex relationships between microbial communities and environmental factors. Nevertheless, they could not determine the direction of the observed effects, which would have shed further light on the the underlying mechanisms. Third, chapter-IV gave a comprehensive understanding of the ecological and evolutionary aspects of root-associated fungal communities. Nonetheless, a more complete characterization of belowground communities could have been achieved with data on bacterial communities. Fourth, functional potential predictions using PICRUSt2 were very useful and cost-effective in uncovering novel insights into the nutrient cycling potential of belowground bacterial and fungal communities and their sub-communities. However, metagenomic and

metatranscriptomic analyses would provide a more robust characterization of genomic potential and gene expression, respectively.

Conclusions and future research perspectives

In the face of escalating climate change events, the importance of multifunctional forests in mitigating negative outcomes such as global warming and desertification is becoming increasingly apparent. My doctoral thesis found that tree mycorrhizal type, tree diversity and site-specific environmental factors play a crucial role in determining the structure and functional potential of forest belowground microbial communities. The results showed that tree mycorrhizal type is a key factor in shaping the diversity and composition of these communities based on the functional demands of the habitat. The study also emphasized the significance of ecological and evolutionary processes between plants and microbes, including co-evolutionary relationships and the influence of spatial distance in promoting enhanced functional stability of belowground microbial communities in multi-tree species mixtures. This research has made a significant contribution to the comprehensive understanding of ecosystem functioning and underscores the need to consider the interplay between tree mycorrhizal type, tree diversity, and environmental factors in the management of forest ecosystems.

My thesis findings present several opportunities for extending research to deepen our understanding of forest ecosystem functioning. There are numerous factors, both above- and belowground, that influence soil ecosystems, necessitating interdisciplinary approaches for a more comprehensive understanding. My thesis was carried out as part of an international research training group, TreeDi, which seeks to synthesize the findings from above- and belowground research to mechanistically understand the role of tree-tree interactions in biodiversity-ecosystem functioning (Trogisch et al., 2020; Trogisch et al., 2021). Soil microbial communities have various attributes, including biomass, physiological potential and taxonomic and functional profiles, which are influenced by factors such as resource availability and quality (Beugnon et al., 2021). The mechanisms behind the convergence of microbial communities, identified in my thesis, could be further explored by linking these attributes with the chemical composition of leaves, litter decomposition rates and soil chemistry at different levels of tree diversity. Additionally, integrating the microbial community data with root exudate data would deepen our understanding of the biotic interactions between plants and microbes. To achieve this, I

recommend leveraging cutting-edge omics techniques, such as metagenomics, metatranscriptomics, metaproteomics and metabolomics, which provide a greater level of resolution on eco-evolutionary processes. Furthermore, examining the relationship between microbial community data and functional traits, such as root diameter and root length density, would shed light on the mechanisms behind complementarity effects in multi-species tree mixtures, such as spatial resource partitioning.

Future studies could also consider incorporating belowground microorganisms, such as protists and viruses, to expand our knowledge of their structure and functioning within the ecosystem. The inclusion of other members of the soil food web, such as meso- and macrofauna, would further enhance our understanding of belowground functioning at a multitrophic level. While plot topography can offer rough insights into microclimatic conditions, future studies should also incorporate measurements of microclimate variables, such as soil temperature and light intensity, to better understand the drivers of microbial community structure and function. Additionally, incorporating aboveground traits, such as branching patterns reflecting the canopy structure, would allow for the study of aboveground complementary mechanisms on belowground microbiota structure and function.

Literature Cited

Aerts, R. (2003) The Role of Various Types of Mycorrhizal Fungi in Nutrient Cycling and Plant Competition. In *Mycorrhizal Ecology*. van der Heijden, M.G.A., and Sanders, I.R. (eds). Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 117-133.

Allaire, J., Gandrud, C., Russell, K., and Yetman, C. (2017) networkD3: D3 javascript network graphs from r. *R package version 04*.

Almagro, M., Ruiz-Navarro, A., Díaz-Pereira, E., Albaladejo, J., and Martínez-Mena, M. (2021) Plant residue chemical quality modulates the soil microbial response related to decomposition and soil organic carbon and nitrogen stabilization in a rainfed Mediterranean agroecosystem. *Soil Biology and Biochemistry* **156**: 108198.

Almario, J., Fabiańska, I., Saridis, G., and Bucher, M. (2022) Unearthing the plant–microbe quid pro quo in root associations with beneficial fungi. *New Phytologist* **234**: 1967-1976.

Amora-Lazcano, E., Vazquez, M., and Azcon, R. (1998) Response of nitrogen-transforming microorganisms to arbuscular mycorrhizal fungi. *Biology and Fertility of Soils* **27**: 65-70.

Arbizu, M. P. pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.0. 1.(2017). In.

Archer, E. (2016) rfPermute: Estimate permutation p-values for Random Forest importance metrics. *R package version* **1**.

Bahram, M., Netherway, T., Hildebrand, F., Pritsch, K., Drenkhan, R., Loit, K. et al. (2020) Plant nutrient-acquisition strategies drive topsoil microbiome structure and function. *New Phytologist*.

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M. et al. (2018) Structure and function of the global topsoil microbiome. *Nature* **560**: 233-237.

Baldrian, P. (2017) Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiology reviews* **41**: 109-130.

Balloffet, N., Deal, R., Hines, S., Larry, B., and Smith, N. (2012) Ecosystem services and climate change. *US Department of Agriculture, Forest Service, Climate Change Resource Center;*

https://wwwfsusdagov/ccrc/topics/ecosystem-services.

Barbera, P., Kozlov, A.M., Czech, L., Morel, B., Darriba, D., Flouri, T., and Stamatakis, A. (2019) EPA-ng: massively parallel evolutionary placement of genetic sequences. *Systematic biology* **68**: 365-369.

Barberan, A., McGuire, K.L., Wolf, J.A., Jones, F.A., Wright, S.J., Turner, B.L. et al. (2015) Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. *Ecology letters* **18**: 1397-1405.

Bardgett, R.D., and Wardle, D.A. (2010) *Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change*: Oxford University Press.

Bardgett, R.D., and Van Der Putten, W.H. (2014) Belowground biodiversity and ecosystem functioning. *Nature* **515**: 505-511.

Barrow, N., Debnath, A., and Sen, A. (2020) Measurement of the effects of pH on phosphate availability. *Plant and Soil* **454**: 217-224.

Battaglia, T. (2018) btools: A suite of R function for all types of microbial diversity analyses. *R package version 00* **1**.

Beidler, K.V., and Pritchard, S.G. (2017) Maintaining connectivity: understanding the role of root order and mycelial networks in fine root decomposition of woody plants. *Plant and soil* **420**: 19-36.

Bender, S.F., and van der Heijden, M.G. (2015) Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. *Journal of Applied Ecology* **52**: 228-239.

Bender, S.F., Wagg, C., and van der Heijden, M.G. (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in ecology & evolution* **31**: 440-452.

Bennett, J.A., Maherali, H., Reinhart, K.O., Lekberg, Y., Hart, M.M., and Klironomos, J. (2017) Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* **355**: 181-184.

Beugnon, R., Ladouceur, E., Sünnemann, M., Cesarz, S., and Eisenhauer, N. (2022) Diverse forests are cool: Promoting diverse forests to mitigate carbon emissions and climate change. *Journal of Sustainable Agriculture and Environment* **1**: 5-8.

Beugnon, R., Du, J., Cesarz, S., Jurburg, S.D., Pang, Z., Singavarapu, B. et al. (2021) Tree diversity and soil chemical properties drive the linkages between soil microbial community and ecosystem functioning. *ISME Communications* **1**: 41.

Bingham, A.H., and Cotrufo, M.F. (2016) Organic nitrogen storage in mineral soil: implications for policy and management. *Science of the Total Environment* **551**: 116-126.

Black, E.M., Chimenti, M.S., and Just, C.L. (2019) Metagenomic analysis of nitrogen-cycling genes in upper Mississippi river sediment with mussel assemblages. *MicrobiologyOpen* **8**: e00739.

Blackwell, M. (2011) The Fungi: 1, 2, 3… 5.1 million species? *American journal of botany* **98**: 426-438.

Bödeker, I.T., Lindahl, B.D., Olson, Å., and Clemmensen, K.E. (2016) Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. *Functional Ecology* **30**: 1967-1978.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R. et al. (2018) Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **6**: 1-17.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A. et al. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature biotechnology* **37**: 852-857.

Bonfante, P., and Anca, I.-A. (2009) Plants, Mycorrhizal Fungi, and Bacteria: A Network of Interactions. *Annual Review of Microbiology* **63**: 363-383.

Bonfante, P., and Genre, A. (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. *Nature Communications* **1**: 48.

Boyce, S., and Tipton, K.F. (2001) Enzyme classification and nomenclature. *e LS*.

Bradstreet, R. (1954) Determination of nitro nitrogen by Kjeldahl method. *Analytical Chemistry* **26**: 235-236.

Brassard, B.W., Chen, H.Y., Cavard, X., Laganiere, J., Reich, P.B., Bergeron, Y. et al. (2013) Tree species diversity increases fine root productivity through increased soil volume filling. *Journal of Ecology* **101**: 210-219.

Bruelheide, H., Nadrowski, K., Assmann, T., Bauhus, J., Both, S., Buscot, F. et al. (2014) Designing forest biodiversity experiments: general considerations illustrated by a new large experiment in subtropical China. *Methods in Ecology and Evolution* **5**: 74-89.

Brundrett, M.C., and Tedersoo, L. (2018) Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist* **220**: 1108-1115.

Buee, M., De Boer, W., Martin, F., Van Overbeek, L., and Jurkevitch, E. (2009) The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. *Plant and Soil* **321**: 189-212.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nature methods* **13**: 581-583.

Camenzind, T., Hättenschwiler, S., Treseder, K.K., Lehmann, A., and Rillig, M.C. (2018) Nutrient limitation of soil microbial processes in tropical forests. *Ecological Monographs* **88**: 4-21.

Canarini, A., Schmidt, H., Fuchslueger, L., Martin, V., Herbold, C.W., Zezula, D. et al. (2021) Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. *Nature communications* **12**: 1-14.

Cao, Q., Sun, X., Rajesh, K., Chalasani, N., Gelow, K., Katz, B. et al. (2021) Effects of Rare Microbiome Taxa Filtering on Statistical Analysis. *Frontiers in Microbiology* **11**.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J. et al. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences* **108**: 4516-4522.

Cardinale, B.J., Srivastava, D.S., Emmett Duffy, J., Wright, J.P., Downing, A.L., Sankaran, M., and Jouseau, C. (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* **443**: 989-992.

Carter, B.J., and Ciolkosz, E.J. (1991) Slope gradient and aspect effects on soils developed from sandstone in Pennsylvania. *Geoderma* **49**: 199-213.

Chai, Y., Jiang, S., Guo, W., Qin, M., Pan, J., Bahadur, A. et al. (2018) The effect of slope aspect on the phylogenetic structure of arbuscular mycorrhizal fungal communities in an alpine ecosystem. *Soil Biology and Biochemistry* **126**: 103-113.

Cheeke, T.E., Phillips, R.P., Brzostek, E.R., Rosling, A., Bever, J.D., and Fransson, P. (2017) Dominant mycorrhizal association of trees alters carbon and nutrient cycling by selecting for microbial groups with distinct enzyme function. *New Phytologist* **214**: 432-442.

Chen, C., Chen, H.Y., Chen, X., and Huang, Z. (2019a) Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nature Communications* **10**: 1-10.

Chen, L., Xiang, W., Wu, H., Ouyang, S., Zhou, B., Zeng, Y. et al. (2019b) Tree species identity surpasses richness in affecting soil microbial richness and community composition in subtropical forests. *Soil Biology and Biochemistry* **130**: 113-121.

Chen, M., Arato, M., Borghi, L., Nouri, E., and Reinhardt, D. (2018) Beneficial services of arbuscular mycorrhizal fungi–from ecology to application. *Frontiers in Plant Science* **9**: 1270.

Chen, Y., Rekha, P., Arun, A., Shen, F., Lai, W.-A., and Young, C.C. (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied soil ecology* **34**: 33-41.

Chen, Y.-L., Xu, T.-L., Veresoglou, S.D., Hu, H.-W., Hao, Z.-P., Hu, Y.-J. et al. (2017) Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. *Soil Biology and Biochemistry* **110**: 12-21.

Cheng, K., and Yu, S. (2020) Neighboring trees regulate the root-associated pathogenic fungi on the host plant in a subtropical forest. *Ecology and evolution* **10**: 3932-3943.

Cheng, Y., Wang, J., Mary, B., Zhang, J.-b., Cai, Z.-c., and Chang, S.X. (2013) Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in central Alberta, Canada. *Soil Biology and Biochemistry* **57**: 848-857.

Churchland, C., and Grayston, S.J. (2014) Specificity of plant-microbe interactions in the tree mycorrhizosphere biome and consequences for soil C cycling. *Frontiers in Microbiology* **5**: 261.

Clemmensen, K., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H. et al. (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* **339**: 1615-1618.

Compant, S., Samad, A., Faist, H., and Sessitsch, A. (2019) A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *Journal of advanced research* **19**: 29-37.

Csardi, G., and Nepusz, T. (2006) The igraph software package for complex network research. *InterJournal, complex systems* **1695**: 1-9.

Czech, L., Barbera, P., and Stamatakis, A. (2020) Genesis and Gappa: processing, analyzing and visualizing phylogenetic (placement) data. *Bioinformatics* **36**: 3263-3265.

Dassen, S., Cortois, R., Martens, H., de Hollander, M., Kowalchuk, G.A., van der Putten, W.H., and De Deyn, G.B. (2017) Differential responses of soil bacteria, fungi, archaea and protists to plant species richness and plant functional group identity. *Molecular Ecology* **26**: 4085-4098.

David, A.S., Seabloom, E.W., and May, G. (2016) Plant host species and geographic distance affect the structure of aboveground fungal symbiont communities, and environmental filtering affects belowground communities in a coastal dune ecosystem. *Microbial Ecology* **71**: 912-926.

Davies, Z.G., Wilson, R.J., Coles, S., and Thomas, C.D. (2006) Changing habitat associations of a thermally constrained species, the silver-spotted skipper butterfly, in response to climate warming. *Journal of Animal Ecology* **75**: 247-256.

Davison, J., Garcia de Leon, D., Zobel, M., Moora, M., Bueno, C.G., Barcelo, M. et al. (2020) Plant functional groups associate with distinct arbuscular mycorrhizal fungal communities. *New Phytologist* **226**: 1117-1128.

Delavaux, C.S., and Bever, J.D. (2022) Evidence for the evolution of native plant response to mycorrhizal fungi in post-agricultural grasslands. *Ecology and evolution* **12**: e9097.

Delgado-Baquerizo, M., and Eldridge, D.J. (2019) Cross-biome drivers of soil bacterial alpha diversity on a worldwide scale. *Ecosystems* **22**: 1220-1231.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D. et al. (2016) Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature communications* **7**: 1-8.

Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D.J., Bardgett, R.D. et al. (2018) A global atlas of the dominant bacteria found in soil. *Science* **359**: 320-325.

Delgado-Baquerizo, M., Bardgett, R.D., Vitousek, P.M., Maestre, F.T., Williams, M.A., Eldridge, D.J. et al. (2019) Changes in belowground biodiversity during ecosystem development. *Proc Natl Acad Sci U S A* **116**: 6891-6896.

Delgado-Baquerizo, M., Grinyer, J., Reich, P.B., and Singh, B.K. (2016) Relative importance of soil properties and microbial community for soil functionality: insights from a microbial swap experiment. *Functional Ecology* **30**: 1862-1873.

Delgado-Baquerizo, M., Eldridge, D.J., Ochoa, V., Gozalo, B., Singh, B.K., and Maestre, F.T. (2017) Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe. *Ecology Letters* **20**: 1295-1305.

Dobzhansky, T. (1973) Nothing in Biology Makes Sense except in the Light of Evolution. *The American Biology Teacher* **35**: 125-129.

Douglas, G.M., Maffei, V.J., Zaneveld, J.R., Yurgel, S.N., Brown, J.R., Taylor, C.M. et al. (2020) PICRUSt2 for prediction of metagenome functions. *Nature biotechnology* **38**: 685-688.

Dray, S., Legendre, P., and Peres-Neto, P.R. (2006) Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecological modelling* **196**: 483-493.

Du, E., Terrer, C., Pellegrini, A.F., Ahlström, A., van Lissa, C.J., Zhao, X. et al. (2020) Global patterns of terrestrial nitrogen and phosphorus limitation. *Nature Geoscience* **13**: 221-226.

E. M., S., J., J., B., S., M., D., and C., K. (2000) Above-ground resource use increases with plant species richness in experimental grassland ecosystems. *Functional Ecology* **14**: 326-337.

Eagar, A.C., Mushinski, R.M., Horning, A.L., Smemo, K.A., Phillips, R.P., and Blackwood, C.B. (2022) Arbuscular mycorrhizal tree communities have greater soil fungal diversity and relative abundances of saprotrophs and pathogens than ectomycorrhizal tree communities. *Applied and environmental microbiology* **88**: e01782-01721.

Eisenhauer, N., Reich, P.B., and Scheu, S. (2012) Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic and Applied Ecology* **13**: 571-578.

Eisenhauer, N., Dobies, T., Cesarz, S., Hobbie, S.E., Meyer, R.J., Worm, K., and Reich, P.B. (2013) Plant diversity effects on soil food webs are stronger than those of elevated CO2 and N deposition in a long-term grassland experiment. *Proceedings of the National Academy of Sciences* **110**: 6889-6894.

Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M.P., and Mommer, L. (2017) Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Scientific reports* **7**: 1-8.

Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A. et al. (2010) Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* **91**: 485-496.

Eisenhauer, N., Schielzeth, H., Barnes, A.D., Barry, K.E., Bonn, A., Brose, U. et al. (2019) A multitrophic perspective on biodiversity–ecosystem functioning research. *Advances in ecological research* **61**: 1-54.

Elser, J.J., Dobberfuhl, D.R., MacKay, N.A., and Schampel, J.H. (1996) Organism size, life history, and N: P stoichiometry: toward a unified view of cellular and ecosystem processes. *BioScience* **46**: 674-684.

Evans, J., and Murphy, M. (2015) rfUtilities: random forests model selection and performance evaluation. *R package version*: 2.1-3.

Fan, F., Yin, C., Tang, Y., Li, Z., Song, A., Wakelin, S.A. et al. (2014) Probing potential microbial coupling of carbon and nitrogen cycling during decomposition of maize residue by 13C-DNA-SIP. *Soil Biology and Biochemistry* **70**: 12-21.

Fanin, N., Alavoine, G., and Bertrand, I. (2020) Temporal dynamics of litter quality, soil properties and microbial strategies as main drivers of the priming effect. *Geoderma* **377**: 114576.

FAO, ITPS, GSBI, SCBD, and EC (2020) State of Knowledge of Soil Biodiversity—Status, Challenges and Potentialities, Report 2020. *Rome Italy: FAO(doi: 104060/cb1928en); https://wwwfaoorg/documents/card/en/c/cb1928en*.

Faust, K., and Raes, J. (2012) Microbial interactions: from networks to models. *Nat Rev Microbiol* **10**: 538-550.

Feng, J., Zeng, X.-M., Zhang, Q., Zhou, X.-Q., Liu, Y.-R., and Huang, Q. (2021) Soil microbial trait-based strategies drive metabolic efficiency along an altitude gradient. *ISME Communications* **1**: 1-9.

Ferlian, O., Goldmann, K., Eisenhauer, N., Tarkka, M.T., Buscot, F., and Heintz-Buschart, A. (2021) Distinct effects of host and neighbour tree identity on arbuscular and ectomycorrhizal fungi along a tree diversity gradient. *ISME Communications* **1**: 40.

Fernandez, C.W., and Kennedy, P.G. (2016) Revisiting the 'Gadgil effect': do interguild fungal interactions control carbon cycling in forest soils? *New phytologist* **209**: 1382-1394.

Fierer, N. (2017) Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology* **15**: 579-590.

Fierer, N., and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences* **103**: 626-631.

Fierer, N., Bradford, M.A., and Jackson, R.B. (2007) Toward an ecological classification of soil bacteria. *Ecology* **88**: 1354-1364.

Finlay, R.D. (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *Journal of experimental botany* **59**: 1115-1126.

Fitter, A., and Garbaye, J. (1994) Interactions between mycorrhizal fungi and other soil organisms. *Plant and soil* **159**: 123-132.

Fox, J., and Weisberg, S. (2018) *An R companion to applied regression*: Sage publications.

Frey, S.D. (2019) Mycorrhizal fungi as mediators of soil organic matter dynamics. *Annual review of ecology, evolution, and systematics* **50**: 237-259.

Gaiero, J.R., Tosi, M., Bent, E., Boitt, G., Khosla, K., Turner, B.L. et al. (2021) Soil microbial communities influencing organic phosphorus mineralization in a coastal dune chronosequence in New Zealand. *FEMS Microbiology Ecology* **97**: fiab034.

Gan, H., Li, X., Wang, Y., Lü, P., Ji, N., Yao, H. et al. (2022) Plants Play Stronger Effects on Soil Fungal than Bacterial Communities and Co-Occurrence Network Structures in a Subtropical Tree Diversity Experiment. *Microbiology Spectrum*: e00134-00122.

Gao, C., Shi, N.-N., Liu, Y.-X., Peay, K.G., Zheng, Y., Ding, Q. et al. (2013) Host plant genus-level diversity is the best predictor of ectomycorrhizal fungal diversity in a Chinese subtropical forest. *Molecular Ecology* **22**: 3403-3414.

Gardes, M., and Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113-118.

Geml, J. (2019) Soil fungal communities reflect aspect-driven environmental structuring and vegetation types in a Pannonian forest landscape. *Fungal Ecology* **39**: 63-79.

Glassman, S.I., Wang, I.J., and Bruns, T.D. (2017) Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales. *Molecular ecology* **26**: 6960-6973.

Graham, E.B., Knelman, J.E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A. et al. (2016) Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? *Frontiers in microbiology* **7**: 214.

Gregor, M., Loehnertz, M., Schröder, C., Aksoy, E., Prokop, G., and Louwagie, G. (2018) *Land Cover Changes and Soil Functions. An approach for integrated accounting*

. European Topic Centre on Urban Land and Soil Systems; https://zenodo.org/record/3785107#.Y-Dkji8w2Ak.

Gu, Z., Eils, R., and Schlesner, M. (2016) Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* **32**: 2847-2849.

Hartman, W.H., Ye, R., Horwath, W.R., and Tringe, S.G. (2017) A genomic perspective on stoichiometric regulation of soil carbon cycling. *The ISME journal* **11**: 2652-2665.

Haug, I., Weber, R., Oberwinkler, F., and Tschen, J. (1994) The mycorrhizal status of Taiwanese trees and the description of some ectomycorrhizal types. *Trees* **8**: 237-253.

Heilmann-Clausen, J., Maruyama, P.K., Bruun, H.H., Dimitrov, D., Læssøe, T., Frøslev, T.G., and Dalsgaard, B. (2016) Citizen science data reveal ecological, historical and evolutionary factors shaping interactions between woody hosts and wood-inhabiting fungi. *New Phytologist* **212**: 1072-1082.

Heklau, H., Schindler, N., Buscot, F., Eisenhauer, N., Ferlian, O., Prada Salcedo, L.D., and Bruelheide, H. (2021) Mixing tree species associated with arbuscular or ectotrophic mycorrhizae reveals dual mycorrhization and interactive effects on the fungal partners. *Ecology and evolution* **11**: 5424-5440.

Herman, D.J., Firestone, M.K., Nuccio, E., and Hodge, A. (2012) Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. *FEMS microbiology ecology* **80**: 236-247.

Hermans, S.M., Buckley, H.L., Case, B.S., Curran-Cournane, F., Taylor, M., and Lear, G. (2020) Using soil bacterial communities to predict physico-chemical variables and soil quality. *Microbiome* **8**: 1-13.

Herzog, C., Hartmann, M., Frey, B., Stierli, B., Rumpel, C., Buchmann, N., and Brunner, I. (2019) Microbial succession on decomposing root litter in a drought-prone Scots pine forest. *The ISME journal* **13**: 2346-2362.

Hiiesalu, I., Bahram, M., and Tedersoo, L. (2017) Plant species richness and productivity determine the diversity of soil fungal guilds in temperate coniferous forest and bog habitats. *Molecular Ecology* **26**: 4846-4858.

Hoeksema, J.D., Bever, J.D., Chakraborty, S., Chaudhary, V.B., Gardes, M., Gehring, C.A. et al. (2018) Evolutionary history of plant hosts and fungal symbionts predicts the strength of mycorrhizal mutualism. *Communications biology* **1**: 1-10.

Högberg, M.N., Bååth, E., Nordgren, A., Arnebrant, K., and Högberg, P. (2003) Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs–a hypothesis based on field observations in boreal forest. *New Phytologist* **160**: 225-238.

Homann, M., Sansjofre, P., Van Zuilen, M., Heubeck, C., Gong, J., Killingsworth, B. et al. (2018) Microbial life and biogeochemical cycling on land 3,220 million years ago. *Nature Geoscience* **11**: 665-671.

Hooper, D.U., Bignell, D.E., Brown, V.K., Brussard, L., Dangerfield, J.M., Wall, D.H. et al. (2000) Interactions between Aboveground and Belowground Biodiversity in Terrestrial Ecosystems: Patterns, Mechanisms, and Feedbacks: We assess the evidence for correlation between aboveground and belowground diversity and conclude that a variety of mechanisms could lead to positive, negative, or no relationship—depending on the strength and type of interactions among species. *BioScience* **50**: 1049-1061.

Horn, S., Caruso, T., Verbruggen, E., Rillig, M.C., and Hempel, S. (2014) Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. *The ISME journal* **8**: 2231-2242.

Horn, S., Hempel, S., Verbruggen, E., Rillig, M.C., and Caruso, T. (2017) Linking the community structure of arbuscular mycorrhizal fungi and plants: a story of interdependence? *The ISME Journal* **11**: 1400-1411.

Huang, W., Liu, J., Wang, Y.P., Zhou, G., Han, T., and Li, Y. (2013) Increasing phosphorus limitation along three successional forests in southern China. *Plant and Soil* **364**: 181-191.

Huang, Y., Ma, Y., Zhao, K., Niklaus, P.A., Schmid, B., and He, J.-S. (2017) Positive effects of tree species diversity on litterfall quantity and quality along a secondary successional chronosequence in a subtropical forest. *Journal of Plant Ecology* **10**: 28-35.

Huang, Y., Chen, Y., Castro-Izaguirre, N., Baruffol, M., Brezzi, M., Lang, A. et al. (2018) Impacts of species richness on productivity in a large-scale subtropical forest experiment. *Science* **362**: 80-83.

Humphreys, C.P., Franks, P.J., Rees, M., Bidartondo, M.I., Leake, J.R., and Beerling, D.J. (2010) Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. *Nature communications* **1**: 1-7.

Huston, M.A. (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* **110**: 449-460.

Ihrmark, K., Bödeker, I., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J. et al. (2012) New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. *FEMS microbiology ecology* **82**: 666-677.

Jacobs, L.M., Sulman, B.N., Brzostek, E.R., Feighery, J.J., and Phillips, R.P. (2018) Interactions among decaying leaf litter, root litter and soil organic matter vary with mycorrhizal type. *Journal of Ecology* **106**: 502-513.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J. et al. (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* **443**: 818-822.

Jansson, J.K., and Hofmockel, K.S. (2020) Soil microbiomes and climate change. *Nature Reviews Microbiology* **18**: 35-46.

Jenkins, M., and Schaap, B. (2018) Forest ecosystem services. In *United Nations Forum on Forests;*

https://wwwunorg/esa/forests/wp-content/uploads/2018/05/UNFF13_BkgdStudy_ForestsEcoServicespdf.

Jiang, F., Zhu, K., Cadotte, M.W., and Jin, G. (2020) Tree mycorrhizal type mediates the strength of negative density dependence in temperate forests. *Journal of Ecology* **108**: 2601-2610.

Jiang, J., Moore, J.A., Priyadarshi, A., and Classen, A.T. (2017) Plant-mycorrhizal interactions mediate plant community coexistence by altering resource demand. *Ecology* **98**: 187-197.

Jiao, J.-Y., Fu, L., Hua, Z.-S., Liu, L., Salam, N., Liu, P.-F. et al. (2021a) Insight into the function and evolution of the Wood–Ljungdahl pathway in Actinobacteria. *The ISME Journal* **15**: 3005-3018.

Jiao, S., and Lu, Y. (2020) Soil pH and temperature regulate assembly processes of abundant and rare bacterial communities in agricultural ecosystems. *Environmental Microbiology* **22**: 1052-1065.

Jiao, S., Peng, Z., Qi, J., Gao, J., and Wei, G. (2021b) Linking bacterial-fungal relationships to microbial diversity and soil nutrient cycling. *Msystems* **6**: e01052-01020.

Johansson, J.F., Paul, L.R., and Finlay, R.D. (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS microbiology ecology* **48**: 1-13.

Jones, P., Garcia, B.J., Furches, A., Tuskan, G.A., and Jacobson, D. (2019) Plant host-associated mechanisms for microbial selection. *Frontiers in Plant Science*: 862.

Kadowaki, K., Yamamoto, S., Sato, H., Tanabe, A.S., Hidaka, A., and Toju, H. (2018) Mycorrhizal fungi mediate the direction and strength of plant-soil feedbacks differently between arbuscular mycorrhizal and ectomycorrhizal communities. *Commun Biol* **1**: 196.

Kappler, A., and Bryce, C. (2017) Cryptic biogeochemical cycles: unravelling hidden redox reactions. *Environmental Microbiology* **19**: 842-846.

Karliński, L., Ravnskov, S., and Rudawska, M. (2020) Soil microbial biomass and community composition relates to poplar genotypes and environmental conditions. *Forests* **11**: 262.

Kaspari, M., Bujan, J., Weiser, M.D., Ning, D., Michaletz, S.T., Zhili, H. et al. (2017) Biogeochemistry drives diversity in the prokaryotes, fungi, and invertebrates of a Panama forest. *Ecology* **98**: 2019-2028.

Kassambara, A. (2021) rstatix: Pipe-friendly framework for basic statistical tests (0.7. 0)[Computer software]. In.

Kassambara, A., and Kassambara, M.A. (2020) Package 'ggpubr'. *R package version 01* **6**.

Keller, A.B., and Phillips, R.P. (2019) Leaf litter decay rates differ between mycorrhizal groups in temperate, but not tropical, forests. *New Phytologist* **222**: 556-564.

Kim, H., Lee, K.K., Jeon, J., Harris, W.A., and Lee, Y.H. (2020) Domestication of Oryza species eco-evolutionarily shapes bacterial and fungal communities in rice seed. *Microbiome* **8**: 20.

Koyama, A., Maherali, H., and Antunes, P.M. (2019) Plant geographic origin and phylogeny as potential drivers of community structure in root-inhabiting fungi. *Journal of Ecology* **107**: 1720-1736.

Kuang, J., Han, S., Chen, Y., Bates, C.T., Wang, P., and Shu, W. (2021) Root-associated fungal community reflects host spatial co-occurrence patterns in a subtropical forest. *ISME Communications* **1**: 1-9.

Kurtz, Z.D., Müller, C.L., Miraldi, E.R., Littman, D.R., Blaser, M.J., and Bonneau, R.A. (2015) Sparse and compositionally robust inference of microbial ecological networks. *PLoS computational biology* **11**: e1004226.

Kuypers, M.M., Marchant, H.K., and Kartal, B. (2018) The microbial nitrogen-cycling network. *Nature Reviews Microbiology* **16**: 263-276.

Lahti, L., and Shetty, S. (2017) microbiome R package.

Lahti, L., Shetty, S., Blake, T., and Salojarvi, J. (2017) Microbiome r package. *Tools Microbiome Anal R*.

Laliberté, E., Grace, J.B., Huston, M.A., Lambers, H., Teste, F.P., Turner, B.L., and Wardle, D.A. (2013) How does pedogenesis drive plant diversity? *Trends in ecology & evolution* **28**: 331-340.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I. et al. (2015) Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications* **6**: 1-8.

Lebreton, A., Zeng, Q., Miyauchi, S., Kohler, A., Dai, Y.-C., and Martin, F.M. (2021) Evolution of the mode of nutrition in symbiotic and saprotrophic fungi in forest ecosystems. *Annual Review of Ecology, Evolution, and Systematics* **52**: 385-404.

Lee Taylor, D., and Bruns, T.D. (1999) Population, habitat and genetic correlates of mycorrhizal specialization in the 'cheating'orchids Corallorhiza maculata and C. mertensiana. *Molecular Ecology* **8**: 1719-1732.

Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L. et al. (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences* **112**: 10967-10972.

Li, Z., Tian, D., Wang, B., Wang, J., Wang, S., Chen, H.Y. et al. (2019) Microbes drive global soil nitrogen mineralization and availability. *Global change biology* **25**: 1078-1088.

Liang, J.-L., Liu, J., Jia, P., Yang, T.-t., Zeng, Q.-w., Zhang, S.-c. et al. (2020) Novel phosphate-solubilizing bacteria enhance soil phosphorus cycling following ecological restoration of land degraded by mining. *The ISME journal* **14**: 1600-1613.

Lin, G., McCormack, M.L., Ma, C., and Guo, D. (2017) Similar below-ground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. *New Phytologist* **213**: 1440-1451.

Liu, X., Tan, N., Zhou, G., Zhang, D., Zhang, Q., Liu, S. et al. (2021) Plant diversity and species turnover co-regulate soil nitrogen and phosphorus availability in Dinghushan forests, southern China. *Plant and Soil* **464**: 257-272.

Liu, Y., Sun, Q., Li, J., and Lian, B. (2018) Bacterial diversity among the fruit bodies of ectomycorrhizal and saprophytic fungi and their corresponding hyphosphere soils. *Scientific reports* **8**: 1-10.

Locey, K.J., and Lennon, J.T. (2016) Scaling laws predict global microbial diversity. *Proceedings of the National Academy of Sciences* **113**: 5970-5975.

López-Mondéjar, R., Zühlke, D., Becher, D., Riedel, K., and Baldrian, P. (2016) Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. *Scientific Reports* **6**: 1-12.

Loreau, M., and Hector, A. (2001) Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**: 72-76.

Louca, S., and Doebeli, M. (2018) Efficient comparative phylogenetics on large trees. *Bioinformatics* **34**: 1053-1055.

Louca, S., Parfrey, L.W., and Doebeli, M. (2016) Decoupling function and taxonomy in the global ocean microbiome. *Science* **353**: 1272-1277.

Luo, G., Xue, C., Jiang, Q., Xiao, Y., Zhang, F., Guo, S. et al. (2020) Soil carbon, nitrogen, and phosphorus cycling microbial populations and their resistance to global change depend on soil C: N: P stoichiometry. *Msystems* **5**: e00162-00120.

Luo, S., Schmid, B., De Deyn, G.B., and Yu, S. (2018) Soil microbes promote complementarity effects among co-existing trees through soil nitrogen partitioning. *Functional Ecology* **32**: 1879-1889.

Lutzoni, F., Kauff, F., Cox, C.J., McLaughlin, D., Celio, G., Dentinger, B. et al. (2004) Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American journal of botany* **91**: 1446-1480.

Ma, B., Wang, H., Dsouza, M., Lou, J., He, Y., Dai, Z. et al. (2016) Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. *The ISME journal* **10**: 1891-1901.

Ma, B., Wang, Y., Ye, S., Liu, S., Stirling, E., Gilbert, J.A. et al. (2020) Earth microbial co-occurrence network reveals interconnection pattern across microbiomes. *Microbiome* **8**: 1-12.

Martikainen, P.J. (2022) Heterotrophic nitrification–An eternal mystery in the nitrogen cycle. *Soil Biology and Biochemistry* **168**: 108611.

Martin, M. (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet journal* **17**: 10-12.

Martinez Arbizu, P. (2017) pairwiseAdonis: Pairwise multilevel comparison using adonis. *R package version 00* **1**.

McGuire, K.L., Fierer, N., Bateman, C., Treseder, K.K., and Turner, B.L. (2012) Fungal community composition in neotropical rain forests: the influence of tree diversity and precipitation. *Microbial Ecology* **63**: 804-812.

McMurdie, P.J., and Holmes, S. (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS one* **8**: e61217.

Midgley, M.G., Brzostek, E., and Phillips, R.P. (2015) Decay rates of leaf litters from arbuscular mycorrhizal trees are more sensitive to soil effects than litters from ectomycorrhizal trees. *Journal of Ecology* **103**: 1454-1463.

Miyauchi, S., Kiss, E., Kuo, A., Drula, E., Kohler, A., Sánchez-García, M. et al. (2020) Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits. *Nature communications* **11**: 1-17.

Mony, C., Gaudu, V., Ricono, C., Jambon, O., and Vandenkoornhuyse, P. (2021) Plant neighbours shape fungal assemblages associated with plant roots: A new understanding of niche-partitioning in plant communities. *Functional Ecology*.

Moore, J.A., Jiang, J., Patterson, C.M., Mayes, M.A., Wang, G., and Classen, A.T. (2015) Interactions among roots, mycorrhizas and free-living microbial communities differentially impact soil carbon processes. *Journal of Ecology* **103**: 1442-1453.

Morris, B.E., Henneberger, R., Huber, H., and Moissl-Eichinger, C. (2013) Microbial syntrophy: interaction for the common good. *FEMS microbiology reviews* **37**: 384-406.

Mothapo, N., Chen, H., Cubeta, M.A., Grossman, J.M., Fuller, F., and Shi, W. (2015) Phylogenetic, taxonomic and functional diversity of fungal denitrifiers and associated N2O production efficacy. *Soil Biology and Biochemistry* **83**: 160-175.

Mushinski, R.M., Payne, Z.C., Raff, J.D., Craig, M.E., Pusede, S.E., Rusch, D.B. et al. (2021) Nitrogen cycling microbiomes are structured by plant mycorrhizal associations with consequences for nitrogen oxide fluxes in forests. *Global change biology* **27**: 1068-1082.

Nagy, L.G., Tóth, R., Kiss, E., Slot, J., Gácser, A., and Kovacs, G.M. (2017) Six key traits of fungi: their evolutionary origins and genetic bases. *Microbiology spectrum* **5**: 5.4. 14.

Naimi, B., Hamm, N.A., Groen, T.A., Skidmore, A.K., and Toxopeus, A.G. (2014) Where is positional uncertainty a problem for species distribution modelling? *Ecography* **37**: 191-203.

Navratilova, D., Tlaskalova, P., Kohout, P., Drevojan, P., Fajmon, K., Chytry, M., and Baldrian, P. (2019) Diversity of fungi and bacteria in species-rich grasslands increases with plant diversity in shoots but not in roots and soil. *FEMS Microbiol Ecol* **95**.

Nawaz, A., Purahong, W., Herrmann, M., Küsel, K., Buscot, F., and Wubet, T. (2019) DNA-and RNA-Derived Fungal Communities in Subsurface Aquifers Only Partly Overlap but React Similarly to Environmental Factors. *Microorganisms* **7**: 341.

Naylor, D., Sadler, N., Bhattacharjee, A., Graham, E.B., Anderton, C.R., McClure, R. et al. (2020) Soil microbiomes under climate change and implications for carbon cycling. *Annual Review of Environment and Resources* **45**: 29-59.

Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F. et al. (2013) Patterns and processes of microbial community assembly. *Microbiology and Molecular Biology Reviews* **77**: 342-356.

Neuenkamp, L., Moora, M., Öpik, M., Davison, J., Gerz, M., Männistö, M. et al. (2018) The role of plant mycorrhizal type and status in modulating the relationship between plant and arbuscular mycorrhizal fungal communities. *New Phytologist* **220**: 1236-1247.

Nguyen, D.Q., Schneider, D., Brinkmann, N., Song, B., Janz, D., Schöning, I. et al. (2020) Soil and root nutrient chemistry structure root-associated fungal assemblages in temperate forests. *Environmental Microbiology* **22**: 3081-3095.

Nguyen, N.H., and Bruns, T.D. (2015) The microbiome of Pinus muricata ectomycorrhizae: community assemblages, fungal species effects, and Burkholderia as important bacteria in multipartnered symbioses. *Microbial ecology* **69**: 914-921.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J. et al. (2016a) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241-248.

Nguyen, N.H., Williams, L.J., Vincent, J.B., Stefanski, A., Cavender-Bares, J., Messier, C. et al. (2016b) Ectomycorrhizal fungal diversity and saprotrophic fungal diversity are linked to different tree community attributes in a field-based tree experiment. *Molecular Ecology* **25**: 4032-4046.

Nielsen, U.N., Osler, G.H., Campbell, C.D., Burslem, D.F., and van der Wal, R. (2010) The influence of vegetation type, soil properties and precipitation on the composition of soil mite and microbial communities at the landscape scale. *Journal of biogeography* **37**: 1317-1328.

Nuccio, E.E., Hodge, A., Pett-Ridge, J., Herman, D.J., Weber, P.K., and Firestone, M.K. (2013) An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. *Environmental Microbiology* **15**: 1870-1881.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D. et al. (2019) vegan: Community Ecology Package. R package version 2.5–6. 2019. In.

Olivares, J., Bedmar, E.J., and Sanjuán, J. (2013) Biological nitrogen fixation in the context of global change. *Molecular Plant-Microbe Interactions* **26**: 486-494.

Öpik, M., Moora, M., Zobel, M., Saks, Ü., Wheatley, R., Wright, F., and Daniell, T. (2008) High diversity of arbuscular mycorrhizal fungi in a boreal herb-rich coniferous forest. *New Phytologist* **179**: 867-876.

Otsing, E., Anslan, S., Ambrosio, E., Koricheva, J., and Tedersoo, L. (2021) Tree species richness and neighborhood effects on ectomycorrhizal fungal richness and community structure in boreal forest. *Frontiers in microbiology*: 264.

Paquette, A., Hector, A., Castagneyrol, B., Vanhellemont, M., Koricheva, J., Scherer-Lorenzen, M., and Verheyen, K. (2018) A million and more trees for science. *Nature ecology & evolution* **2**: 763-766.

Paradis, E., Claude, J., and Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289-290.

Peay, K.G., Baraloto, C., and Fine, P.V. (2013) Strong coupling of plant and fungal community structure across western Amazonian rainforests. *The ISME journal* **7**: 1852-1861.

Penn, C.J., and Camberato, J.J. (2019) A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture* **9**: 120.

Phillips, R.P., Brzostek, E., and Midgley, M.G. (2013) The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytologist* **199**: 41-51.

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B.D., Clemmensen, K.E., Kauserud, H. et al. (2020) FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity* **105**: 1-16.

Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S. et al. (2015) Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology letters* **18**: 85-95.

Purahong, W., Krüger, D., Buscot, F., and Wubet, T. (2016) Correlations between the composition of modular fungal communities and litter decomposition-associated ecosystem functions. *Fungal Ecology* **22**: 106-114.

Purschke, O., Michalski, S.G., Bruelheide, H., and Durka, W. (2017) Phylogenetic turnover during subtropical forest succession across environmental and phylogenetic scales. *Ecology and Evolution* **7**: 11079-11091.

Püschel, D., Janoušková, M., Voříšková, A., Gryndlerová, H., Vosátka, M., and Jansa, J. (2017) Arbuscular mycorrhiza stimulates biological nitrogen fixation in two Medicago spp. through improved phosphorus acquisition. *Frontiers in plant science* **8**: 390.

Rambelli, A. (1973) The rhizosphere of mycorrhizae. *Ectomycorrhizae: Their ecology and physiology*: 299-343.

Read, D., and Perez-Moreno, J. (2003) Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance? *New phytologist* **157**: 475-492.

Rivers, A.R., Weber, K.C., Gardner, T.G., Liu, S., and Armstrong, S.D. (2018) ITSxpress: software to rapidly trim internally transcribed spacer sequences with quality scores for marker gene analysis. *F1000Research* **7**.

Rivest, M., Whalen, J.K., and Rivest, D. (2019) Tree diversity is not always a strong driver of soil microbial diversity: a 7-yr-old diversity experiment with trees. *Ecosphere* **10**: e02685.

Röttjers, L., and Faust, K. (2018) From hairballs to hypotheses–biological insights from microbial networks. *FEMS microbiology reviews* **42**: 761-780.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G. et al. (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME journal* **4**: 1340-1351.

Rudnick, M., Van Veen, J., and De Boer, W. (2015) Baiting of rhizosphere bacteria with hyphae of common soil fungi reveals a diverse group of potentially mycophagous secondary consumers. *Soil Biology and Biochemistry* **88**: 73-82.

Schöps, R., Goldmann, K., Herz, K., Lentendu, G., Schöning, I., Bruelheide, H. et al. (2018) Land-Use Intensity Rather Than Plant Functional Identity Shapes Bacterial and Fungal Rhizosphere Communities. *Frontiers in Microbiology* **9**.

Schuldt, A., Bruelheide, H., Buscot, F., Assmann, T., Erfmeier, A., Klein, A.M. et al. (2017) Belowground top-down and aboveground bottom-up effects structure multitrophic community relationships in a biodiverse forest. *Sci Rep* **7**: 4222.

Semple, C., and Steel, M. (2003) *Phylogenetics*: Oxford University Press on Demand.

Shi, Y., Delgado-Baquerizo, M., Li, Y., Yang, Y., Zhu, Y.-G., Peñuelas, J., and Chu, H. (2020) Abundance of kinless hubs within soil microbial networks are associated with high functional potential in agricultural ecosystems. *Environment International* **142**: 105869.

Simard, S.W., Beiler, K.J., Bingham, M.A., Deslippe, J.R., Philip, L.J., and Teste, F.P. (2012) Mycorrhizal networks: mechanisms, ecology and modelling. *Fungal Biology Reviews* **26**: 39-60.

Sing, T., Sander, O., Beerenwinkel, N., and Lengauer, T. (2005) ROCR: visualizing classifier performance in R. *Bioinformatics* **21**: 3940-3941.

Singavarapu, B., Beugnon, R., Bruelheide, H., Cesarz, S., Du, J., Eisenhauer, N. et al. (2021) Tree mycorrhizal type and tree diversity shape the forest soil microbiota. *Environmental Microbiology*.

Smith, G.R., Finlay, R.D., Stenlid, J., Vasaitis, R., and Menkis, A. (2017) Growing evidence for facultative biotrophy in saprotrophic fungi: data from microcosm tests with 201 species of wood-decay basidiomycetes. *New Phytologist* **215**: 747-755.

Smith, S., and Read, D. (2008) Mineral nutrition, toxic element accumulation and water relations of arbuscular mycorrhizal plants. *Mycorrhizal symbiosis* **3**: 145-118.

Smith, S.E., and Read, D.J. (2010) *Mycorrhizal symbiosis*: Academic press.

Soudzilovskaia, N.A., van der Heijden, M.G., Cornelissen, J.H., Makarov, M.I., Onipchenko, V.G., Maslov, M.N. et al. (2015) Quantitative assessment of the differential impacts of arbuscular and ectomycorrhiza on soil carbon cycling. *New Phytologist* **208**: 280-293.

Soudzilovskaia, N.A., Vaessen, S., Barcelo, M., He, J., Rahimlou, S., Abarenkov, K. et al. (2020) FungalRoot: global online database of plant mycorrhizal associations. *New Phytologist* **227**: 955-966.

Spatafora, J.W., and Blackwell, M. (1993) Molecular systematics of unitunicate perithecial ascomycetes: The Clavicipitales-Hypocreales connection. *Mycologia* **85**: 912-922.

Statnikov, A., Henaff, M., Narendra, V., Konganti, K., Li, Z., Yang, L. et al. (2013) A comprehensive evaluation of multicategory classification methods for microbiomic data. *Microbiome* **1**: 11.

Stohr, W.G. (2013) Belowground ecosystems: the foundation for forest health, restoration and sustainable management. *Journal of Environmental Assessment Policy and Management* **15**: 1350019.

Strassert, J.F., and Monaghan, M.T. (2022) Phylogenomic insights into the early diversification of fungi. *Current Biology*.

Su, Y., He, Z., Yang, Y., Jia, S., Yu, M., Chen, X., and Shen, A. (2020) Linking soil microbial community dynamics to straw-carbon distribution in soil organic carbon. *Scientific reports* **10**: 1-12.

Sun, Y.-P., Unestam, T., Lucas, S.D., Johanson, K.J., Kenne, L., and Finlay, R. (1999) Exudation-reabsorption in a mycorrhizal fungus, the dynamic interface for interaction with soil and soil microorganisms. *Mycorrhiza* **9**: 137-144.

Swift, M.J., Heal, O.W., Anderson, J.M., and Anderson, J. (1979) *Decomposition in terrestrial ecosystems*: Univ of California Press.

Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., and Zuberer, D.A. (2005) *Principles and applications of soil microbiology*: Pearson.

Tajik, S., Ayoubi, S., and Lorenz, N. (2020) Soil microbial communities affected by vegetation, topography and soil properties in a forest ecosystem. *Applied Soil Ecology* **149**: 103514.

Talbot, J.M., Bruns, T.D., Taylor, J.W., Smith, D.P., Branco, S., Glassman, S.I. et al. (2014) Endemism and functional convergence across the North American soil mycobiome. *Proc Natl Acad Sci U S A* **111**: 6341-6346.

Tansley, A.G. (1935) The Use and Abuse of Vegetational Concepts and Terms. *Ecology* **16**: 284-307.

Taylor, D.L., and Bruns, T.D. (1997) Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. *Proceedings of the National Academy of Sciences* **94**: 4510-4515.

Tedersoo, L., and Nara, K. (2010) General latitudinal gradient of biodiversity is reversed in ectomycorrhizal fungi. *New Phytologist*: 351-354.

Tedersoo, L., and Bahram, M. (2019) Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes. *Biological Reviews* **94**: 1857-1880.

Tedersoo, L., Bahram, M., and Zobel, M. (2020a) How mycorrhizal associations drive plant population and community biology. *Science* **367**.

Tedersoo, L., Mett, M., Ishida, T.A., and Bahram, M. (2013) Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. *New Phytologist* **199**: 822-831.

Tedersoo, L., Pärtel, K., Jairus, T., Gates, G., Põldmaa, K., and Tamm, H. (2009) Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the Helotiales. *Environmental Microbiology* **11**: 3166-3178.

Tedersoo, L., Anslan, S., Bahram, M., Drenkhan, R., Pritsch, K., Buegger, F. et al. (2020b) Regional-scale in-depth analysis of soil fungal diversity reveals strong pH and plant species effects in Northern Europe. *Frontiers in microbiology* **11**: 1953.

Tedersoo, L., Bahram, M., Cajthaml, T., Polme, S., Hiiesalu, I., Anslan, S. et al. (2016) Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. *The ISME journal* **10**: 346-362.

Tedersoo, L., Bahram, M., Toots, M., Diedhiou, A.G., Henkel, T.W., Kjoller, R. et al. (2012) Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology* **21**: 4160-4170.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R. et al. (2014) Global diversity and geography of soil fungi. *Science* **346**: 1256688.

Teste, F.P., Jones, M.D., and Dickie, I.A. (2020) Dual-mycorrhizal plants: their ecology and relevance. *New Phytologist* **225**: 1835-1851.

Tian, Y., Takanashi, K., Toda, H., Haibara, K., and Ding, F. (2013) pH and substrate regulation of nitrogen and carbon dynamics in forest soils in a karst region of the upper Yangtze River basin, China. *Journal of forest research* **18**: 228-237.

Tilman, D. (1999) The ecological consequences of changes in biodiversity: a search for general principles. *Ecology* **80**: 1455-1474.

Tilman, D., Isbell, F., and Cowles, J.M. (2014) Biodiversity and ecosystem functioning. *Annual review of ecology, evolution, and systematics* **45**: 471-493.

Tipton, L., Muller, C.L., Kurtz, Z.D., Huang, L., Kleerup, E., Morris, A. et al. (2018) Fungi stabilize connectivity in the lung and skin microbial ecosystems. *Microbiome* **6**: 12.

Toju, H., Sato, H., and Tanabe, A.S. (2014) Diversity and spatial structure of belowground plant-fungal symbiosis in a mixed subtropical forest of ectomycorrhizal and arbuscular mycorrhizal plants. *PLoS One* **9**: e86566.

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hamonts, K., Anderson, I.C., and Singh, B.K. (2017) Keystone microbial taxa regulate the invasion of a fungal pathogen in agro-ecosystems. *Soil Biology and Biochemistry* **111**: 10-14.

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C. et al. (2016) Microbial regulation of the soil carbon cycle: evidence from gene–enzyme relationships. *The ISME journal* **10**: 2593-2604.

Trogisch, S., Albert, G., Du, J., Wang, Y., Xue, K., and Bruelheide, H. (2020) Promoting resilience of large international collaborative research programs in times of global crisis. *Ecology and Evolution* **10**: 12549-12554.

Trogisch, S., Liu, X., Rutten, G., Xue, K., Bauhus, J., Brose, U. et al. (2021) The significance of tree-tree interactions for forest ecosystem functioning. *Basic and Applied Ecology*.

UNEP, F. (2020) The State of the World's Forests 2020: Forests, Biodiversity and People. *FAO UNEP, Rome; https://wwwfaoorg/3/ca8642en/ca8642enpdf*.

Urbanová, M., Šnajdr, J., and Baldrian, P. (2015) Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biology and Biochemistry* **84**: 53-64.

Uroz, S., Calvaruso, C., Turpault, M.-P., Pierrat, J.-C., Mustin, C., and Frey-Klett, P. (2007) Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. *Applied and environmental microbiology* **73**: 3019-3027.

Van Der Heijden, M.G., Bardgett, R.D., and Van Straalen, N.M. (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters* **11**: 296-310.

Verbruggen, E., Pena, R., Fernandez, C.W., and Soong, J.L. (2017) Mycorrhizal interactions with saprotrophs and impact on soil carbon storage. In *Mycorrhizal mediation of soil*: Elsevier, pp. 441-460.

Vick-Majors, T.J., Priscu, J.C., and A Amaral-Zettler, L. (2014) Modular community structure suggests metabolic plasticity during the transition to polar night in ice-covered Antarctic lakes. *The ISME journal* **8**: 778-789.

Vieira, S., Sikorski, J., Dietz, S., Herz, K., Schrumpf, M., Bruelheide, H. et al. (2020) Drivers of the composition of active rhizosphere bacterial communities in temperate grasslands. *The ISME journal* **14**: 463-475.

Wagg, C., Bender, S.F., Widmer, F., and van der Heijden, M.G. (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences* **111**: 5266-5270.

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E., and van der Heijden, M.G.A. (2019) Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nature Communications* **10**: 4841.

Waldrop, M.P., Zak, D.R., Blackwood, C.B., Curtis, C.D., and Tilman, D. (2006) Resource availability controls fungal diversity across a plant diversity gradient. *Ecology Letters* **9**: 1127-1135.

Wallander, H., Lindahl, B.D., and Nilsson, L.O. (2006) Limited transfer of nitrogen between wood decomposing and ectomycorrhizal mycelia when studied in the field. *Mycorrhiza* **16**: 213-217.

Wang, B., and Qiu, Y.-L. (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**: 299-363.

Wang, D.Y.-C., Kumar, S., and Hedges, S.B. (1999) Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proceedings of the Royal Society of London Series B: Biological Sciences* **266**: 163-171.

Wang, G., Koziol, L., Foster, B.L., and Bever, J.D. (2022) Microbial mediators of plant community response to long-term N and P fertilization: Evidence of a role of plant responsiveness to mycorrhizal fungi. *Global Change Biology* **28**: 2721-2735.

Wang, J., Wu, Y., Zhou, J., Bing, H., and Sun, H. (2016) Carbon demand drives microbial mineralization of organic phosphorus during the early stage of soil development. *Biology and fertility of soils* **52**: 825-839.

Wang, Z., Jiang, Y., Deane, D.C., He, F., Shu, W., and Liu, Y. (2019) Effects of host phylogeny, habitat and spatial proximity on host specificity and diversity of pathogenic and mycorrhizal fungi in a subtropical forest. *New Phytologist* **223**: 462-474.

Wang, Z., Wang, Z., Li, T., Wang, C., Dang, N., Wang, R. et al. (2021) N and P fertilization enhanced carbon decomposition function by shifting microbes towards an r-selected community in meadow grassland soils. *Ecological Indicators* **132**: 108306.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., Van Der Putten, W.H., and Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *science* **304**: 1629-1633.

Warmink, J., Nazir, R., and Van Elsas, J. (2009) Universal and species-specific bacterial 'fungiphiles' in the mycospheres of different basidiomycetous fungi. *Environmental Microbiology* **11**: 300-312.

Watkinson, S.C. (2016) Mutualistic symbiosis between fungi and autotrophs. In *The fungi*: Elsevier, pp. 205-243.

Wehner, J., Powell, J.R., Muller, L.A., Caruso, T., Veresoglou, S.D., Hempel, S., and Rillig, M.C. (2014) Determinants of root-associated fungal communities within A steraceae in a semi-arid grassland. *Journal of Ecology* **102**: 425-436.

Wei, Z., Gu, Y., Friman, V.-P., Kowalchuk, G.A., Xu, Y., Shen, Q., and Jousset, A. (2019) Initial soil microbiome composition and functioning predetermine future plant health. *Science advances* **5**: eaaw0759.

Weißbecker, C., Buscot, F., and Wubet, T. (2017) Preservation of nucleic acids by freeze-drying for next generation sequencing analyses of soil microbial communities. *Journal of Plant Ecology* **10**: 81-90.

Weißbecker, C., Heintz-Buschart, A., Bruelheide, H., Buscot, F., and Wubet, T. (2019) Linking Soil Fungal Generality to Tree Richness in Young Subtropical Chinese Forests. *Microorganisms* **7**: 547.

Weissbecker, C., Heintz-Buschart, A., Bruelheide, H., Buscot, F., and Wubet, T. (2019) Linking Soil Fungal Generality to Tree Richness in Young Subtropical Chinese Forests. *Microorganisms* **7**.

Weißbecker, C., Wubet, T., Lentendu, G., Kühn, P., Scholten, T., Bruelheide, H., and Buscot, F. (2018) Experimental evidence of functional group-dependent effects of tree diversity on soil fungi in subtropical forests. *Frontiers in Microbiology* **9**: 2312.

White, T.J., Bruns, T., Lee, S., and Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* **18**: 315-322.

Wickham, H., Chang, W., and Wickham, M.H. (2016) Package 'ggplot2'. *Create elegant data visualisations using the grammar of graphics Version* **2**: 1-189.

Wiens, J.J., and Graham, C.H. (2005) Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual review of ecology, evolution, and systematics*: 519-539.

Woese, C.R., Kandler, O., and Wheelis, M.L. (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences* **87**: 4576-4579.

Wu, J., and Yu, S. (2019) Effect of root exudates of Eucalyptus urophylla and Acacia mearnsii on soil microbes under simulated warming climate conditions. *BMC microbiology* **19**: 1-12.

Wubet, T., Kottke, I., Teketay, D., and Oberwinkler, F. (2009) Arbuscular mycorrhizal fungal community structures differ between co-occurring tree species of dry Afromontane tropical forest, and their seedlings exhibit potential to trap isolates suited for reforestation. *Mycological Progress* **8**: 317-328.

Xu, S., Eisenhauer, N., Ferlian, O., Zhang, J., Zhou, G., Lu, X. et al. (2020) Species richness promotes ecosystem carbon storage: evidence from biodiversity-ecosystem functioning experiments. *Proceedings of the Royal Society B* **287**: 20202063.

Xu, W., Liu, L., He, T., Cao, M., Sha, L., Hu, Y. et al. (2016) Soil properties drive a negative correlation between species diversity and genetic diversity in a tropical seasonal rainforest. *Scientific Reports* **6**: 1-8.

Yamamura, T., Schwendenmann, L., and Lear, G. (2013) Tree species identity has little impact on the structure of soil bacterial communities in a 10-year-old tropical tree plantation. *Biology and fertility of soils* **49**: 819-828.

Yang, B., Liang, Y., Schmid, B., Baruffol, M., Li, Y., He, L. et al. (2022) Soil Fungi Promote Biodiversity–Productivity Relationships in Experimental Communities of Young Trees. *Ecosystems* **25**: 858-871.

Yarwood, S.A. (2018) The role of wetland microorganisms in plant-litter decomposition and soil organic matter formation: a critical review. *FEMS Microbiology Ecology* **94**: fiy175.

Yuan, M.M., Kakouridis, A., Starr, E., Nguyen, N., Shi, S., Pett-Ridge, J. et al. (2021) Fungal-Bacterial Cooccurrence Patterns Differ between Arbuscular Mycorrhizal Fungi and Nonmycorrhizal Fungi across Soil Niches. *Mbio* **12**: e03509-03520.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., and Tilman, D. (2003) Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology* **84**: 2042-2050.

Zang, X., Liu, M., Fan, Y., Xu, J., Xu, X., and Li, H. (2018) The structural and functional contributions of β-glucosidase-producing microbial communities to cellulose degradation in composting. *Biotechnology for biofuels* **11**: 1-13.

Zhang, L., Xu, M., Liu, Y., Zhang, F., Hodge, A., and Feng, G. (2016) Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. *New Phytologist* **210**: 1022-1032.

Zhao, K., Kong, W., Wang, F., Long, X.-E., Guo, C., Yue, L. et al. (2018) Desert and steppe soils exhibit lower autotrophic microbial abundance but higher atmospheric CO2 fixation capacity than meadow soils. *Soil Biology and Biochemistry* **127**: 230-238.

Zhou, J., Deng, Y., Shen, L., Wen, C., Yan, Q., Ning, D. et al. (2016) Temperature mediates continental-scale diversity of microbes in forest soils. *Nature communications* **7**: 1-10.

Zhu, C., Wang, Z., Luo, W., Feng, J., Chen, Y., He, D. et al. (2022) Fungal phylogeny and plant functional traits structure plant–rhizosphere fungi networks in a subtropical forest. *Oikos*: e08992.

Appendices

Author Contributions

Chapter II

Conceptualization - TW, HB, BS; field sampling - BS, RB; data generation - BS, RB, JD; bioinformatic analysis and data curation - BS, AN, TW; statistical analysis and visualization - BS; writing the original manuscript draft - BS; review and editing of the manuscript – All authors. My overall contribution was 80%.

Chapter III

Conceptualization – BS, TW, HB; data generation - BS; bioinformatic analysis and data curation - BS, TW; statistical analysis and visualization - BS; writing the original manuscript draft - BS; review and editing of the manuscript – All authors. My overall contribution was 85%.

Chapter IV

Conceptualization – BS, TW, HB; field sampling - BS, FD, RB; data generation – FD, BS, AN; bioinformatic analysis and data curation – TW, BS; statistical analysis and visualization – TW, FD, BS; writing the original manuscript draft – BS, HU; BS led the writing; review and editing of the manuscript – All authors. My overall contribution was 60%.

Acknowledgements

It is a nostalgic feeling to look back on the past four and a half years of this arduous journey and to acknowledge the people who were with me along the way. This is a deeply satisfying moment, and I am humbled and grateful to those who accompanied me on this long voyage towards obtaining my doctoral degree. I could not have made this journey without the love, support, and encouragement of my family, friends, and colleagues.

I am deeply grateful to my supervisors, Dr. Tesfaye Wubet and Prof. Dr. Helge Bruelheide, for their outstanding guidance and support throughout my PhD journey. Thank you for entrusting me with such an exciting and challenging project that helped me grow as a researcher. Tesfaye, I cannot thank you enough for your invaluable support as my supervisor, mentor, and friend. Your care and attention to detail ensured the project progressed without mistakes, while your patience and understanding towards my inevitable errors were appreciated. Thanks to you, publishing my first paper was a transformative experience that propelled my growth as a researcher. Your suggestions and talks, not just limited to science but also personal matters, were highly appreciated. Helge, your constant support and encouragement have been invaluable to me throughout my journey. I am deeply grateful for your brilliant ideas and suggestions in data analysis and manuscript editing, which have greatly benefited my work. Despite your busy schedule, I appreciate the time and understanding you have shown me, and your positive attitude has always been uplifting. Thank you for your unwavering support and for always being there with a smile.

I felt a warm and welcoming sense of belonging at the Community Ecology Department of UFZ. I am extremely grateful for the incredible support and friendship of all my colleagues. Ali, your unwavering support and our conversations throughout this journey have been invaluable. Ellen and Julia, I can't thank you enough for your assistance with all the bureaucratic work. To all my lovely colleagues, including Eleonore, Anna, Mar, Vicki, Johannes, Qun, Chong, Hafeez, Amelie, Friedrich, Lotte, Marina, Chris and other alumni, thank you for being there and for your friendly support. I am also grateful to Walter, Stefan, Ina, Martina, and Antja for your support.

I would like to express my gratitude to the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation – 319936945/GRK2324), German Centre for Integrative Biodiversity Research (iDiv), funded by the German Research Foundation (DFG– FZT

118, 202548816), and UFZ HiGRADE for their funding. Special thanks to Stefan for his support and coordination. I also want to thank all the members of TreeDi and BEF China platform for their support. All of my amazing colleagues and friends from TreeDi – Mariem, Pertu, Andrea, Remy, Georg, Maria, Florain, Gonzalo, LiYi, Huiyun Gan, Sirong, Jianqing, Tobi, Sue and others – Thank you.

To my father, Singavarapu Uma MaheswaraRao, my sister Lavanya, and other family members and personal friends, your constant love and support has been indispensable. I extend my heartfelt gratitude to each and every one of you. To my dear wife, Ramyahta, I cannot thank you enough for your support and patience in this journey. Your support and patience have been remarkable, especially during my unavailability and frustrations. Thank you for always taking care of me.

- Bala

Curriculum Vitae

Personal Information
Name: Bala Veera Venkata Apparao Singavarapu
Email: balu.singavarapu@gmail.com

Education

06/2018 – 07/2023	**Ph.D. student**, at Helmholtz-Centre for Environmental Research - UFZ, Department Community Ecology & Martin Luther University Halle-Wittenberg (Institute for Biology, Geobotany) Research topic: "Tree mycorrhizal type and tree diversity effects on the structure and functional potential of forest belowground microbial communities" Supervisors: Prof. Dr. Helge Bruelheide, Dr. Tesfaye Wubet
10/2014-12/2017	**M.Sc AgriGenomics**, Christian-Albrechts-Universität, Kiel, Germany Title master thesis: "Metaprofiling of wheat phylloplane microbial endophyte communities" Supervisor: Prof. Dr. Eva Stukenbrock
06/2007-04/2009	**M.Sc Molecular Genetics**, Andhra University, Visakhapatnam, India Key subjects: Molecular biology, Biostatistics, Bioinformatics, Molecular and Population genetics
06/2003-03/2006	**Bachelor of Science**, Andhra University, Visakhapatnam, India Key subjects: Chemistry, Biochemistry, Biotechnology

Scientific & Professional Employment

Since 01/2023	**Researcher (**Postdoc position**)** on the project "Using Bioinformatics to understand Ecosystem Health" at Institute for Biodiversity, Chair of Aquatic Geomicrobiology, Friedrich Schiller University Jena and German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Germany. PI: Prof. Dr. Kirsten Küsel
03/2013 - 08/2013	**DNA Sequencing Executive**, Eurofins Genomics, Bengaluru, India
07/2010 - 01/2013	**Molecular Biologist**, Vimta labs, Hyderabad, India

Scientific Memberships and Awards

Since 2015	Member of the German Genetics Society (GfG)
2011- 2012	Outstanding Performance Award in the Cell and Molecular biology division, Vimta, Hyderabad, India

Publications and Conference Contributions

Publications

Publications in international peer-reviewed journals:

Beugnon, R., …, **Singavarapu, B**., …, Cesarz S (2023). Tree diversity effects on litter decomposition are mediated by litterfall and microbial processes. Oikos. doi.org/10.1111/oik.09751.

Singavarapu B, Du J, Beugnon R, Cesarz S, Eisenhauer N, Xue K, Wang Y, Bruelheide H & Wubet T (2023). Functional Potential of Soil Microbial Communities and Their Subcommunities Varies with Tree Mycorrhizal Type and Tree Diversity. Microbiology spectrum, e0457822. https://doi.org/10.1128/spectrum.04578-22**.**

Beugnon, R., …, **Singavarapu, B**., …, Eisenhauer, N. (2023). Abiotic and Biotic Drivers of Tree Trait Effects on Soil Microbial Biomass and Soil Carbon Concentration." Ecological Monographs e1563. https://doi.org/10.1002/ecm.1563

Singavarapu, B., Beugnon, R., Bruelheide, H., Cesarz, S., Du, J., Eisenhauer, N., Guo, L.-D., Nawaz, A., Wang, Y., Xue, K. and Wubet, T. (2022), Tree mycorrhizal type and tree diversity shape the forest soil microbiota. Environ Microbiol, 24: 4236-4255. https://doi.org/10.1111/1462-2920.15690

Al Naggar, Y., **Singavarapu, B**., Paxton, R. J., & Wubet, T. (2022), Bees under interactive stressors: the novel insecticides flupyradifurone and sulfoxaflor along with the fungicide azoxystrobin disrupt the gut microbiota of honey bees and increase opportunistic bacterial pathogens. Science of The Total Environment. 2022.157941. https://doi.org/10.1016/j.scitotenv.2022.157941

Beugnon, R., …, **Singavarapu, B**., …, Eisenhauer, N. (2021). Tree diversity and soil chemical properties drive the linkages between soil microbial community and ecosystem functioning. ISME COMMUN. 1, 41 (2021). https://doi.org/10.1038/s43705-021-00040-0

Submitted:

Slabbert, EL., …, **Singavarapu, B**., …, Knight, TM. (submitted). Large- and small-scale factors driving multi-trophic communities in agroecosystems. *Landscape Ecology*

Singavarapu, B., Ul Haq, H., Darnstaedt, F., Nawaz, A., Eisenhauer, N., ... Bruelheide, H and Wubet, T. (submitted). Influence of Tree Mycorrhizal Type, Tree Species Identity, and Diversity on Forest Root-Associated Mycobiomes. *New phytologist*

Conference contributions

Singavarapu, B., Wubet, T. *Tree mycorrhizal type and tree diversity effects on rhizosphere interaction zone microbiomes in sub-tropical forests.* (Oral contribution). TreeDì-BEF China Seminar Series, online, 2020.

Singavarapu, B., Wubet, T. *TSP Rhizosphere microbiome – progress report.* (Oral contribution). TreeDì Doctoral Conference, Beijing, China, 2019.

Singavarapu, B., Nawaz, A., Bruelheide, H & Wubet, T. *Tree neighbor diversity and mycorrhizal trait affect the rhizosphere microbiome assemblages of tree species*. (Poster). International conference on Mycorrhiza, ICOM10, Mexico, 2019

Singavarapu, B., Nawaz, A., Bruelheide, H & Wubet, T. *Neighbor diversity affects the rhizosphere microbiome assemblages of tree species pairs in subtropical forest ecosystem*. (Poster). International conference on Rhizosphere, Rhizosphere5, Canada, 2019.

Declaration of Independence / Eigenständigkeitserklärung

I hereby declare that I have written this dissertation entitled "Tree mycorrhizal type and tree diversity effects on the structure and functional potential of forest belowground microbial communities" independently and without outside help and that I have not used any sources or aids other than those indicated in the text. Text passages, which were taken over from used works literally or in contents, were marked by me as such. I further declare that I have never applied for a doctoral degree before. This doctoral thesis has not been submitted to the Faculty of Natural Sciences I - Biosciences of the Martin Luther University Halle-Wittenberg or any other scientific institution for the purpose of a doctorate.

Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit mit dem Titel „Tree mycorrhizal type and tree diversity effects on the structure and functional potential of forest belowground microbial communities“ eigenständig und ohne fremde Hilfe verfasst sowie keineanderen als die im Text angegebenen Quellen und Hilfsmittel verwendet habe. Textstellen, welche aus verwendeten Werken wörtlich oder inhaltlich übernommenwurden, wurden von mir als solche kenntlich gemacht. Ich erkläre weiterhin, dass ichmich bisher noch nie um einen Doktorgrad beworben habe. Die vorliegendeDoktorarbeit wurde bis zu diesem Zeitpunkt weder bei der NaturwissenschaftlichenFakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg nocheiner anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt.

Halle (Saale),

Bala Veera Venkata Apparao Singavarapu