Assembly of root-associated fungi in different soil layers and nitrogen uptake by ectomycorrhizae in temperate forests

Dissertation

for the award of the degree

"Doctor rerum naturalium" (Dr. rer. nat.)

of the Georg-August-Universität Göttingen

within the doctoral program Forest Science and Forest Ecology of the Graduate School Forest and Agricultural Sciences (GFA)

Submitted by

Anis Mahmud Khokon

From Tangail, Bangladesh

Göttingen, July 2021

Referee: Prof. Dr. Andrea Polle

Co-referee: Prof. Dr. Christian Ammer

Date of oral examination: 01.07.2021

Table of Contents

List of Abbreviation

a.m.	Ante meridiem
Acer	Acer pseudoplatanus
AIC	Akaike's information criterion
ALB	Schwäbische Alb
ANOSIM	Analyses of similarity
ANOVA	Analysis of variance
APE	Atomic percent excess
ASV	Amplicon sequence variants
ATP	Adenosine triphosphate
AU	Autumn
Avg.	Average
Beech	*Fagus sylvatica*
BLAST	Basic Local Alignment Search Tools
bp	Base pairs
C	Carbon
Ca	Calcium
$CaCl_2$	Calcium chloride
DFG	German Research Foundation
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide
DW	Dry weight
EMF	Ectomycorrhizal fungi
EMRT	Ectomycorrhizal root tips
et al.	and others
Eqn.	Equation
FDR	False discovery rate
Fe	Iron
$FeSO_4$	Iron (II) sulfate
Fraxinus	Fraxinus excelsior
FW	Fresh weight
g	Gram
GAMLSS	Generalized additive model for location, scale and shape
glm	Generalized linear model

glmer	Generalized linear mixed-effects models
h	hour
H'	Shannon diversity index
HAI	Hainich-Dün
HCl	Hydrogen chloride
HNO_3	Nitric acid
HSD	Honestly significant difference
IPCC	Intergovernmental Panel on Climate Change
ITS	Internal transcribed spacer
K	Potassium
K_2HPO_4	Dipotassium phosphate
Kb	Kilobyte
km	Kilometer
L	Litre
LASSO	Least absolute shrinkage and selection operator
lm	Linear model
lmer	Linear mixed-effects models
m	Metre
M	Molar concentration
mg	Milligram
Mg	Magnesium
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulfate
min	Minutes
Mio	Million
ml	Millilitre
mmol	Millimoles
Mn	Manganese
$MnCl_2$	Manganese (II) chloride
MT	Morphotypes
N	Nitrogen
n	Number of replicates
NaCl	Sodium chloride
NADP	Nicotinamide adenine dinucleotide phosphate

NCBI	National Center for Biotechnology Information
NH_4^+	Ammonium
NM	Non-mycorrhizal
NMDS	Non-metric multidimensional scaling
NO_3^-	Nitrate
^oc	Degree Celsius
OR	Odds ratio
OTU	Operational taxonomic units
P	Phosphorous
p	probability of error
p.m.	Post meridiem
PAT	Pathotrophic
PCR	Polymerase chain reaction
pH	Negative log of the activity of the hydrogen ion
Picea	*Picea abies*
Pinus	*Pinus sylvestris*
Psol	Plant available phosphorous
Quercus	*Quercus robur*
RAF	Root-associated fungi
RDA	Redundancy analysis
rpm	Revolutions per minute
RRI	Root resource index
RT	Root tips
s	Second
S	Sulphur
SAP	Saprotrophic
SCH	Schorfheide-Chorin
SE	Standard error
SP	Spring
SRI	Soil resource index
SU	Summer
SYM	Symbiotrophic
Tilia	*Tilia sp.*
TSR	Tree species richness

USA	Unites States of America
v.	Version
VIF	Variance inflation factors
VRT	Vital root tips
β-diversity	Beta diversity
β_{SIM}	Turnover
β_{SNE}	Nestedness
β_{SOR}	Total beta diversity
µg	Microgram
µl	Microliter
µmol	Micromole
µmol	Micromoles
%	Percentage

List of Figures

Supplement Figures

List of Tables

Supplement Tables

Summary

Fungi are a remarkably highly diverse group of organisms on Earth, playing a pivotal role in ecosystem functioning. Belowground, they act as decomposers, pathogens or symbionts. Members of these functional categories are colonizing roots and have been defined as root-associated fungi. The abundance and distribution patterns of fungal communities are determined by various environmental factors, including soil and root properties, vegetation and climatic conditions. Soil fungal communities are known to be vertically stratified across different soil layers but our knowledge about root-associated fungal assemblages in different soil layers is still limited. Ectomycorrhizal fungi are an important functional group in the root-associated fungal assemblage that can enhance host nutrient uptake. Nitrogen (N) is an essential nutrient for plant growth but often a growth-limiting factor in temperate forest ecosystems. To date, functional diversity of ectomycorrhizal fungi for plant N uptake under natural forest system is not fully understood.

Here, I studied the vertical differentiation of root-associated fungi in the organic layer (Oe and Oa) and mineral topsoil (0-10 cm) under the framework of the Biodiversity Exploratories that includes 150 experimental forest plots across three biogeographical regions in Germany. These three biogeographic regions are located in the Schwäbische Alb in south-western, Hainich-Dün in central and Schorfheide-Chorin in north-eastern Germany. Furthermore, I used beech (*Fagus sylvatica*) as a host to investigate the contribution of distinct ectomycorrhizal fungal taxa in their natural assemblage for N uptake.

This thesis is structured in three main research chapters. Chapter one aimed to enhance our understanding of fungal niche partitioning by studying commonalities and differences of assembly processes of root-associated fungi in the organic layer and mineral topsoil. I hypothesised that: i) species richness and relative abundance of symbiotrophic fungi are higher than those of saprotrophic fungi, irrespective of the soil layer; ii) the taxonomic community composition of symbiotrophic and saprotrophic fungi differ significantly between the organic layer and mineral soil and shows lower turnover for symbiotrophic than for saprotrophic fungi between the soil layers; iii) root-associated fungal patterns indicate selection either to soil strata or to climatic-edaphic factors. The results revealed a clear separation of root-associated fungal community composition between soil layers. Saprotrophic fungi showed the highest richness in organic layer and symbiotrophic in mineral soil. Still, symbiotrophic fungi exhibited higher relative sequence abundances than saprotrophic fungi in both soil layers. β-diversity of root-associated fungi was mainly due to turnover between the organic layer and mineral soil and

showed regional differences for symbiotrophic and saprotrophic fungi. Regional differences were also found for different phylogenetic levels, i.e., fungal orders and indicator species in the organic layer and mineral soil, supporting that habitat conditions strongly influence the differentiation of root-associated fungal assemblages. Important exceptions were fungal orders that occurred irrespective of the habitat conditions in distinct soil layers across the biogeographic gradient: Russulales and Cantharellales (ectomycorrhizal fungi) were enriched in root-associated fungal assemblages in mineral soil, whereas saprotrophic Polyporales and Sordariales and ectomycorrhizal Boletales were enriched in the organic layer. These results underpin phylogenetic signature for niche partitioning at the rank of fungal orders and suggest that root-associated fungal assembly entails two strategies encompassing flexible and territorial habitat colonization by different fungal taxa.

Chapter two aimed to uncover the environmental factors that drive root-associated fungi in the organic layer and mineral soil. Soil and root chemistry as well as tree species were included as potential environmental factors in the analyses. I tested the following hypotheses in the organic layer and mineral topsoil separately: i) tree species identity influences symbiotrophic fungal richness due to fungal host preferences but not that of saprotrophic fungal richness; therefore symbiotrophic fungal richness increases with tree species richness, while saprotrophic fungi remain unaffected; ii) symbiotrophic fungal richness increases with increasing root nutrient resources, while saprotrophic fungal richness increases with soil nutrient resources. The results revealed that tree species richness positively influenced the richness of all fungi, symbiotrophic and saprotrophic in mineral soil but not in the organic layer. Among the tree species, only *Tilia* showed significant influence on saprotrophic fungal richness in the organic layer. In contrast, *Fagus sylvatica*, *Fraxinus excelsior*, *Picea abies* and *Quercus robur* showed significant positive effect on symbiotrophic and *Fraxinus excelsior* on saprotrophic fungal richness in mineral soil. Root and soil resource index showed positive relationships with the richness of symbiotrophic and saprotrophic fungi in mineral soil. Variance partitioning showed that only 5% of the variation was explained for symbiotrophic and saprotrophic fungal richness in the organic layer but about 68% was explained for symbiotrophic richness and 24% for saprotrophic richness in the mineral soil with the significant contributions of the variables soil resource index, root resource index, tree species richness and main tree species on the plot. Overall, these results suggest that the relationship of root-associated fungal richness with vegetation and nutrient resources vary in different habitat conditions. Forest floor strongly overrules the effects of tree species and nutrient resources on root-associated fungal richness in temperate forests.

Chapter three aimed to investigate whether functional diversity of ectomycorrhizal fungi determines beech N uptake in temperate forest. I tested the following hypotheses: i) ectomycorrhizal taxon-specific identity drives beech N uptake; alternatively, ectomycorrhizal fungal diversity plays a significant role in beech N uptake (ii) intraspecific ^{15}N enrichment of ectomycorrhizal fungal species shows significant differences for NH_4^+ and NO_3^-. The results indicated that both beech ectomycorrhizal root tips and root segments do not discriminate between the offered inorganic N sources. NH_4^+ derived ^{15}N was always more enriched compared to NO_3^- derived ^{15}N in ectomycorrhizal root tips and beech root segments. Significant differences in interspecific ^{15}N enrichment occurred among different ectomycorrhizal fungal species for both NH_4^+ and NO_3^-. Despite a strong interspecific variation in ^{15}N enrichment among the ectomycorrhizal fungi species, no species were identified that had substantial effects on beech N uptake but beech root N uptake increased with increasing ectomycorrhizal fungal diversity. Overall, these results suggest that it is more beneficial for beech N uptake to have a high diversity of ectomycorrhizal fungi than few species fostering high N uptake.

Zusammenfassung

Pilze sind eine bemerkenswert vielfältige Gruppe von Organismen auf der Erde, die eine zentrale Rolle für das Funktionieren von Ökosystemen spielen. Unterirdisch agieren sie als Zersetzer, Krankheitserreger oder Symbionten. Mitglieder dieser Funktionskategorien besiedeln Wurzeln und wurden als wurzelassoziierte Pilze definiert. Die Abundanz und die Verteilungsmuster von Pilzgemeinschaften werden durch verschiedene Umweltfaktoren bestimmt, darunter Boden- und Wurzeleigenschaften, Vegetation und klimatische Bedingungen. Es ist bekannt, dass Pilzgemeinschaften im Boden über verschiedene Bodenschichten hinweg vertikal verteilt sind, aber unser Wissen über wurzelassoziierte Pilzgemeinschaften in verschiedenen Bodenschichten ist noch begrenzt. Ektomykorrhizapilze sind eine wichtige funktionelle Gruppe in der wurzelassoziierten Pilze, die die Nährstoffaufnahme des Wirts verbessern kann. Stickstoff (N) ist ein essentieller Nährstoff für das Wachstum und die Entwicklung von Pflanzen, aber oft ein limitierender Faktor in Waldökosystemen. Bis heute ist die funktionelle Diversität von Ektomykorrhizapilzen für die pflanzliche N-Aufnahme in natürlichen Waldsystemen nicht vollständig verstanden.

Hier habe ich die vertikale Differenzierung von wurzelassoziierten Pilzen in der organischen Schicht (Oe und Oa) und im mineralischen Oberboden (0-10 cm) im Rahmen der Biodiversitätsexploratorien untersucht, die 150 Waldparzellen in drei biogeographischen Regionen in Deutschland umfassen. Diese Regionen befinden sich auf der Schwäbischen Alb im Südwesten, im Hainich-Dün in der Mitte und in der Schorfheide-Chorin im Nordosten Deutschlands. Außerdem habe ich Buche (Fagus sylvatica) als Wirt verwendet, um den Beitrag verschiedener Ektomykorrhizapilz-Taxa in ihrer natürlichen Zusammensetzung zur N-Aufnahme zu untersuchen.

Diese Arbeit ist in drei Hauptforschungskapitel gegliedert. Kapitel eins zielte darauf ab, unser Verständnis der Nischenaufteilung von Pilzen zu verbessern, indem ich Gemeinsamkeiten und Unterschiede der Zusammensetzungsprozesse von wurzelassoziierten Pilzen im organischen und mineralischen Oberboden untersuchte. Ich stellte die Hypothese auf, dass: i) der Artenreichtum und die relative Abundanz symbiotischer Pilze höher sind als die saprotropher Pilze, unabhängig von der Bodenschicht; ii) die taxonomische Gemeinschaftszusammensetzung symbiotischer und saprotropher Pilze sich signifikant zwischen organischer Schicht und Mineralboden unterscheidet und einen geringeren Umsatz für symbiotrophe als für saprotrophe Pilze zwischen den Bodenschichten zeigt; iii) wurzelassoziierte Pilzmuster weisen auf eine Selektion entweder auf Bodenschichten oder auf

klimatisch-edaphische Faktoren hin. Die Ergebnisse zeigten eine klare Trennung der Zusammensetzung der Wurzel-assoziierten Pilzgemeinschaft zwischen den Bodenschichten. Saprotrophe Pilze zeigten die höchste Abundanz in organischen und symbiotrophe in mineralischen Böden. Dennoch wiesen symbiotrophe Pilze in beiden Bodenschichten höhere relative Sequenzhäufigkeiten auf als saprotrophe Pilze. Die β-Diversität der wurzelassoziierten Pilze war hauptsächlich auf den Umsatz zwischen organischem und mineralischem Boden zurückzuführen und zeigte regionale Unterschiede für symbiotrophe und saprotrophe Pilze. Regionale Unterschiede wurden auch für verschiedene phylogenetische Ebenen, d.h. Pilzordnungen und Indikatorarten im organischen und mineralischen Boden gefunden, was dafür spricht, dass die Habitatbedingungen die Differenzierung der wurzelassoziierten Pilzgefüge stark beeinflussen. Wichtige Ausnahmen waren Pilzordnungen, die unabhängig von den Habitatbedingungen in verschiedenen Bodenschichten über den biogeographischen Gradienten hinweg auftraten: Russulales und Cantharellales (Ektomykorrhizapilze) waren in wurzelassoziierten Pilzgefügen in Mineralböden angereichert, während saprotrophe Polyporales und Sordariales und ektomykorrhizierende Boletales in der organischen Schicht angereichert waren. Diese Ergebnisse untermauern die phylogenetische Signatur für die Nischenaufteilung auf der Ebene der Pilzordnungen und deuten darauf hin, dass wurzelassoziierte Pilzansammlungen zwei Strategien beinhalten, die eine flexible und territoriale Habitatbesiedlung durch verschiedene Pilztaxa umfassen.

Kapitel zwei zielte darauf ab, die Umweltfaktoren aufzudecken, die wurzelassoziierte Pilze in den organischen Schichten und im Mineralboden antreiben. Die Boden- und Wurzelchemie sowie die Baumart wurden als potenzielle Umweltfaktoren in die Analysen einbezogen. Ich testete die folgenden Hypothesen in der organischen Schicht und im mineralischen Oberboden getrennt: i) die Baumartenidentität beeinflusst den symbiotrophen Pilzreichtum aufgrund der Wirtspräferenzen der Pilze, nicht aber den saprotrophen Pilzreichtum; daher steigt der symbiotrophe Pilzreichtum mit dem Baumartenreichtum, während der saprotrophe Pilzreichtum unbeeinflusst bleibt; ii) der symbiotrophe Pilzreichtum steigt mit zunehmenden Nährstoffressourcen der Wurzeln, während der saprotrophe Pilzreichtum mit den Nährstoffressourcen des Bodens steigt. Die Ergebnisse zeigten, dass der Baumartenreichtum den Reichtum aller Pilze, symbiotropher und saprotropher, im Mineralboden positiv beeinflusst, nicht aber in der organischen Schicht. Unter den Baumarten zeigte nur *Tilia* einen signifikanten Einfluss auf den saprotrophen Pilzreichtum in der organischen Schicht. Im Gegensatz dazu zeigten *Fagus sylvatica*, *Fraxinus excelsior*, *Picea abies* und *Quercus robur* einen signifikant positiven Effekt auf den symbiotrophen und *Fraxinus excelsior* auf den

saprotrophen Pilzreichtum im Mineralboden. Wurzel- und Bodenressourcenindex zeigten positive Beziehungen mit dem Reichtum an symbiotrophen und saprotrophen Pilzen im Mineralboden. Die Varianzaufteilung zeigte, dass nur 5% der Variation für den Reichtum an symbiotrophen und saprotrophen Pilzen in der organischen Schicht erklärt wurde, aber 68% für den Reichtum an symbiotrophen Pilzen und 24% für den Reichtum an saprotrophen Pilzen in der mineralischen Schicht mit den signifikanten Beiträgen der Variablen Bodenressourcenindex, Wurzelressourcenindex, Baumartenreichtum und Baumidentität. Insgesamt deuten diese Ergebnisse darauf hin, dass die Beziehung zwischen dem Reichtum an wurzelassoziierten Pilzen und den Vegetations- und Nährstoffressourcen in verschiedenen Habitatbedingungen variiert. Der Waldboden setzt die Auswirkungen von Baumarten und Nährstoffressourcen auf den wurzelassoziierten Pilzreichtum in gemäßigten Wäldern außer Kraft.

In Kapitel drei sollte untersucht werden, ob die funktionelle Diversität von Ektomykorrhizapilzen die N-Aufnahme von Buchen in gemäßigten Wäldern bestimmt. Ich testete die folgenden Hypothesen: i) Ektomykorrhiza-Taxon-spezifische Identität treibt die N-Aufnahme der Buche an; alternativ spielt die Ektomykorrhiza-Pilz-Diversität eine signifikante Rolle bei der N-Aufnahme der Buche; ii) intraspezifische ^{15}N-Anreicherung der Ektomykorrhiza-Pilzarten zeigt signifikante Unterschiede für NH_4+ und NO_3-. Die Ergebnisse zeigten, dass sowohl ektomykorrhizischen Wurzelspitzen als auch Wurzelsegmente nicht zwischen den angebotenen anorganischen N-Quellen unterscheiden. NH_4^+ abgeleitetes ^{15}N war in den ektomykorrhizischen Wurzelspitzen und Buchen wurzelsegmenten immer stärker angereichert als NO_3^- abgeleitetes ^{15}N. Signifikante Unterschiede in der interspezifischen ^{15}N-Anreicherung traten zwischen verschiedenen Ektomykorrhiza-Pilzarten sowohl für NH_4+ als auch für NO_3- auf. Trotz einer starken interspezifischen Variation in der ^{15}N-Anreicherung unter den Ektomykorrhizapilzarten wurden keine Arten identifiziert, die wesentliche Auswirkungen auf die N-Aufnahme der Buche hatten, aber die N-Aufnahme der Buchenwurzeln stieg mit zunehmender Ektomykorrhizapilz-Diversität. Insgesamt deuten diese Ergebnisse darauf hin, dass es für die N-Aufnahme der Buche vorteilhafter ist, eine hohe Diversität an Ektomykorrhizapilzen zu haben als wenige Arten, die eine hohe N-Aufnahme fördern.

CHAPTER 1

General Introduction

1.1 Temperate forest ecosystem

Forests harbour about two-third of terrestrial plants (Fichtner and Härdtle, 2021; Hobohm, 2021) and offer many ecosystem services (Gamfeldt et al., 2013; Wardle et al., 2004). Forest ecosystems have always been a fundamental part of human existence by providing timber, energy, food, and recreation (Fichtner and Härdtle, 2021; Hobohm, 2021). A total of 10.4 million km^2 areas are covered by temperate forest, which is about 6% of all the Earth's ecosystems (de Gouvenain and Silander, 2017; McCarragher and Rigg, 2017). Temperate forests dominate in North America, England, and northern Europe, eastern China and Japan (de Gouvenain and Silander, 2017; McCarragher and Rigg, 2017). The climatic characteristics in these temperate forest regions show distinct cyclic and temporal changes pattern involving periods of growth and dormancy (de Gouvenain and Silander, 2017; Gilliam, 2016; McCarragher and Rigg, 2017). Such seasonal changing patterns may differ in different temperate region, latitude and topographic features (de Gouvenain and Silander, 2017; Gilliam, 2016; McCarragher and Rigg, 2017). For example, temperate forests at high latitudes usually have shorter growing seasons than those at low latitudes (de Gouvenain and Silander, 2017; Gilliam, 2016; McCarragher and Rigg, 2017). The biodiversity of temperate forests has changed compared to the past decades due to the historic extensive use and land conversion by human activities (Franklin, 1988; Gilliam, 2016).

1.1.2 Temperate forest in Germany

The temperate forest area covers over 11.4 million hectares (~32% of the total area) in Germany (Friedrich et al., 2015). The key climatic characteristics of these regions are relatively warm summers without frost (~ 30^{o} C) and quite cold winter (~ -20^{o} C); plant receives enough rainfall throughout the growing season and year-round (Leuschner and Ellenberg, 2017; Schröter et al., 2006). Such climatic characteristics are beneficial for the growth of broadleaved deciduous trees (Bréda et al., 2006; Frelich et al., 2015). In the past years, sustainable forest management practices were applied in Germany (Häusler and Scherer-Lorenzen, 2001), introducing a higher proportion of deciduous tree species such as European beech (*Fagus sylvatica*) and oak (*Quercus sp.*) (Friedrich et al., 2015; Leuschner and Ellenberg, 2017). As a result, German forests are now more diversely structured than in the past decades (Friedrich et al., 2015). In 2012, the European beech forested area was 1,680,072 hectares and accounted for about 21% of the total forested areas in Germany (Friedrich et al., 2015; Wühlisch and Muhs, 2010). Today, beech is one of the most abundant tree species as well as preferred by forest practitioners and governments in afforestation programmes due to its high economic value (Bolte et al., 2007; Friedrich et al., 2015; Leuschner and Ellenberg, 2017; Wühlisch and Muhs, 2010). However,

beech is relatively sensitive to drought (Bolte et al., 2007; Leuschner and Ellenberg, 2017) and, thus, the predicted climate change with longer periods of drought might favour other tree species in the future. There are several other tree species that co-occur with beech in temperate forest, including maple (*Acer pseudoplatanus*), pine (*Pinus sylvestris*) and spruce (*Picea abies*) (Leuschner and Ellenberg, 2017) but some like spruce are also drought susceptible.

1.2 Nitrogen in temperate forests

Nitrogen (N) is an essential nutrient for plant growth and development (Ollivier et al., 2011; Rennenberg and Dannenmann, 2015), but often a growth-limiting factor in temperate forests (Rennenberg et al., 2009). To encounter low N availability in soil, plant developed symbiotic and mutualistic interactions with below-ground microbes to turn inaccessible N into simple and readily available N (Becquer et al., 2019; Chalot and Plassard, 2011; Courty et al., 2015). In forest soil, NH_4^+ and NO_3^- are the main inorganic N forms that are easily accessible to ectomycorrhizal fungi and transfer to their host (Courty et al., 2015; Gobert and Plassard, 2008; Fleck et al., 2019). The concentration of NH_4^+ and NO_3^- showed strong seasonal and regional fluctuations in forest soil solution (Dannenmann et al., 2009; Nacry et al., 2013). Plant N availability is strongly influenced by the activities of roots and ectomycorrhizal fungi (Courty et al., 2015; Chalot and Plassard, 2011). In the past decade, atmospheric N deposition has increased worldwide due to anthropogenic activities and irregular summer drought (Galloway et al., 2004). Increased N deposition can negatively affect below-ground microbial community composition in forest ecosystems (de Witte et al., 2017; Lilleskov et al., 2002).

1.3 Root-associated fungi and their role in ecosystem functioning

Fungi are highly diverse microorganism on Earth and play an important role in terrestrial ecosystem functioning (Blackwell, 2011; Heijden et al., 2008). In forest ecosystem, fungi are in intimate association with the roots of the tree and have been defined as root-associated fungi (RAF) (Vandenkoornhuyse et al., 2002). RAF are taxonomically and functionally highly diverse and perform multiple ecological functions (Baldrian, 2017; Vandenkoornhuyse et al., 2015). RAF communities consist of different functional groups that can be classified according to their lifestyles as symbiotrophic (SYM), saprotrophic (SAP) and pathotrophic (PAT) fungi (Baldrian, 2017). These ecological functional groups have different but distinct roles in biogeochemical cycling (Baldrian, 2017). While SAP fungi are mainly responsible for decomposing organic materials and for maintaining carbon (C) cycling (Baldrian, 2017), SYM fungi are promoting mineral nutrition to the plants in exchange for photosynthetically derived

carbohydrate (Heijden et al., 2015). PAT fungi cause disease and negatively affect plant growth and development (García-Guzmán and Heil, 2014).

Because of their important roles in biogeochemical cycling in forests, the assembly processes of soil fungi have received considerable attention in ecological studies. In general, the assembly of fungal communities is governed by two ecological processes, e.g. deterministic processes, (i.e. environmental filtering) (Kraft et al., 2015) and stochastic processes, (i.e. random changes) (Hubbel, 2001). There is little evidence that stochastic processes have strong impact on fungal assemblages (Cline and Zak, 2014). Rather environmental filtering, including factors such as temperature, precipitation, soil pH, vegetation types, soil and root chemistry are strongly determined the fungal assemblages (Bahram et al., 2018; Birkhofer et al., 2012; Goldmann et al., 2015; Nguyen et al., 2020; Schröter et al., 2019; Tedersoo et al., 2014; Wang et al., 2020; Wubet et al., 2012). RAF, which are a sub-community of soil fungi underlie only partly the same assembly processes as soil fungi (Goldmann et al., 2016), but the drivers of RAF communities are still not well understood.

1.4. Ectomycorrhizal fungi and their role in tree nutrition

Ectomycorrhizal fungi (EMF) are an important component of RAF and form the most significant tree symbionts in many boreal and temperate forest ecosystems (Blackwell, 2011; Heijden et al., 2008). Approximately 95% of the boreal and temperate forest tree roots are colonized with EMF, which can supply mineral nutrient (e.g. N and P) to the host (Becquer et al., 2019; Courty et al., 2010). Therefore, EMF plays pivotal roles in tree nutrition, especially in forests with low nutrient availability (Becquer et al., 2019). Ectomycorrhizal fungal taxa are morphologically heterogeneous and functionally differ in their ability in N acquisition (Lilleskov et al., 2002; Pena and Polle, 2014). Thus, EMF species are an important factor for plant nutrient uptake. Species diversity of EMF is linked to functional diversity (Pena et al., 2013). For example, high diversity of EMF leads to enhanced phosphorous uptake efficiency (Köhler et al., 2018). High EMF species richness is positively related to soil peroxidase activity (Phillips et al., 2014; Talbot et al., 2013), which may enhance access to N from organic material. EMF diversity further benefited the uptake of inorganic N under drought stress (Pena and Polle, 2014). However, it is unknown how variation in the species composition of natural fungal communities affects root N uptake.

1.5 The Biodiversity Exploratory research project

To uncover the influence of forest management and environmental variation on ecosystem functions, the Biodiversity Exploratories were established in 2006 across three biogeographic

regions in Germany (www.biodiversity-exploratories.de) (Fischer et al., 2010). Geographically these regions are located: Schorfheide-Chorin in northeast, Hainich-Dün in central and Schwäbische-Alb in southwest of Germany (Fischer et al., 2010). Each region has unique characteristics that distinguish it from the other regions, and together they reflect various landscapes, climatic conditions, soil types, land-use intensities, and forest management types. Many scientific groups are working in these research regions covering various research topics, including microbiology, zoology and botany, which allow comprehensive interdisciplinary biological research. The combined results are used to investigate the interconnectedness between different species and the influence of their diversity on ecosystem functioning (Felipe-Lucia et al., 2018).

With respect to soil science, a joint soil sampling campaign has taken place every three years since 2008 across all three Exploratories, which enables the researchers to compare the results from the various scientific groups. The collected research data are easily accessible to all the participating scientist, enabling multidisciplinary data analyses and underpin the benefits of having the Biodiversity Exploratories platform (Felipe-Lucia et al., 2018). Moreover, the organization of the consortium provides a well-established platform to perform a large spatial scale and long-term functional biodiversity research under changing land use and biogeographic regions. Therefore, the Biodiversity Exploratories have been chosen for the current PhD study, which is focused on increasing knowledge on the assembly processes of RAF and their role in tree nutrition.

1.6 Goal of the thesis

This thesis was aimed to investigate the assembly patterns of RAF with different functional groups in the organic layer and mineral topsoil, to uncover the factors that drive RAF vertical assemblages, and to investigate whether functional diversity of EMF determines beech N uptake in temperate forests (Figure 1.1).

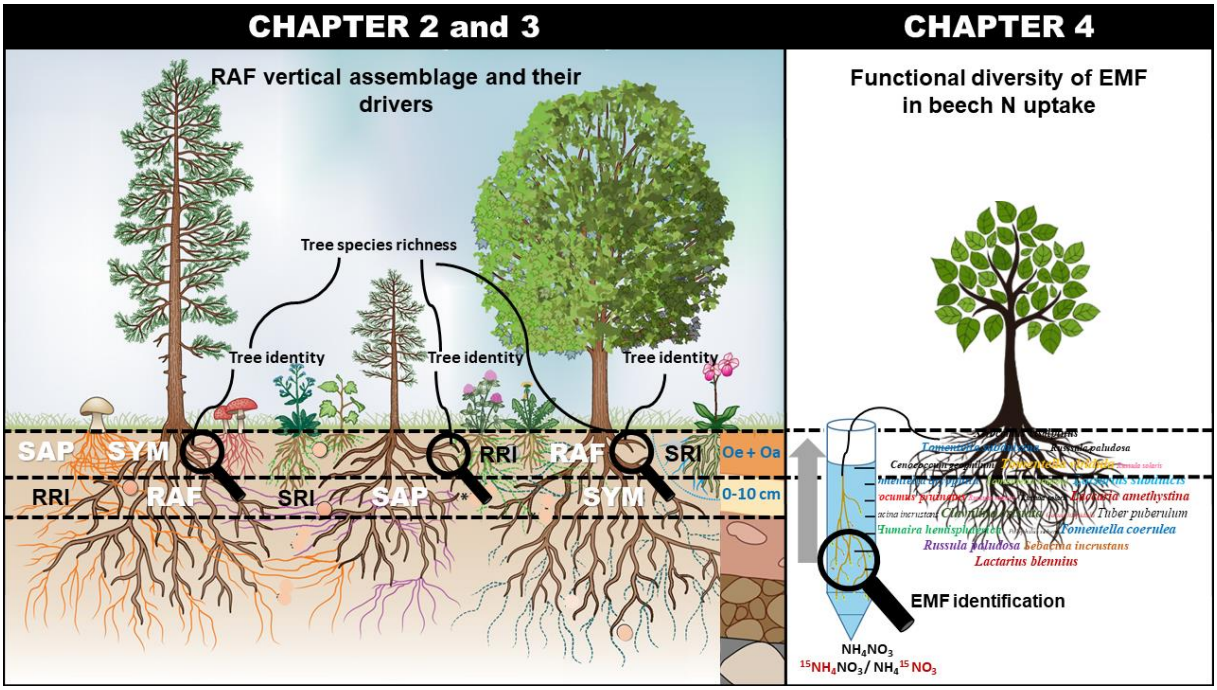


Figure 1.1: Schematic overview of the three research chapters presented in this doctoral thesis. The studies were conducted in mixed forest stands (chapter 2 and 3) and in pure beech forest stands (Chapter 4) in temperate forests. Chapter 2 investigated the vertical assembly patterns of root-associated fungi (RAF-all fungi), symbiotrophic (SYM) and saprotrophic (SAP) fungi in organic layers (Oe and Oa) and mineral topsoil (0-10 cm). Chapter 3 uncovered the factors that drive RAF, SYM and SAP fungal richness in organic layers and mineral topsoil. Environmental facrots that were used in the chapters 3 are tree species identiy, tree species richness, root resources (RRI) and soil resources (SRI). Chapter 4 investigated the functional diversity of ectomycorrhizal fungi (EMF) determines beech N uptake under field conditions. Figure 1.1 was modified from van der Heijden et al., (2015).

The following specific aims were addressed:

i. To investigate the assembly patterns of RAF with different functional groups in different soil layers (Chapter 2), I analysed the habitat and regional preference of different functional groups and different phylogenetic levels, i.e. fungal orders and indicator taxa in the organic layer and mineral topsoil and in different biogeographic regions.

ii. To uncover the environmental factors that drive RAF vertical assemblages (Chapter 3), I tested the influence of soil chemistry, root chemistry and tree species on RAF richness in the organic layer and mineral topsoil.

iii. To investigate whether functional diversity of EMF determines beech N uptake in temperate forests. I tested the influence of EMF taxon-specific and EMF diversity on beech root N uptake using stable isotope experiments in natural forest system.

1.7 References

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., Bengtsson-Palme, J., Anslan, S., Coelho, L.P., Harend, H., Huerta-Cepas, J., Medema, M.H., Maltz, M.R., Mundra, S., Olsson, P.A., Pent, M., Põlme, S., Sunagawa, S., Ryberg, M., Tedersoo, L., Bork, P., 2018. Structure and function of the global topsoil microbiome. Nature 560, 233–237. https://doi.org/10.1038/s41586-018-0386-6

Baldrian, P., 2017. Microbial activity and the dynamics of ecosystem processes in forest soils. Current Opinion in Microbiology, Environmental microbiology, 9 37, 128–134. https://doi.org/10.1016/j.mib.2017.06.008

Becquer, A., Guerrero-Galán, C., Eibensteiner, J.L., Houdinet, G., Bücking, H., Zimmermann, S.D., Garcia, K., 2019. The ectomycorrhizal contribution to tree nutrition, Advances in Botanical Research, Molecular Physiology and Biotechnology of Trees. Academic Press, pp. 77–126. https://doi.org/10.1016/bs.abr.2018.11.003

Birkhofer, K., Schöning, I., Alt, F., Herold, N., Klarner, B., Maraun, M., Marhan, S., Oelmann, Y., Wubet, T., Yurkov, A., Begerow, D., Berner, D., Buscot, F., Daniel, R., Diekötter, T., Ehnes, R.B., Erdmann, G., Fischer, C., Foesel, B., Groh, J., Gutknecht, J., Kandeler, E., Lang, C., Lohaus, G., Meyer, A., Nacke, H., Näther, A., Overmann, J., Polle, A., Pollierer, M.M., Scheu, S., Schloter, M., Schulze, E.-D., Schulze, W., Weinert, J., Weisser, W.W., Wolters, V., Schrumpf, M., 2012. General relationships between abiotic soil properties and soil biota across spatial scales and different land-use types. PLOS ONE 7, e43292. https://doi.org/10.1371/journal.pone.0043292

Blackwell, M., 2011. The Fungi: 1, 2, 3 … 5.1 million species? American Journal of Botany 98, 426–438. https://doi.org/10.3732/ajb.1000298

Bolte, A., Czajkowski, T., Kompa, T., 2007. The north-eastern distribution range of European beech—a review. Forestry: An International Journal of Forest Research 80, 413–429. https://doi.org/10.1093/forestry/cpm028

Bréda, N., Huc, R., Granier, A., Dreyer, E., 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. Ann. For. Sci. 63, 625–644. https://doi.org/10.1051/forest:2006042

Chalot, M., Plassard, C., 2011. Ectomycorrhiza and nitrogen provision to the host tree, Ecological aspects of nitrogen metabolism in plants. John Wiley & Sons, Ltd, pp. 69–94. https://doi.org/10.1002/9780470959404.ch4

Cline, L.C., Zak, D.R., 2014. Dispersal limitation structures fungal community assembly in a long-term glacial chronosequence. Environmental Microbiology 16, 1538–1548. https://doi.org/10.1111/1462-2920.12281

Courty, P.-E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.-P., Uroz, S., Garbaye, J., 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biology and Biochemistry 42, 679–698. https://doi.org/10.1016/j.soilbio.2009.12.006

Courty, P.E., Smith, P., Koegel, S., Redecker, D., Wipf, D., 2015. Inorganic nitrogen uptake and transport in beneficial plant root-microbe interactions. Critical Reviews in Plant Sciences 34, 4–16. https://doi.org/10.1080/07352689.2014.897897

de Gouvenain, R.C., Silander, J.A., 2017. Temperate forests, reference module in life sciences. Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.02310-4

Dannenmann, M., Simon, J., Gasche, R., Holst, J., Naumann, P.S., Kögel-Knabner, I., Knicker, H., Mayer, H., Schloter, M., Pena, R., Polle, A., Rennenberg, H., Papen, H., 2009. Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biology and Biochemistry 41, 1622–1631. https://doi.org/10.1016/j.soilbio.2009.04.024

Felipe-Lucia, M.R., Soliveres, S., Penone, C., Manning, P., van der Plas, F., Boch, S., Prati, D., Ammer, C., Schall, P., Gossner, M.M., Bauhus, J., Buscot, F., Blaser, S., Blüthgen, N., de Frutos, A., Ehbrecht, M., Frank, K., Goldmann, K., Hänsel, F., Jung, K., Kahl, T., Nauss, T., Oelmann, Y., Pena, R., Polle, A., Renner, S., Schloter, M., Schöning, I., Schrumpf, M., Schulze, E.-D., Solly, E., Sorkau, E., Stempfhuber, B., Tschapka, M., Weisser, W.W., Wubet, T., Fischer, M., Allan, E., 2018. Multiple forest attributes underpin the supply of multiple ecosystem services. Nature Communications 9, 4839. https://doi.org/10.1038/s41467-018-07082-4

Fichtner, A., Härdtle, W., 2021. Forest Ecosystems: A functional and biodiversity perspective, Perspectives for Biodiversity and Ecosystems. Springer International Publishing, Cham, pp. 383–405. https://doi.org/10.1007/978-3-030-57710-0_16

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.-D., Weisser, W.W., 2010. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic and Applied Ecology 11, 473–485. https://doi.org/10.1016/j.baae.2010.07.009

Fleck, S., Eickenscheidt, N., Ahrends, B., Evers, J., Grüneberg, E., Ziche, D., Höhle, J., Schmitz, A., Weis, W., Schmidt-Walter, P., Andreae, H., Wellbrock, N., 2019. Nitrogen status and dynamics in German forest soils. Springer International Publishing, Cham, pp. 123–166. https://doi.org/10.1007/978-3-030-15734-0_5

Franklin, J.F., 1988. Structural and functional diversity in temperate forests. In: Wilson, E. O., ed. Biodiversity. Washington, DC: National Academy Press: 166-175.

Frelich, L.E., Montgomery, R.A., Oleksyn, J., 2015. Northern temperate forests. Routledge Handbook of Forest Ecology 30–45. https://doi.org/10.4324/9781315818290

Friedrich S, Polley H, Hennig P, Kroiher F, Marks A, Riedel T, Schmidt U, Schwitzgebel F, Stauber T (2015) The forests in Germany: Selected results of the third national forest inventory. Federal Ministry of Food and Agriculture.

Gamfeldt, L., Snäll, T., Bagchi, R., Jonsson, M., Gustafsson, L., Kjellander, P., Ruiz-Jaen, M.C., Fröberg, M., Stendahl, J., Philipson, C.D., Mikusiński, G., Andersson, E.,

Westerlund, B., Andrén, H., Moberg, F., Moen, J., Bengtsson, J., 2013. Higher levels of multiple ecosystem services are found in forests with more tree species. Nature Communications 4, 1340. https://doi.org/10.1038/ncomms2328

García-Guzmán, G., Heil, M., 2014. Life histories of hosts and pathogens predict patterns in tropical fungal plant diseases. New Phytologist 201, 1106–1120. https://doi.org/10.1111/nph.12562

Gilliam, F.S., 2016. Forest ecosystems of temperate climatic regions: from ancient use to climate change. New Phytologist 212, 871–887. https://doi.org/10.1111/nph.14255

Goldmann, K., Schöning, I., Buscot, F., Wubet, T., 2015. Forest management type influences diversity and community composition of soil fungi across temperate forest ecosystems. Front. Microbiol. 6. https://doi.org/10.3389/fmicb.2015.01300

Häusler A, Michael SL (2001) Sustainable forest management in Germany: The ecosystem approach of the biodiversity convention reconsidered, German Federal Agency for Nature Conservation.

Heijden, M.G.A.V.D., Bardgett, R.D., Straalen, N.M.V., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Heijden, M.G.A. van der, Martin, F.M., Selosse, M.-A., Sanders, I.R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist 205, 1406–1423. https://doi.org/10.1111/nph.13288

Hobohm, C., 2021. Perspectives for biodiversity and ecosystems, Environmental Challenges and Solutions. Springer International Publishing. https://doi.org/10.1007/978-3-030-57710-0

Hubbel, S.P., 2001. The unified neutral theory of biodiversity and biogeography (MPB-32).

Köhler, J., Yang, N., Pena, R., Raghavan, V., Polle, A., Meier, I.C., 2018. Ectomycorrhizal fungal diversity increases phosphorus uptake efficiency of European beech. New Phytologist 220, 1200–1210. https://doi.org/10.1111/nph.15208

Kraft, N.J.B., Adler, P.B., Godoy, O., James, E.C., Fuller, S., Levine, J.M., 2015. Community assembly, coexistence and the environmental filtering metaphor. Functional Ecology 29, 592–599. https://doi.org/10.1111/1365-2435.12345

Leuschner, C., Ellenberg, H., 2017. Ecology of central European forests: vegetation ecology of central Europe, Volume I. Springer International Publishing. https://doi.org/10.1007/978-3-319-43042-3

Lilleskov, E.A., Fahey, T.J., Horton, T.R., Lovett, G.M., 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83, 104–115. https://doi.org/10.1890/0012-9658(2002)083[0104:BEFCCO]2.0.CO;2

McCarragher, S., Rigg, L.S., 2017. Temperate forest ecosystems, International encyclopedia of geography. American Cancer Society, pp. 1–14. https://doi.org/10.1002/9781118786352.wbieg0508

Nacry, P., Bouguyon, E., Gojon, A., 2013. Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant Soil 370, 1–29. https://doi.org/10.1007/s11104-013-1645-9

Nguyen, D.Q., Schneider, D., Brinkmann, N., Song, B., Janz, D., Schöning, I., Daniel, R., Pena, R., Polle, A., 2020. Soil and root nutrient chemistry structure root-associated fungal assemblages in temperate forests. Environmental Microbiology 22, 3081–3095. https://doi.org/10.1111/1462-2920.15037

Ollivier, J., Töwe, S., Bannert, A., Hai, B., Kastl, E.-M., Meyer, A., Su, M.X., Kleineidam, K., Schloter, M., 2011. Nitrogen turnover in soil and global change. FEMS Microbiology Ecology 78, 3–16. https://doi.org/10.1111/j.1574-6941.2011.01165.x

Pena, R., Polle, A., 2014. Attributing functions to ectomycorrhizal fungal identities in assemblages for nitrogen acquisition under stress. The ISME Journal 8, 321–330. https://doi.org/10.1038/ismej.2013.158

Pena, R., Tejedor, J., Zeller, B., Dannenmann, M., Polle, A., 2013. Interspecific temporal and spatial differences in the acquisition of litter-derived nitrogen by ectomycorrhizal fungal assemblages. New Phytologist 199, 520–528. https://doi.org/10.1111/nph.12272

Phillips, L.A., Ward, V., Jones, M.D., 2014. Ectomycorrhizal fungi contribute to soil organic matter cycling in sub-boreal forests. The ISME Journal 8, 699–713. https://doi.org/10.1038/ismej.2013.195

Rennenberg, H., Dannenmann, M., Gessler, A., Kreuzwieser, J., Simon, J., Papen, H., 2009. Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. Plant Biology 11, 4–23. https://doi.org/10.1111/j.1438-8677.2009.00241.x

Rennenberg, H., Dannenmann, M., 2015. Nitrogen nutrition of trees in temperate forests—The significance of nitrogen availability in the pedosphere and atmosphere. Forests 6, 2820–2835. https://doi.org/10.3390/f6082820

Schröter, K., Wemheuer, B., Pena, R., Schöning, I., Ehbrecht, M., Schall, P., Ammer, C., Daniel, R., Polle, A., 2019. Assembly processes of trophic guilds in the root mycobiome of temperate forests. Molecular Ecology 28, 348–364. https://doi.org/10.1111/mec.14887

Schröter, D., Zebisch, M., Grothmann, T., 2006. Climate Change in Germany—Vulnerability and Adaptation of Climate-Sensitive Sectors. pp. 44–56.

Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Peay, K.G., 2013. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil Biology and Biochemistry 57, 282–291. https://doi.org/10.1016/j.soilbio.2012.10.004

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., Kesel, A.D., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science 346. https://doi.org/10.1126/science.1256688

Vandenkoornhuyse, P., Baldauf, S.L., Leyval, C., Straczek, J., Young, J.P.W., 2002. Extensive fungal diversity in plant roots. Science 295, 2051–2051. https://doi.org/10.1126/science.295.5562.2051

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Van, A.L., Dufresne, A., 2015. The importance of the microbiome of the plant holobiont. New Phytologist 206, 1196–1206. https://doi.org/10.1111/nph.13312

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Putten, W.H. van der, Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633. https://doi.org/10.1126/science.1094875

Wang, Y.-L., Gao, C., Chen, L., Ji, N.-N., Wu, B.-W., Lü, P.-P., Li, X.-C., Qian, X., Maitra, P., Babalola, B.J., Zheng, Y., Guo, L.-D., 2020. Community assembly of endophytic fungi in ectomycorrhizae of betulaceae plants at a regional scale. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.03105

Wubet, T., Christ, S., Schöning, I., Boch, S., Gawlich, M., Schnabel, B., Fischer, M., Buscot, F., 2012. Differences in soil fungal communities between European beech (Fagus sylvatica L.) dominated forests are related to soil and understory vegetation. PLOS ONE 7, e47500. https://doi.org/10.1371/journal.pone.0047500

Wühlisch, G. von, Muhs, H.J., 2010. Current state of European beech (*Fagus sylvatica* L.) forests in Germany. Communicationes Instituti Forestalis Bohemicae 25, 113–121.

CHAPTER 2

Soil layers matter: vertical stratification of root-associated fungal assemblages in temperate forest reveals phylogenetic signature

2.1 Introduction

Fungi are a remarkably highly diverse group of organisms on Earth, playing significant roles in terrestrial biogeochemical cycles (Blackwell, 2011; Heijden et al., 2008). In temperate forests, saprotrophic, symbiotrophic, and pathotrophic fungi have distinct tasks in carbon and nutrient cycling (Baldrian, 2017). Saprotrophic fungi are mainly responsible for decomposing plant litter in the forest floor and for maintaining carbon cycling, whereas symbiotrophic fungi such as mycorrhizal fungi colonize roots and mine the soil for mineral nutrients, which they deliver to the plant in exchange for photosynthetically derived carbohydrates (Baldrian, 2017). Pathogenic fungi cause diseases and thereby may eventually structure the composition of the vegetation (García-Guzmán and Heil, 2014). A small change in these microbial structures can significantly impact matter fluxes and ecosystem functioning (Orwin et al., 2011).

Because of their eminent roles in nutrient cycling in forests, the assembly processes of belowground fungal communities have received increasing attention. In general, stochastic processes such as dispersal and random changes (Hubbel, 2001) have little impact on fungal assemblages (Cline and Zak, 2014). The composition of soil fungal communities is mainly driven by habitat filtering, i.e., deterministic processes (Bahram et al., 2018; Kraft et al., 2015; Tedersoo et al., 2014). Environmental factors with strong effects on the fungal community composition in soil of temperate forests include temperature, precipitation, soil properties, vegetation, etc., (Birkhofer et al., 2012; Goldmann et al., 2015; Tedersoo et al., 2014; Wang et al., 2014; Wubet et al., 2012).

In addition to forest soil, roots are an important habitat for belowground fungi (Vandenkoornhuyse et al., 2015). The fungi colonize the rhizoplane and grow saprotrophically or interact with living tissue as endophytes, pathogens or symbionts (Vandenkoornhuyse et al., 2015). Based on their habitat, these fungi have been defined as root-associated fungi (RAF) (Vandenkoornhuyse et al., 2002) and are considered as critical components of the plant microbiome (Vandenkoornhuyse et al., 2015). Like soil fungal assemblages, the RAF composition in temperate forests is shaped by various biotic and abiotic environmental conditions including tree species, soil pH, soil moisture, availability of mineral nitrogen and phosphorus and the soil C/N ratio (Carteron et al., 2020; Clausing et al., 2021; Goldmann et al., 2016; Nguyen et al., 2020; Schröter et al., 2019). However, along large-scale environmental gradients these factors have a lower impact on the composition of RAF than on the composition of fungi in soil supporting specific recruiting patterns for RAF (Goldmann et al., 2016). The composition of RAF is also associated with root properties, especially root N contents (Nguyen

et al., 2020; Schröter et al., 2019). The impact of soil and root chemistry structures RAF assembly and was evident by distinct effects on phylogenetic groups at the rank of fungal orders (Nguyen et al., 2020).

In addition to biogeographic conditions that influence the habitat properties, soil depth is a further important factor structuring fungal communities (Asplund et al., 2019; Carteron et al., 2020; Clemmensen et al., 2015; Lindahl et al., 2007; Schlatter et al., 2018; Toju et al., 2016). The forest floor is characterized by high organic carbon (C) and N contents and a wider C/N ratio, whereas the mineral topsoil contains narrower C/N ratios and generally decreasing availabilities of nutrient resources (Herold et al., 2014; Jobbágy and Jackson, 2001). The transition from the organic layer to mineral topsoil often shows a sharp border associated a drastic shift in soil chemistry (Herold et al., 2014; Jobbágy and Jackson, 2001). The structuring gradient of soil layers has mainly been studied for soil fungi, showing that saprotrophic fungi were more abundant in the uppermost soil layers composed of fresh litter and decomposing organic material, whereas mycorrhizal fungi dominated deeper in the mineral soil (Carteron et al., 2020; Clemmensen et al., 2013a; Lindahl et al., 2007; Rosling et al., 2003). The influence of soil horizons on fungal composition was even stronger than that of other environmental factors (Asplund et al., 2019; Carteron et al., 2020). Fine-scale analyses of vertical distribution of fungi have also been conducted in different soil depth and shown vertical niche partitioning of ectomycorrhizal fungi on roots (Taylor and Bruns, 1999), as fungal hyphae (Dickie et al., 2002), or both (Genney et al., 2006). Despite the huge impact of soil horizons on structuring fungal assemblages, studies on the stratification of RAF in organic layer and mineral soil are scarce and entirely lacking for large-scale biogeographic regions.

The goal of this study was to enhance our understanding of fungal niche partitioning by studying commonalities and differences of assembly processes of RAF in the organic layer and mineral top soil across different biogeographic regions. Therefore, we used 150 experimental forest plots of a large-scale infrastructure for biodiversity studies (Biodiversity Exploratories) in typical European forests mainly composed of beech (*Fagus sylvatica*) and conifers (*Picea abies, Pinus sylvestris*) (Seidel et al., 2020). The Biodiversity Exploratories are located in the northeast, the middle and the southwest of Germany, encompassing areas of about 450 to 1,300 km^2. The northeast is characterized by drier and warmer climate and sandy soils, whereas the middle and the southwest have lower temperatures, higher soil moisture and silty or loamy soils (Fischer et al., 2010; Gan et al., 2020; Solly et al., 2014). We used this setup to investigate fungal assemblages on roots in organic layer and mineral soil. Since beech and conifer roots in

the forest plots of the current experiment are massively colonized by ectomycorrhizal fungi (Pena et al., 2017), we hypothesized that i) species richness and relative abundance of symbiotrophic fungi is higher than that of saprotrophic fungi in RAF assemblages, irrespective of the soil layer. We further hypothesized that ii) the taxonomic community composition of symbiotrophic and saprotrophic fungi differs significantly between organic layer and mineral soil and shows lower turnover for symbiotrophic than for saprotrophic fungi between the soil layers. We hypothesized that iii) RAF patterns indicate selection either to soil strata or to regional habitat conditions. To test the latter hypothesis, we compared the responses of phylogenetically related taxa (at the rank of fungal orders) to mineral and organic soil across different biogeographic regions. Phylogenetic signature to mineral or organic soil is expected to result in consistent RAF patterns in different biogeographic regions, while varying patterns of RAF orders in different regions indicate flexible adjustment to divergent climatic-edaphic factors. Moreover, we expected to discover root-associated indicator species for distinct soil layers and for different biogeographic regions representing territorial or flexible behaviour.

2.2 Materials and methods

2.2.1 Study sites characteristics

This study was conducted in 150 experimental forest plots in the Biodiversity Exploratories (http://www.biodiversity-exploratories.de, Fischer et al., 2010). The Biodiversity Exploratories are located in three biogeographic regions: Schorfheide-Chorin (SCH) in the northeast, Hainich-Dün (HAI) in the centre and Schwäbische Alb (ALB) in the southwest region of Germany. The soil types vary among the regions, with silty soils in ALB, loamy soils in HAI and sandy soils in SCH (Gan et al., 2020). The plots are located in an average range of about 53 to 141 years-old forest stands mainly composed of Fagaceae (*Fagus sylvatica* or *Quercus sp.*) and Pinaceae (*Picea abies* and *Pinus sylvestris*) (Seidel et al., 2020). Additional soil and climatic characteristics have been compiled in Supplementary Table S2.1.

2.2.2 Root and soil sampling from organic and mineral soil layers

In each region, roots and soil were sampled in 50 experimental plots (Fischer et al., 2010) in May 2017. In each plot, two transects of 40 m length from north to south and from east to west were established, and soil was collected at a distance of 4.5, 10.5, 16.5, 22.5, 28.5, 34.5 and 40.5 m along the transects as described previously (Nguyen et al., 2020). At each sampling point, organic layer (Oe and Oa horizons) and mineral topsoil (to a depth of 10 cm) were sampled separately, resulting in 14 samples per soil layer. In each plot, the samples per layer were combined to one sample of the organic and one sample of the mineral soil. Subsequently,

the soil was sieved. Fine roots (< 2 mm in diameter) were collected and washed in tap water. Approximately 1 g of fine roots were frozen in liquid nitrogen in the field and stored at -80 °C. The remaining roots and aliquots of soil samples were stored at +4 °C.

2.2.3 Determination of soil chemical properties

2.2.3.1 Carbon and nitrogen:

Soil samples were dried at 40 °C for two weeks and ground to a homogenous fine powder with a ball mill (Retsch, Type MM400, Haan, Germany). Aliquots of dry soil powder (10 mg organic soil, and 30 mg mineral soil) were weighed into in 4 mm x 6 mm tin cartouches (IVA Analysentechnik, Meerbusch, Germany) using a super-micro balance (S4, Sartorius, Goettingen, Germany). Total soil carbon (C) and nitrogen (N) were measured by dry combustion in a CN analyzer "Vario Max" (Elementar Analysensysteme GmbH, Hanau, Germany). We used Acetanilide (71.09% C, 10.36% N) as the standard.

2.2.3.2 Phosphorus and basic cations:

Potentially plant-available phosphorus (P_{sol}) was extracted according to the method of Bray and Kurtz (Bray and Kurtz, 1945). Approximately 100 mg of dry soil powder was mixed with 150 ml of Bray extraction solution (0.03 N NH_4F, 0.025 N HCl). The suspension was shaken slowly for 60 min on a rotary shaker and subsequently filtered through phosphate-free paper filters (MN 280 1/4 125 mm, Macherey–Nagel, Düren, Germany). P_{sol} was measured in the filtrate by inductively coupled plasma–optical emission mass spectroscopy (ICP-OES) (iCAP 7000 Series ICP–OES, Thermo Fisher Scientific, Dreieich, Germany). To determine the potassium (K), calcium (Ca) and magnesium (Mg) contents, approximately 40 to 50 mg soil powder was extracted in 25 ml of 65% HNO_3 (Merck, Darmstadt, Germany) for 12 h at 160 °C (Heinrichs et al., 1986). The suspension was filtered (filter papers MN 640 w, width 90 mm, Macherey–Nagel, Düren, Germany) and the filtrate used for element determination by ICP-OES (iCAP 7000 Series, Thermo Fischer Scientific). The calibration was performed with standards of 1 g L^{-1} (Einzelstandards, Bernd Kraft, Duisburg, Germany). The cations per sample (µmol g^{-1} dry soil) were added and the sum of cation was used in further analyses.

2.2.4 DNA extraction and polymerase chain reaction

Frozen fine roots were milled with a Retsch ball mill (Type MM400, Retsch GmbH, Haan, Germany) at a frequency of 30 s^{-1} for 3 min in liquid nitrogen. The genomic DNA was extracted from the frozen root powder with the innuPREP Plant DNA Kit (Analytik Jena AG, Jena, Germany), following the instructions of the manufacturer. We used the internal transcribed spacer (ITS) region 2 for fungal identification as recommended by Horton and Bruns (Horton

and Bruns, 2001). To amplify the fungal ribosomal ITS2 region in roots, we used ITS3KYO2 (5′-GATGAAGAACGYAGYRAA-3′) as the forward primer (Toju et al., 2012) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as the reverse primer (White et al., 1990). The primers contained adapter sequences for MiSeq sequencing (Illumina Inc., San Diego, USA). The polymerase chain reactions (PCR) were conducted as described elsewhere (Nguyen et al., 2020). The size of the PCR products was determined in agarose gel electrophoresis (2% agarose gels, Biozym Scientific GmbH, Hessisch Oldendorf, Germany) with a DNA standard (Thermo Scientific™ GeneRuler™ 1kb DNA Ladder, Life Technologies GMbH, Darmstadt, Germany). The PCR products were purified with a magnetic bead-based Magsi-NGS*PREP* kit (Steinbrenner Laborsysteme GmbH, Wiesenbach, Germany) according to the manufacturer's instructions. The PCR products were stained using GelRed (0.01 µl ml^{-1}, GelRed™ Nucleic Acid, Biotium Inc., VWR International GmbH, Darmstadt, Germany), visualized with a laser scanner (FLA-5100 Fluorescence Laser Scanner, Raytest GmbH, Straubenhardt, Germany) and an image analyser (Aida Image Analyser v. 4.27, Raytest GmbH, Straubenhardt, Germany). The purified PCR products were then quantified using a Qubit dsDNA HS assay Kit in a Qubit 3.0 Fluorometer (Thermo Fischer Scientific, Dreieich, Germany). Amplicon sequencing was conducted on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 (Illumina Inc., San Diego, USA) at the Göttingen Genomics Laboratory (G2L), Germany.

2.2.5 Bioinformatics processing and analyses

The raw paired-end reads were quality filtered with fastp v0.20.0 using the following settings: phredscore of threshold 20, overlapping base pair (bp) correction, sliding window size of 4 and a minimum length of 50 bp (von Hoyningen-Huene et al., 2019). The resulting paired-end ITS sequences were merged (*--fastq_mergepairs*) using PEAR v0.9.11 (Zhang et al., 2014). Reverse and forward primers were clipped (*-g for_primer –a rev_primer-trim-n*) by employing cutadapt v2.5 with default settings (Martin, 2011). To generate amplicon sequence variants (ASVs) (Callahan et al., 2017), high quality ITS sequence reads were processed with VSEARCH v2.14.1 (Edgar, 2010), which included the following steps in order: size sorting and filter (*-sortbylength*) that discarded ITS sequences shorter than 140 bp, dereplication (*-derep_fulllength-sizeout*), and denoising (*-cluster_unoise-minsize* 8) (Edgar, 2016). The operational taxonomic units (OTUs) were generated by clustering the ASVs at 97% sequence identity (*-sortbysize* & *-cluster_size*). All quality-filtered merged reads were mapped to chimera-free OTUs, and an OTU abundance table was created using VSEARCH (*-usearch_global-id 0.97*). The taxonomic classification of the OTUs was extracted from the UNITE database v8.2 (Kõljalg et al., 2013) by employing BLASTn, version 2.9.0+. Taxonomic

information was then added to the OTU abundance table with BIOM tools (McDonald et al., 2012). All unidentified fungal ASVs were searched (BLASTn) (Altschul et al., 1990) against the nt database (2020-01-17) to remove non-fungal OTUs and only fungal OTUs were kept in the OTUs abundance table.

Here, we obtained a total number of 2.6 and 4.0 million sequences for the root samples from the organic layer and mineral soil, respectively. The OTU table was rarefied to 3890 reads per sample (minimum number of counts in a sample) by employing the function *amp_subset_samples()* from the 'ampvis2' package implemented in R (Andersen et al., 2018). Rarefaction analysis resulted in a total number of 2046 distinct OTUs from the organic layer and 2147 OTUs from mineral soil. Fungal OTUs were further functionally annotated as symbiotroph (SYM), saprotroph (SAP) and pathotroph (PAT) using the program 'FunGuild' (Nguyen et al., 2016).

2.2.6 Data processing and statistical analysis

All the statistical analyses were conducted in R v4.0.3 (R Development Core Team, 2020). Data normal distribution and homogeneity of the variances were inspected visually using histograms and residual plots. Data were further logarithmically transformed when necessary to meet the criteria of normal distribution and homogeneity of variances when necessary. Generalized linear models (Poisson regression, chi-square test) were used with the function *glm()* from 'lme4' package to investigate datasets with count data (fungal species richness, sequences read abundance) (Bates et al., 2015). Linear models were used to investigate the datasets with continuous data (C, N, C/N, P_{sol}, sum of cations) with the function *lm()* from 'lme4' package (Bates et al., 2015). Differences among variables were calculated using the *Anova()* function from the 'car' package. Pairwise differences between different groups were compared with a *post hoc test* (HSD Tukey's honestly significant difference) using the function *glht()* from the 'multcomp' package (Hothorn et al., 2008). Differences were considered to be significant when $p \leq 0.05$.

Non-metric multidimensional scaling (NMDS) ordination of the root fungal community composition was performed using function *metaMDS()* in 'vegan' package (Oksanen et al., 2013) with three dimensions, 100 iterations and Bray-Curtis as the dissimilarity measure. Effects of the soil layers on fungal community composition were tested using Analysis of Similarities (ANOSIM with 999 permutation steps) with the function *anosim()* as implemented in 'vegan' package (Oksanen et al., 2013).

We used "generalized additive models for location scale and shape" (GAMLSS) with a zero-inflated beta (BEZI) family (GAMLSS-BEZI) from the 'metamicrobiomeR' package (Ho et al., 2019) to compare the relative abundance of the fungal orders between organic layer and mineral soil. Fungal orders were filtered applying the threshold of > 0.5% of the mean sequence abundances using the function *taxa.filter()* to exclude low-abundant fungal orders from further analysis. The relative abundances of the root fungal orders from the organic layer and mineral soil were compared using the function *taxa.compare()*. The p values were adjusted using the default method 'False Discovery Rate (FDR). The analyses were conducted for each of the three study regions separately and then a meta-analysis was performed using the function *meta.taxa()* to estimate the overall effects across the three regions with "region" as a random factor. In the meta-analysis, the model pooled adjusted estimates and standard errors with inverse variance weighting and the DerSimonian-Laird estimator from each region to determine the overall effects across three regions (Ho et al., 2019). Heatmap and forest plot were generated using the function *meta.niceplot()*. Data were displayed as log-odds ratio (log(OR)) heatmaps to indicate enrichment or depletion of a RAF between organic layer and mineral soil. The selected fungal orders were further annotated as SYM or SAP, using a threshold of 95% of sequences per order being annotated to only one distinct functional mode (Supplement Table S2.4). If a fungal order could not be assigned to SYM or SAP, it was classified as multiple functional mode such as SAP+PAT, SAP+SYM, etc. (Supplement Table S2.4).

Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/) were used to identify exclusive and shared OTUs between organic layer and mineral soil. To determine the β-diversity (β_{SOR}, Sorensen dissimilarity), the abundance-based OTU data matrices were transformed into presence-absence (1 or 0) matrices with the function *beta.temp()* from the 'betapart' package (Baselga and Orme, 2012). Total β-diversity was further partitioned into species turnover (β_{SIM}) and nestedness (β_{SNE}) using 'betapart'. Pairwise differences of each response variable (e.g. overall β-diversity, nestedness and turnover components) and each fungal group (e.g. all fungi, SYM and SAP) were compared separately among the regions, using a *post hoc* test (HSD Tukey's honestly significant difference). Further, paired rank-sum test was used to compare the different fungal functional group in each region.

Bipartite networks were built to evaluate associations of fungal taxa (OTUs) with organic layer and mineral soil, using the 'bipartite' package (Dormann et al., 2009). We included OTUs with relative abundances >0.1% of the fungal sequences in this analysis (Supplement Table S2.5). The *plotweb()* function was used to visualize bipartite network plots. To determine fungal

indicator taxa for roots in the organic layer and mineral soil, we used the *multipatt()* function from the 'indicspecies' package (Cáceres and Legendre, 2009). Further, the significant difference of the enrichment of the SYM and SAP taxa between the soil layers were tested using the function *fischer.test()*. Data were visualized using 'ggplot2´ package (Wickham, 2009) in R.

2.3 Results

2.3.1 Differences in soil chemistry among different biogeographic regions are larger in the mineral topsoil than in the organic layer

Across the three regions, which spanned a geographic distance of about >800 km, the organic soil was characterized by higher concentrations of carbon, nitrogen, basic cations and potentially soluble P than the mineral topsoil (Table 2.1). In the organic layer, C/N ratios exhibited relatively stable values of approximately 22 across the three regions (Table 2.1). In the mineral soil, C/N ratios varied among the regions and were approximately 30% lower in ALB and HAI than in SCH (Table 2.1). Mean P_{sol} concentrations varied among the regions, about 1.5-fold in the organic and 2-fold in the mineral soil (Table 2.1). The basic cation concentrations varied among the regions, about 3.6-fold in the organic and 7.7-fold in the mineral soil (Table 2.1). Overall, regional differences were stronger in the mineral topsoil than in the organic layer (Table 2.1).

Table 2.1: Soil chemical properties in the different soil layers and different regions. ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin. Carbon (C) (g kg^{-1} DW); nitrogen (N) (g kg^{-1} DW); ratio of CN; phosphorous (P_{sol}) (mg kg^{-1} DW); Cations: sum of potassium (K), calcium (Ca) and magnesium (Mg) (mmol kg^{-1} DW). Data indicate means ± SE (n = 50). Linear models were used to compare the means of the element between the regions. Significant differences of the means are shown in bold. Pairwise differences of the nutrient elements between the organic layer and mineral soil were compared using a *post hoc test* (HSD Tukey's honestly significant difference). Different letters denote significant differences between soil layers and within the regions.

	ALB		**HAI**		**SCH**		**Organic**		**Mineral**	
	Organic	**Mineral**	**Organic**	**Mineral**	**Organic**	**Mineral**	**F**	**p**	**F**	**p**
C	315.47 ± 6.65 (e)	58.53 ± 1.88 (c)	358.57 ± 7.80 (f)	40.86 ± 1.72 (b)	268.81 ± 8.64 (d)	22.93 ± 0.71 (a)	33.62	< **0.001**	135.97	< **0.001**
N	14.20 ± 0.34 (e)	4.33 ± 0.13 (c)	15.94 ± 0.37 (e)	2.98 ± 0.12 (b)	11.80 ± 0.27 (d)	1.21 ± 0.05 (a)	39.93	< **0.001**	211.22	< **0.001**
C:N	22.39 ± 0.34 (c)	13.54 ± 0.14 (a)	22.62 ± 0.30 (c)	13.74 ± 0.16 (a)	22.71 ± 0.43 (c)	19.48 ± 0.46 (b)	0.21	0.810	133.57	< **0.001**
P_{sol}	230.22 ± 11.44 (d)	87.00 ± 8.06 (ab)	248.01 ± 11.88 (d)	58.03 ± 8.40 (a)	168.49 ± 9.67 (c)	119.87 ± 6.22 (b)	14.58	< **0.001**	16.31	< **0.001**
Cations	506.90 ± 24.95 (d)	370.14 ± 18.80 (c)	485.26 ± 14.76 (d)	334.77 ± 27.18 (c)	138.68 ± 7.01 (b)	48.38 ± 2.06 (a)	144.01	< **0.001**	85.18	< **0.001**

2.3.2 Strong taxonomic differentiation of root-associated fungal assemblages between the organic layer and mineral soil

Our analyses of RAF in 300 root samples from two different soil layers in three biogeographic regions resulted in a rarefied data set containing 1.2 Mio fungal sequences, which clustered into 2537 different fungal OTUs. The mean OTU richness per plot ranged from 158 to 188 for the roots in the organic layer and from 106 to 157 in roots from the mineral topsoil (Supplement Table S2.2). SAP and PAT fungi on the roots exhibited higher OTU richness in the organic layer than the mineral soil (Figure 2.1 a,b,c), while the richness of the SYM fungi was higher in the mineral than in the organic soil (Figure 2.1 a,b,c). Similarly to OTU richness, the number of SYM sequences was higher on roots in the mineral than in the organic layer, while SAP and PAT sequences were enriched on roots from the organic compared to the mineral layer (Figure 2.1 d,e,f). Overall, OTU richness of PAT and their relative sequence abundances (3% of the total number of sequences) were low compared to SYM (54%) or SAP (31%) (Figure 2.1).

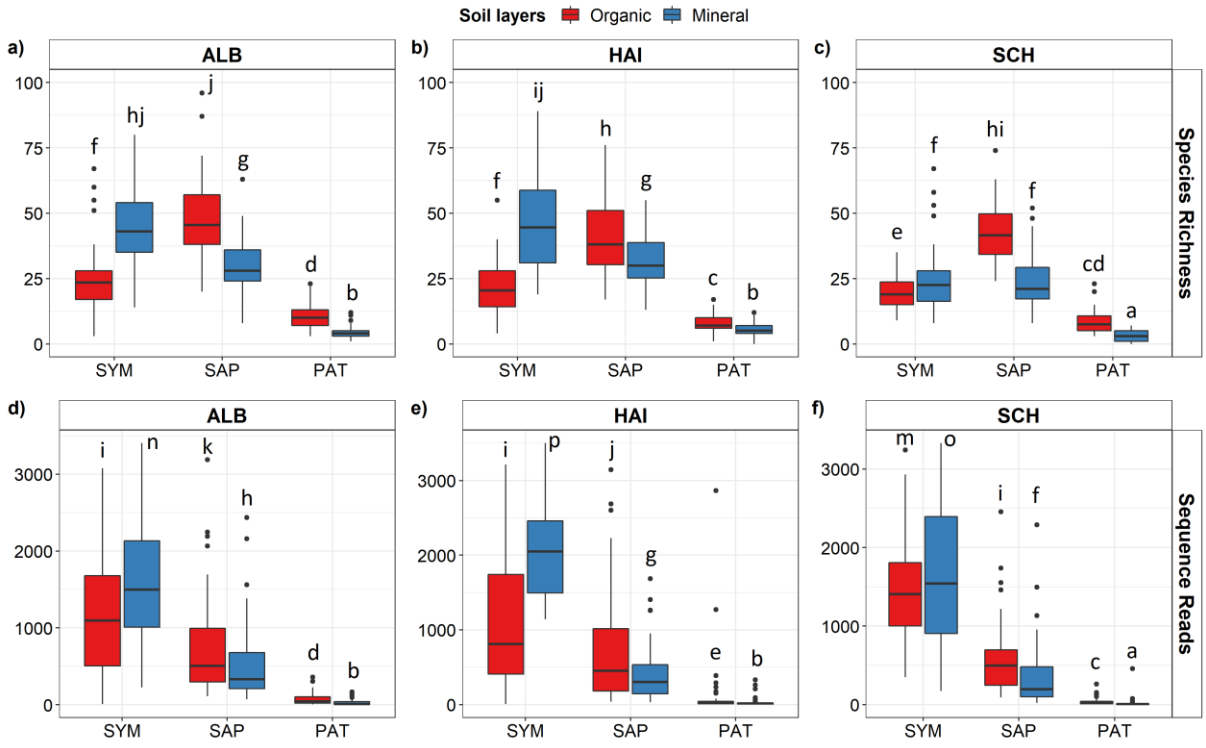


Figure 2.1: Species richness and sequence read abundance of the root-associated fungi in organic layer and mineral topsoil in three biogeographic regions. ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin. SYM = Symbiotroph, SAP = Saprotroph and PAT = Pathotroph. Data indicate n = 50. Generalized linear model with Poisson regression and chi-square test was used to compare the fungal groups between soil layers. Pairwise differences of the functional groups of root-associated fungi between organic layer and mineral soil were compared using a *post hoc test* (HSD Tukey's honestly significant difference). Different letters indicate the significant differences of the means at $p \leq 0.05$.

In each region, the RAF assemblage in the organic layer was clearly separated from that in the mineral soil (Figure 2.2 a-c), showing dissimilarities of fungal communities on roots in different soil layers. These separations were significant (ANOSIM, p and r values in Figure 2.2 a-c).

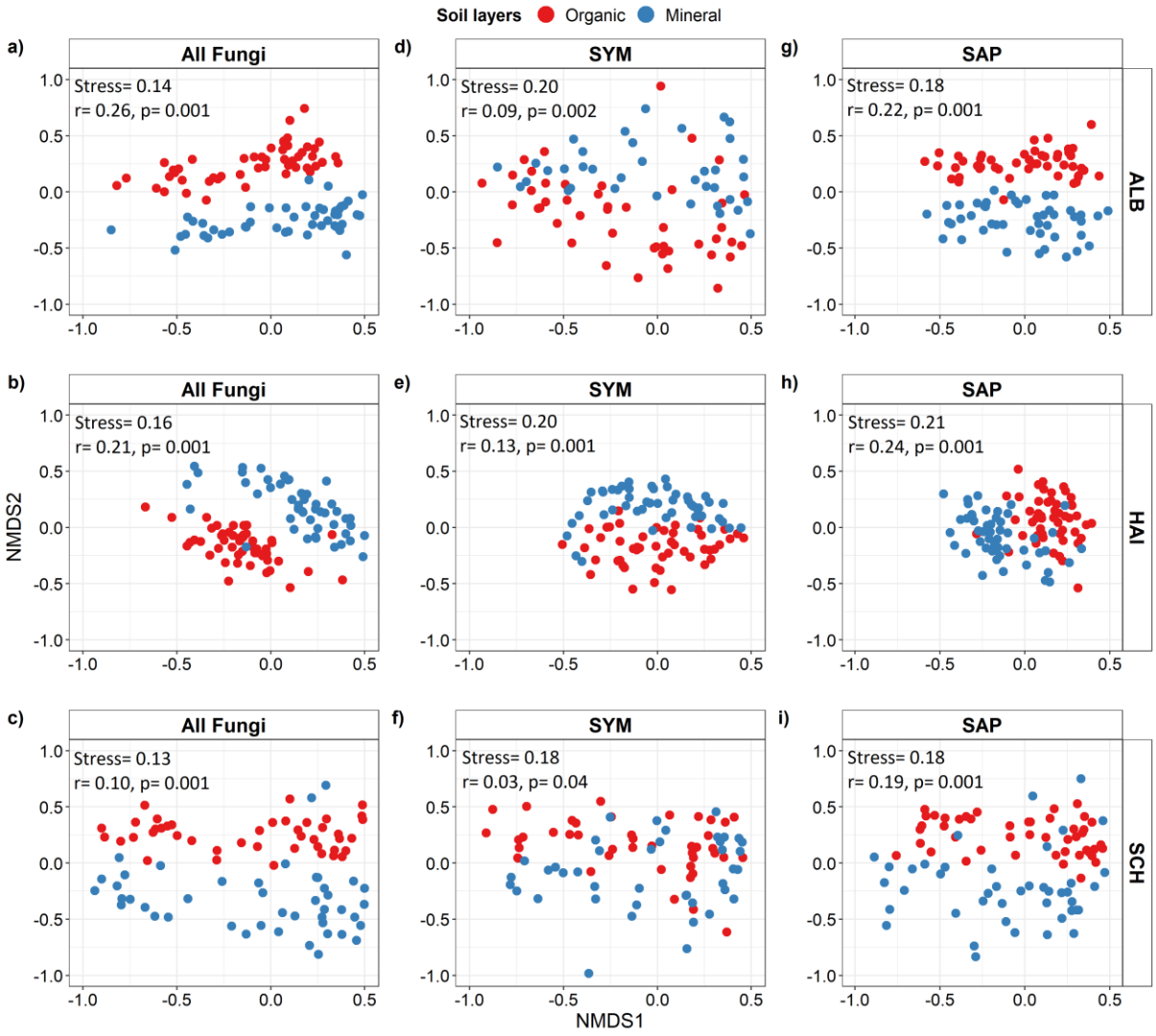


Figure 2.2: Non-metric multidimensional scaling (NMDS) of root-associated fungal community composition dissimilarity of all fungi (all OTUs), symbiotrophic (SYM) and saprotrophic (SAP) fungi in three biogeographic regions. ALB = Schwäbische Alb, HAI = Hainich-Dün and SCH = Schorfheide-Chorin. Data indicate n = 50. Red and blue colours refer to organic layer and mineral soil, respectively. Significance differences of root-associated fungal community composition between soil layers were tested with analysis of similarities (ANOSIM) using 999 permutations and 'bray' distance method.

Significant separations between the organic layer and mineral soil were also found for SYM and SAP, the main functional fungal groups colonizing roots (SYM: Figure 2.2 d-f, SAP: Figure 2.2 g-i). The goodness-of-fit (r values in Figure 2.2 d-i) was greater for SAP than for SYM assemblages, indicating stronger dissimilarities for SAP than for SYM assemblages on roots in

different soil layer. Overall, our data show a strong vertical differentiation of the taxonomic and functional composition of the RAF between organic and mineral soil layers (Figure 2.2).

2.3.3 β-diversity of root-associated fungal taxa between organic and mineral soil shows regional differences

We studied the exchange of root fungi between the organic and mineral soil. Taking all fungal OTUs per region together, we found that approximately 50% of the OTUs on roots were shared between the mineral and organic layer and the remaining OTUs were relatively equally distributed between the two soil layers (Supplement Figure S2.2). However, overall species richness was lower in SCH than in the other two regions (Supplement Table S2.2).

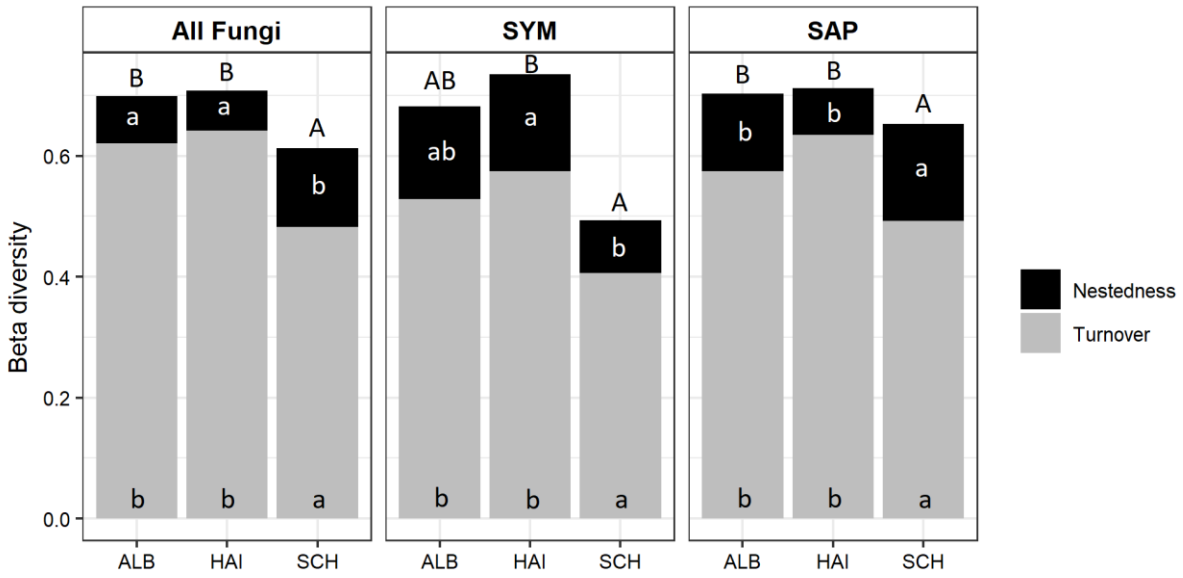


Figure 2.3: Variability of root-associated fungal community composition of all fungi (all OTUs), symbiotrophic (SYM), and saprotrophic (SAP) fungi between organic layer and mineral soil. Data indicates n = 50. The stacked bars refer to the overall β-diversity (β_{SOR}) for the three biogeographic regions. ALB = Schwäbische Alb, HAI = Hainich-Dün and SCH = Schorfheide-Chorin. The Grey coloured of the stack bars refer to the mean fungal turnover (β_{SIM}), and black coloure to the mean fungal nestedness (β_{SNE}). Pairwise differences of each variable were compared separately among the regions using a *post hoc test* (HSD Tukey's honestly significant difference). The paired rank-sum test was used to compare the different fungal groups in each region. Different letters represent the significant differences of the means at $p \leq 0.05$. Capital letters refer to the significant differences for the overall β-diversity and small letters for the turnover and nestedness components of the β-diversity.

We found significant differences for the β-diversity of the fungal OTUs among the three regions, which were caused by lower β-diversity of RAF in SCH than in the other two regions (Figure 2.3; Supplement Table S2.3). We partitioned total β-diversity into the turnover component, i.e. fungal OTU replacement between organic and mineral soil layers, and nestedness, i.e., loss of OTUs between the organic layer and mineral soil. We found significant

differences in RAF turnover among the regions, irrespective of the fungal group analysed with higher turnover in ALB and HAI than in SCH (Figure 2.3; Supplement Table S2.3). Nestedness of all fungi was higher in SCH than ALB or HAI (Fig. 2.3) and due to higher nestedness of SAP (Figure 2.3). In general, the extent of the turnover accounted for approximately 80% of the β-diversity of RAF assemblages (Figure 2.3). We further compared the turnover and nestedness components of SYM and SAP fungi in each distinct region. We found significant differences of SYM and SAP fungal turnover and nestedness in HAI (turnover: test statistics = 2.10, p = 0.04; nestedness: test statistics = 3.21, p = 0.001) and SCH (turnover: test statistics = 3.21, p = 0.001; nestedness: test statistics = 3.20, p = 0.001) but not in ALB (turnover: test statistics = 1.65, p = 0.10; nestedness: test statistics = 1.01, p = 0.31).

2.3.4 Phylogenetically related fungal groups show divergent responses to organic and mineral soil

We classified OTUs according to fungal orders and selected 19 orders (each representing at least 0.5 % or more of the total sequences) (Supplement Table S2.4), which accounted together for 85 % of the total sequences (Supplement Figure S2.1). Eight of the 19 fungal orders were assigned as SYM, six as SAP, two as SYM-SAP and three as SAP-PAT guilds (Supplement Table S2.5). In most regions, we found significant differences in the relative abundance of the selected RAF orders between organic and mineral soil layers (Figure 2.4a). However, the responses did not always show the same direction in different regions (Figure 2.4a), thus, resulting only in eight significantly affected orders across all study regions (Figure 2.4b). The orders of Polyporales, and Sordariales which contained only SAP fungi, were enriched on roots from the organic layer compared to those from the mineral soil (Figure 2.4 a,b). Significant enrichment on roots from the organic layer was also observed for the Hypocreales and Pleosporales, orders, which were composed of SAP and PAT fungi (Figure 2.4). Russulales and Cantharellales, orders containing only SYM fungi, were enriched on roots in the mineral soil layer (Figure 2.4 a,b). In contrast to Russulales and Cantharellales, Boletales, also a SYM order, were enriched on the roots from the organic layer (Figure 2.4 a,b).

Heterogeneous enrichment patterns between different soil layers were observed for SYM fungal orders (Thelephorales, Atheliales, Pezizales and Mytilinidales) as well as for SAP fungal orders (Chaetothyriales and Tubeufiales). Most orders, which showed contrasting enrichment in different regions, were enriched on roots from the organic layers in ALB and SCH but depleted in HAI (Figure 2.4a).

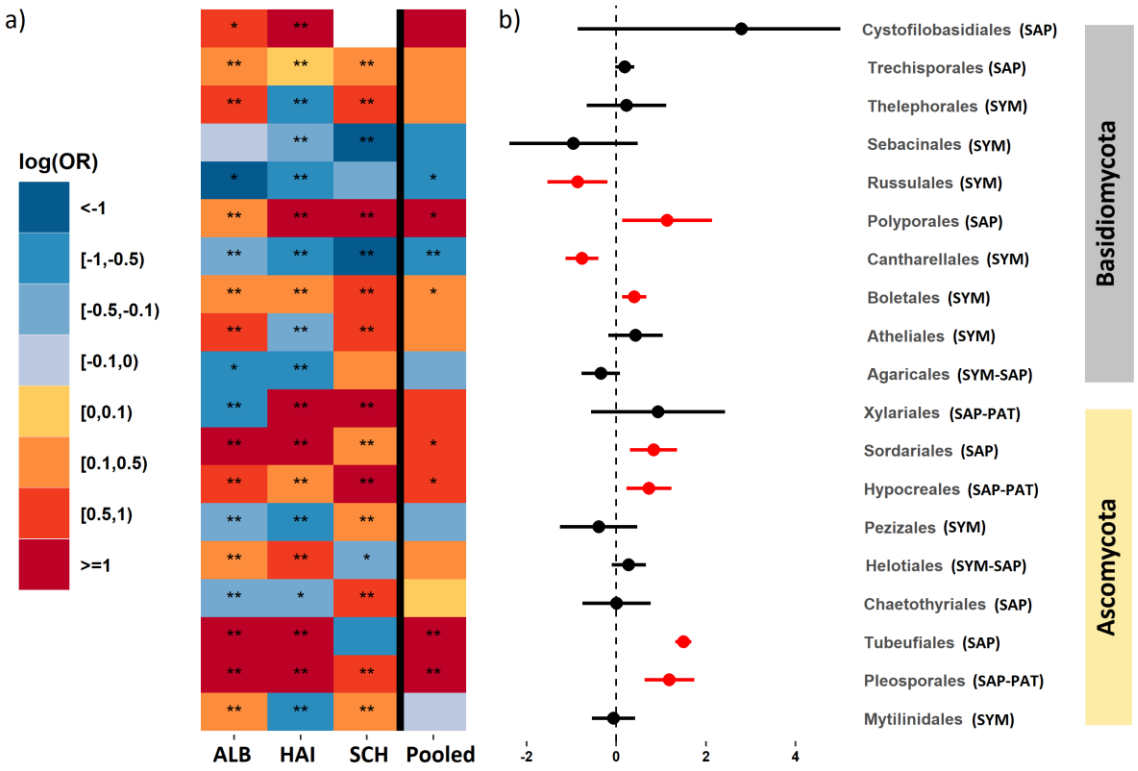


Figure 2.4: Ratio of the most abundant root-associated fungal orders between organic layer to mineral soil. Fungal order > 0.5% of the total abundance are shown in the graph. Data are displayed as a heatmap of log-odds ratio (log(OR)) of relative abundance between organic layer to mineral soil for three regions (a) and forest plot of pooled estimates across all three regions with 95% confidence intervals (b). ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin. Red and blue colours indicate the enrichment of the root-associated fungal order in the organic layer and mineral soil, respectively. Missing (unavailable) values are shown in white colours. Differential relative abundance of the fungal order with $p < 0.05$ are denoted with * and $p < 0.0001$ are denoted with **. Pooled log-odds ratio (OR) estimates with pooled $p < 0.05$ are in red colour in the forest plot (b).

2.3.5 Fungal indicator taxa in organic and mineral soil layers

Bipartite network association analysis was performed to determine the degree of habitat preference of distinct taxa (OTU based) in the organic layer and mineral soil. In ALB, 82 fungal taxa were significantly associated with roots, and equal numbers of taxa (41) being significantly associated with the organic and mineral soil (Figure 2.5a). A similar pattern was observed for HAI (OTUs: organic = 44 and mineral = 43, Figure 2.5b) but not in the SCH region (OTUs: organic = 31 and mineral = 18, Figure 2.5c). Across the regions, we found that 30 of 39 SAP species (77 %) were indicator taxa on roots from the organic layer and that 52 of 63 SYM species (81 %) were indicator taxa on roots in the mineral soil (Supplement Table S2.5). The enrichment of SYM in the mineral and SAP indicator taxa in the organic layer was significant ($p < 0.001$, Fisher's exact test).

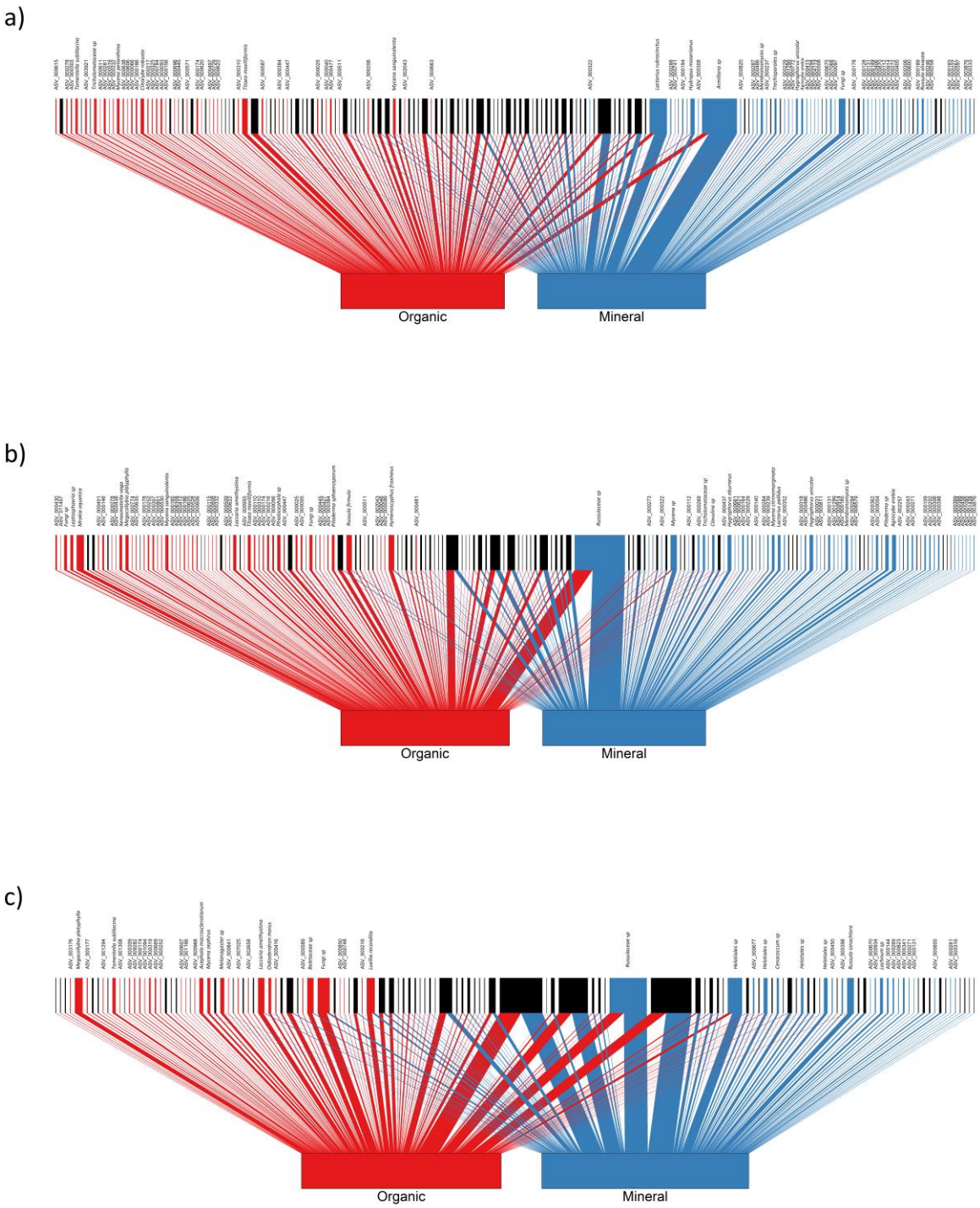


Figure 2.5: Bipartite networks associations of root-associated fungi between organic layer and mineral soil in three biogeographic regions. ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin. Upper nodes refer to fungal OTUs, and lower nodes refer to organic layer (red) and mineral soil (blue). The width of the upper nodes reflects the relative abundance of fungal OTUs from both soil layers. Line widths represent the relative abundance of the fungal OTUs from the respective soil layer. The statistically significant association of root-associated fungal OTUs are shown in red for organic layer and blue for mineral soil in the upper nodes. Black coloured upper nodes represent non-significant fungal OTUs in association between soil layers.

Only three taxa were present as indicator species in the three study regions: *Calycellina fagina* (Heliotales, SAP) and *Laccaria amethystina* (Agaricales, SYM) in the organic layer and a member of the *Russulaceae* family (ASV_000131, SYM) in the mineral soil (Supplement Table S2.5). Five SAP taxa (*Megacollybia platyphylla, Titaea maxilliformis, Luellia recondita, Apodus sp., Cladophialophora sp.*) and two SYM taxa (*Melanogaster sp, Tomentella sublilacina*) occurred in two regions as indicator species for roots from the organic layer (Supplement Table S2.5). Seven SYM taxa (*Hygrophorus unicolor, Tuber sp., Lactarius pallidus, Amanita sp., Russula foetens, Russula sp., Glomeraceae sp.*) and three SAP taxa (*Lachnum sp., Hydropus moserianus, Agrocybe erebia*) occurred in two regions as indicator species for roots in the mineral soil (Supplement Table S2.5). In most cases, regions with shared indicator species were between HAI and ALB (Supplement Table S2.5). ALB and SCH rarely shared indicator species (Supplement Table S2.5).

2.4 Discussion

This study significantly extends our knowledge on vertical RAF distribution patterns in temperate forests. We investigated RAF assembly patterns between the organic layer and mineral topsoil across three biogeographic regions spanning a distance of about >800 km. Previous studies in boreal and temperate forest focused on soil-localized fungi between different soil profiles (Asplund et al., 2019; Bödeker et al., 2016; Brabcová et al., 2016; Clemmensen et al., 2013b; Lindahl et al., 2007; Peršoh et al., 2018; Santalahti et al., 2016; Voříšková et al., 2014), while only few studies investigated RAF community composition in vertical soil layers (Carteron et al., 2020; Mrak et al., 2020; Rosling et al., 2003; Tedersoo et al., 2003). Moreover, the studies across the vertical soil layer focused on ectomycorrhizal fungi applying traditional morphotyping (Mrak et al., 2020; Rosling et al., 2003; Tedersoo et al., 2003) or were conducted at a small spatial scale (Carteron et al., 2020). Therefore, our study fills a knowledge gap characterizing RAF vertical assembly patterns in different biogeographic regions to distinguish local and regional effects.

2.4.1 Vertical stratification of root-associated fungi between the organic layer and mineral topsoil

Similar to other previous studies, we found that vertical soil layers represent distinct environmental conditions with multiple edaphic factors (e.g. soil C, N, C/N ratio, P_{sol} and basic cations) that decrease in mineral soil compared to organic layer (Carteron et al., 2020; Herold et al., 2014; Jobbágy and Jackson, 2001). In this study, the functional groups of the RAF assemblages mainly composed of SYM and SAP fungi that actively involved in carbon and nutrient cycling in forest ecosystems (Baldrian, 2017). In contrast to other fungal guilds, PAT

fungi were significantly less abundant in both soil layers. Schröter et al. (2019) provided evidence that PAT fungi occurred by chance in the RAF community.

In general, high N deposition results in declining the richness of ectomycorrhizal fungi species (de Witte et al., 2017; Lilleskov et al., 2002), while nutrient-rich conditions are favourable for SAP fungi (Carteron et al., 2020; Clemmensen et al., 2013; Lindahl et al., 2007). Here, we found that the relative abundance of SYM is higher than that of SAP fungi in RAF assemblages in both soil layers. RAF taxonomic community composition of SYM and SAP fungi is vertically differentiated between the organic layer and mineral soil. Further, we observed that species richness of SYM fungi is dominant in nutrient-poor conditions (mineral soil) and SAP fungi in nutrient-rich conditions (organic layer). These results are in line with other studies of soil fungi in the boreal forest (Clemmensen et al., 2013b; Lindahl et al., 2007) and RAF in the temperate forest (Carteron et al., 2020). SAP fungi usually prefer to reside in the organic layer, where they degrade organic material to obtain C and contribute to the decomposition of organic substance in forest floor (Baldrian, 2008; Grinhut et al., 2007; Lindahl et al., 2007). Unlike SAP fungi, SYM usually mine in the mineral soil for inorganic nutrients and directly depends on host-derived C, and therefore may reside in older litter layers and mineral topsoil (Colpaert and Laere, 1996). These patterns also indicate different nutritional acquisition strategies of SAP and SYM fungi. Another alternative explanation for the predominance of the SAP and SYM fungi in different soil layers might reflect the antagonistic relationship hypothesis between these functional groups proposed by Gadgil and Gadgil (Gadgil and Gadgil, 1971). Since these functional groups compete for the same resources, they might vertically differentiate to avoid competition.

Besides soil layers, RAF community composition variation could be further attributed to environmental differences among the regions (i.e. regional effect). For example, the overall RAF abundance and β-diversity were lower in a region with drier, more acidic and nutrient-poor forest soil conditions than regions with cooler, moist climate and nutrient-rich soil. This can be explained by the classical ecological theory that environmental stress results in decline of species richness and diversity, separating out more similar species that can tolerate harsh environmental conditions (Chase, 2007). In addition, it has been reported that naturally N-rich soil can also result in increased ectomycorrhizal fungal diversity (Kranabetter et al., 2009). Here, SYM species richness was higher in N-rich regions than N-poor region. Ectomycorrhizal fungi are also sensitive to high temperature and drought stress (Marx et al., 1970; Sánchez et al., 2001; Lazarević et al., 2016; Leberecht et al., 2016; Mucha et al., 2018, Taniguchi et al.,

2018), suggesting the lower SYM fungal richness in drier SCH region than the other two regions.

Furthermore, we observed a low nestedness and high turnover of RAF at the plot level, and a particularly lower turnover of SYM than SAP fungi between soil layers. This result was expected since mycorrhizal fungi colonizing roots can be directly influenced by various biotic and abiotic factors, including factors related to vegetation (Goldmann et al., 2015; Pena et al., 2017; Wubet et al., 2012), tree identity (Ishida et al., 2007; Tedersoo et al., 2003; van der Linde et al., 2018), root and soil chemistry (Ballauff et al., 2021; Nguyen et al., 2020), while SAP fungi may be influenced indirectly. However, each fungal functional group comprises different fungal orders and is characterized by different (positive and negative) response patterns of its individual members to abiotic factors and roots traits (Nguyen et al., 2020). Therefore, certain root traits and soil chemical properties may drive SYM fungi differently than SAP fungi and thus, explain lower turnover of SYM fungi than of SAP in the RAF communities.

2.4.2 Indicator root-associated fungal orders and taxa in organic and mineral soil

One of the important goals of this study was to identify the indicator RAF orders and taxa in distinct soil layers across temperate forests. Phylogenetic structures are known to carry information on ecological community assemblages due to the relatedness among the members of the community, suggesting similar ecological requirements and functions of phylogenetically related taxa (Cavender-Bares et al., 2009; Pausas and Verdú, 2010; Treseder and Lennon, 2015). For example, Pena et al. (2017) observed a stronger phylogenetic clustering of SYM fungi in drier and acidic soil conditions compared to cool and moist soil in temperate forests. In a similar line, Treseder and Lennon (2005) reported that the highest variance of fungal traits related to the transformation of nitrogen and phosphorous could be explained at the level of the subphylum or phylum. They showed that traits assessed on the basis of genes counts were more conserved in phylogenetically closely related taxa than in the distant ones. Here, we investigated the responses of RAF at different phylogenetic levels, i.e. at the level of fungal orders and at the level of individual taxa in response to the nutrient-rich organic layer and nutrient-poor mineral soil conditions.

In agreement with existing research in the same studied forest, we found that members of the Agaricales, Russulales, Helotiales, Thelephorales and Sebacinales formed a dominant group of RAF assemblages (Nguyen et al., 2020). SYM fungi orders such as Russulales and Cantharellales significantly varied between soil layers and were dominant in mineral compared to organic soil in all studied regions. Previous studies reported a significant decrease of

Russulales after P fertilization in low P temperate forest (Mason et al., 2021), and decreases in Russulales and Cantharellales in combination with N+P addition in temperate forest soil (Clausing et al., 2021). Other studies reported no significant effects of N fertilization on the relative abundance of Russulales and Cantharellales in boreal forests (Allison et al., 2007; Nicolás et al., 2017). As mentioned earlier, the concentration of N and P was lower in mineral soil than organic layer in this study which might be the reason of higher relative abundance of Russulales and Cantharellales in mineral soil compared to organic layer. All known members of the Russulales are ectomycorrhizal fungi with hydrophilic exploration type, absorb nutrients from their surrounding soil and are capable of producing extracellular enzymes that could degrade organic matter in litter and soil (Agerer, 2001). However, their involvement in decomposition processes is still questionable since several members of the Russulales order were inactive during the organic matter decomposition period experiments (Baldrian et al., 2012). At the taxon level, we found that several members of the Russulales, such as *Russula sp. and Russlaceae sp.* were indicator taxa in mineral soil across all regions. All known members of Russula are widespread and highly colonizing roots of beech trees in temperate forests (Buée et al., 2005; Lang et al., 2011; Pena et al., 2017). Members of Cantharellales contain class II peroxidase enzymes that are capable of degrading lignin and facilitating wood decomposition processes (Kohler et al., 2015; Sinsabaugh, 2010).

Boletales was the only SYM fungal order that showed significantly higher abundance in organic layer than in mineral soil across all regions. It has been reported that the relative abundance of Boletales increased twofold after P or P+N fertilization in temperate forest soil (Clausing et al., 2021). In our studied forest soils, P_{sol} and N were higher in organic layer than mineral soil. Several members of the Boletales are characterised by long-distance hyphae and may explore regions beyond their surrounding rhizosphere (Agerer, 2001). Therefore, Boletales are considered as being beneficial in forest ecosystems with low nutrient availability to access more distant nutrient resources (Hobbie and Agerer, 2010) and meet the P demand of trees (Almeida et al., 2019). Almeida et al. (2019) found increases in the abundance of Boletales (e.g. *Imleria badia),* after adding apatite (a recalcitrant P source) to the ingrowth mesh bag in control and N plots but not when it was amended with available P sources, further underpinning the role of these taxa for P nutrition.

At the species level, we found *Laccaria amethystina* was indicator taxa in organic layer across all three regions, suggesting that this ectomycorrhizal fungus spread across temperate forest ecosystems and can be considered as a marker species to the organic soil layer. *Laccaria*

amethystina is known as an ammonia fungus (lmamura, 2001) and the most common species that is highly colonizing roots of oak and beech tree species in temperate deciduous and deciduous-coniferous forest ecosystem in Europe (Mueller and History, 1992). Besides *Laccaria amethystina*, several other taxa such as *Laccaria maritima*, *Laccaria subdulcis* and *Laccaria blennius* were found to highly colonize the roots of beech trees in the same studied forest (Lang et al., 2011; Pena et al., 2017). Several species from the Laccaria genus (e.g. *Laccaria bicolor*) have little capacity to degrade organic matter (Martin et al., 2008). Therefore, we speculate the *Laccaria* species in the organic layer may benefit from N released by the degradation of organic material.

In contrast to SYM fungi, SAP fungal orders, including Trechisporales, Polyporales, and Sordariales showed significant differences between the soil layers and were enriched in the organic layer across all regions. Previous studies also showed that the relative abundances of Trechisporales and Sordariales were higher in the litter and organic soil surface than mineral soil in temperate forests (Clausing et al., 2021; Peršoh et al., 2018; Žifčáková et al., 2016). Trechisporales (white-rot decomposer) and Polyporales (partly brown-rot) are well known for their efficient ligninolytic capabilities to degrade the deadwood in the forest floor (Binder et al., 2013; Nagy et al., 2016). These SAP Basidiomycota were also found to highly colonizing fresh litter and incubated litter in a field experiment in boreal forest (Bödeker et al., 2016).

At the species level, SAP fungal taxa such as *Calycellina fagina* and *Cladophialophora sp.* were indicator taxa in the organic layer, whereas no single indicator SAP taxon was found in the mineral soil layer across all regions. Members of the Calycellina and Cladophialophora genera are usually growing on dead wood and plant matter in forest floors and play a role in decomposition processes (Badali et al., 2008; Cannon and Kirk, 2007).

The consistent enrichment patterns of the RAF orders and species in response to distinct soil layers across all three regions, indicating that ecological niche partitioning strongly influences the differentiation of RAF community structure. Furthermore, we observed several fungal orders and indicator taxa that were particular to a distinct soil layer or region or shared among at least two regions in the organic and mineral soil. Most of these indicator SYM taxa in mineral soil were shared among those regions where nutrient resources and environmental conditions are more similar (ALB and HAI) than the more dissimilar one (SCH). From this result, we can only speculate that RAF assembly may entail two strategies encompassing flexible and territorial habitat colonization by different fungal taxa.

2.5 Conclusion

Our results support the ecological concept that resource partitioning and phylogenetically conserved properties determine the ecological communities. A distinct response of RAF fungal orders and indicator taxa that were specific to soil layer and region indicated that habitat conditions strongly influence the differentiation of the RAF community structure. The results also support a phylogenetic signature for niche partitioning since several fungal orders were enriched in distinct soil layers across the biogeographic gradient, irrespective of the habitat conditions. Divergent response patterns of the different fungal orders from functional trophic mode group regarding habitat conditions suggest widely conserved properties may dominate the response. However, our knowledge is still limited on fungal traits, fungal nutritional preferences and their functions for tree nutrition in forest ecosystems. To better understanding the role of RAF in aboveground and belowground relationships, further studies are required.

2.6 Declaration

I contributed to the joined soil sampling 2017, organized by soil and microbe core project of the Biodiversity Exploratories. I analysed root and soil chemistry measurements (C and N), provided the materials for ICP and Illumina sequencing. I analysed and synthesised the data using advanced statistical methods. Illumina amplicon sequencing, DNA sequence processing and annotation were conducted by Dominik Schneider and Rolf Daniel at the Department of Genomic and Applied Microbiology, University of Göttingen.

2.7 Data availability

All data have been deposited in the BExIS database (https://www.bexis.uni-jena.de) under the following accession numbers (data owner): Soil chemistry - 26228 and 26229 (Polle) and root-associated fungal taxonomy - 30973 and 30974 (Polle).

2.8 References

Agerer, R., 2001. Exploration types of ectomycorrhizae. Mycorrhiza 11, 107–114. https://doi.org/10.1007/s005720100108

Allison, S.D., Hanson, C.A., Treseder, K.K., 2007. Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. Soil Biology and Biochemistry 39, 1878–1887. https://doi.org/10.1016/j.soilbio.2007.02.001

Almeida, J.P., Rosenstock, N.P., Forsmark, B., Bergh, J., Wallander, H., 2019. Ectomycorrhizal community composition and function in a spruce forest transitioning between nitrogen and phosphorus limitation. Fungal Ecology, Ecology of Mycorrhizas in the Anthropocene 40, 20–31. https://doi.org/10.1016/j.funeco.2018.05.008

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. Journal of Molecular Biology 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Andersen, K.S., Kirkegaard, R.H., Karst, S.M., Albertsen, M., 2018. ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. bioRxiv 299537. https://doi.org/10.1101/299537

Asplund, J., Kauserud, H., Ohlson, M., Nybakken, L., 2019. Spruce and beech as local determinants of forest fungal community structure in litter, humus and mineral soil. FEMS Microbiology Ecology 95. https://doi.org/10.1093/femsec/fiy232

Badali, H., Gueidan, C., Najafzadeh, M.J., Bonifaz, A., van den Ende, A.H.G.G., de Hoog, G.S., 2008. Biodiversity of the genus Cladophialophora. Studies in Mycology, Black fungal extremes 61, 175–191. https://doi.org/10.3114/sim.2008.61.18

Bahram, M., Hildebrand, F., Forslund, S.K., Anderson, J.L., Soudzilovskaia, N.A., Bodegom, P.M., Bengtsson-Palme, J., Anslan, S., Coelho, L.P., Harend, H., Huerta-Cepas, J., Medema, M.H., Maltz, M.R., Mundra, S., Olsson, P.A., Pent, M., Põlme, S., Sunagawa, S., Ryberg, M., Tedersoo, L., Bork, P., 2018. Structure and function of the global topsoil microbiome. Nature 560, 233–237. https://doi.org/10.1038/s41586-018-0386-6

Baldrian, P., 2017. Microbial activity and the dynamics of ecosystem processes in forest soils. Current Opinion in Microbiology, Environmental microbiology 37, 128–134. https://doi.org/10.1016/j.mib.2017.06.008

Baldrian, P., 2008. Chapter 2 Enzymes of saprotrophic basidiomycetes, British Mycological Society Symposia Series, Ecology of Saprotrophic Basidiomycetes. Academic Press, pp. 19–41. https://doi.org/10.1016/S0275-0287(08)80004-5

Baldrian, P., Kolařík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. The ISME Journal 6, 248–258. https://doi.org/10.1038/ismej.2011.95

Ballauff, J., Schneider, D., Edy, N., Irawan, B., Daniel, R., Polle, A., 2021. Shifts in root and soil chemistry drive the assembly of belowground fungal communities in tropical land-use systems. Soil Biology and Biochemistry 154, 108140. https://doi.org/10.1016/j.soilbio.2021.108140

Baselga, A., Orme, C.D.L., 2012. betapart: an R package for the study of beta diversity. Methods in Ecology and Evolution 3, 808–812. https://doi.org/10.1111/j.2041-210X.2012.00224.x

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Binder, M., Justo, A., Riley, R., Salamov, A., Lopez-Giraldez, F., Sjökvist, E., Copeland, A., Foster, B., Sun, H., Larsson, E., Larsson, K.-H., Townsend, J., Grigoriev, I.V., Hibbett, D.S., 2013. Phylogenetic and phylogenomic overview of the Polyporales. Mycologia 105, 1350–1373. https://doi.org/10.3852/13-003

Birkhofer, K., Schöning, I., Alt, F., Herold, N., Klarner, B., Maraun, M., Marhan, S., Oelmann, Y., Wubet, T., Yurkov, A., Begerow, D., Berner, D., Buscot, F., Daniel, R., Diekötter, T., Ehnes, R.B., Erdmann, G., Fischer, C., Foesel, B., Groh, J., Gutknecht, J., Kandeler, E., Lang, C., Lohaus, G., Meyer, A., Nacke, H., Näther, A., Overmann, J., Polle, A., Pollierer, M.M., Scheu, S., Schloter, M., Schulze, E.-D., Schulze, W., Weinert, J., Weisser, W.W., Wolters, V., Schrumpf, M., 2012. General relationships between abiotic soil properties and soil biota across spatial scales and different land-use types. PLOS ONE 7, e43292. https://doi.org/10.1371/journal.pone.0043292

Blackwell, M., 2011. The Fungi: 1, 2, 3 … 5.1 million species? American Journal of Botany 98, 426–438. https://doi.org/10.3732/ajb.1000298

Bödeker, I.T.M., Lindahl, B.D., Olson, Å., Clemmensen, K.E., 2016. Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. Functional Ecology 30, 1967–1978. https://doi.org/10.1111/1365-2435.12677

Brabcová, V., Nováková, M., Davidová, A., Baldrian, P., 2016. Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community. New Phytologist 210, 1369–1381. https://doi.org/10.1111/nph.13849

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Science 59, 39–46. https://doi.org/10.1097/00010694-194501000-00006

Buée, M., Vairelles, D., Garbaye, J., 2005. Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus silvatica*) forest subjected to two thinning regimes. Mycorrhiza 15, 235–245. https://doi.org/10.1007/s00572-004-0313-6

Cáceres, M.D., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90, 3566–3574. https://doi.org/10.1890/08-1823.1

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal 11, 2639–2643. https://doi.org/10.1038/ismej.2017.119

Cannon, P.F., Kirk, P.M., 2007. Fungal Families of the World. CABI.

Carteron, A., Beigas, M., Joly, S., Turner, B.L., Laliberté, E., 2020. Temperate forests dominated by arbuscular or ectomycorrhizal fungi are characterized by strong shifts from saprotrophic to mycorrhizal fungi with increasing soil depth. Microb Ecol. https://doi.org/10.1007/s00248-020-01540-7

Cavender-Bares, J., Kozak, K.H., Fine, P.V.A., Kembel, S.W., 2009. The merging of community ecology and phylogenetic biology. Ecology Letters 12, 693–715. https://doi.org/10.1111/j.1461-0248.2009.01314.x

Chase, J.M., 2007. Drought mediates the importance of stochastic community assembly. PNAS 104, 17430–17434. https://doi.org/10.1073/pnas.0704350104

Clausing, S., Likulunga, L.E., Janz, D., Feng, H.Y., Schneider, D., Daniel, R., Krüger, J., Lang, F., Polle, A., 2021. Impact of nitrogen and phosphorus addition on resident soil and root mycobiomes in beech forests. bioRxiv 2020.12.29.424645. https://doi.org/10.1101/2020.12.29.424645

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615–1618. https://doi.org/10.1126/science.1231923

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A., Lindahl, B.D., 2015. Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. New Phytologist 205, 1525–1536. https://doi.org/10.1111/nph.13208

Cline, L.C., Zak, D.R., 2014. Dispersal limitation structures fungal community assembly in a long-term glacial chronosequence. Environmental Microbiology 16, 1538–1548. https://doi.org/10.1111/1462-2920.12281

Colpaert, J.V., Laere, A.V., 1996. A comparison of the extracellular enzyme activities of two ectomycorrhizal and a leaf-saprotrophic basidiomycete colonizing beech leaf litter. New Phytologist 134, 133–141. https://doi.org/10.1111/j.1469-8137.1996.tb01153.x

de Witte, L.C., Rosenstock, N.P., van der Linde, S., Braun, S., 2017. Nitrogen deposition changes ectomycorrhizal communities in Swiss beech forests. Science of The Total Environment 605–606, 1083–1096. https://doi.org/10.1016/j.scitotenv.2017.06.142

Dickie, I.A., Xu, B., Koide, R.T., 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytologist 156, 527–535. https://doi.org/10.1046/j.1469-8137.2002.00535.x

Dormann, C.F., Fründ, J., Blüthgen, N., Gruber, B., 2009. Indices, graphs and null models: analyzing bipartite ecological networks. The Open Ecology Journal 2.

Edgar, R.C., 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv 081257. https://doi.org/10.1101/081257

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.-D., Weisser, W.W., 2010. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic and Applied Ecology 11, 473–485. https://doi.org/10.1016/j.baae.2010.07.009

Gadgil, R.L., Gadgil, P.D., 1971. Mycorrhiza and litter decomposition. Nature 233, 133–133. https://doi.org/10.1038/233133a0

Gan, H.Y., Schöning, I., Schall, P., Ammer, C., Schrumpf, M., 2020. Soil organic matter mineralization as driven by nutrient stoichiometry in soils under differently managed forest stands. Front. For. Glob. Change 3. https://doi.org/10.3389/ffgc.2020.00099

García-Guzmán, G., Heil, M., 2014. Life histories of hosts and pathogens predict patterns in tropical fungal plant diseases. New Phytologist 201, 1106–1120. https://doi.org/10.1111/nph.12562

Genney, D.R., Anderson, I.C., Alexander, I.J., 2006. Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. New Phytologist 170, 381–390. https://doi.org/10.1111/j.1469-8137.2006.01669.x

Goldmann, K., Schöning, I., Buscot, F., Wubet, T., 2015. Forest management type influences diversity and community composition of soil fungi across temperate forest ecosystems. Front. Microbiol. 6. https://doi.org/10.3389/fmicb.2015.01300

Goldmann, K., Schröter, K., Pena, R., Schöning, I., Schrumpf, M., Buscot, F., Polle, A., Wubet, T., 2016. Divergent habitat filtering of root and soil fungal communities in temperate beech forests. Scientific Reports 6, 31439. https://doi.org/10.1038/srep31439

Grinhut, T., Hadar, Y., Chen, Y., 2007. Degradation and transformation of humic substances by saprotrophic fungi: processes and mechanisms. Fungal Biology Reviews 21, 179–189. https://doi.org/10.1016/j.fbr.2007.09.003

Heijden, M.G.A.V.D., Bardgett, R.D., Straalen, N.M.V., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Heinrichs, H., Brumsack, H.-J., Loftfield, N., König, N., 1986. Verbessertes Druckaufschlußsystem für biologische und anorganische Materialien. Zeitschrift für Pflanzenernährung und Bodenkunde 149, 350–353. https://doi.org/10.1002/jpln.19861490313

Herold, N., Schöning, I., Berner, D., Haslwimmer, H., Kandeler, E., Michalzik, B., Schrumpf, M., 2014. Vertical gradients of potential enzyme activities in soil profiles of European beech, Norway spruce and Scots pine dominated forest sites. Pedobiologia 57, 181–189. https://doi.org/10.1016/j.pedobi.2014.03.003

Ho, N.T., Li, F., Wang, S., Kuhn, L., 2019. metamicrobiomeR: an R package for analysis of microbiome relative abundance data using zero-inflated beta GAMLSS and meta-analysis across studies using random effects models. BMC Bioinformatics 20, 188. https://doi.org/10.1186/s12859-019-2744-2

Hobbie, E.A., Agerer, R., 2010. Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. Plant Soil 327, 71–83. https://doi.org/10.1007/s11104-009-0032-z

Horton, T.R., Bruns, T.D., 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10, 1855–1871. https://doi.org/10.1046/j.0962-1083.2001.01333.x

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical Journal 50, 346–363. https://doi.org/10.1002/bimj.200810425

Hubbel, S.P., 2001. The unified neutral theory of biodiversity and biogeography (MPB-32).

Ishida, T.A., Nara, K., Hogetsu, T., 2007. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer–broadleaf forests. New Phytologist 174, 430–440. https://doi.org/10.1111/j.1469-8137.2007.02016.x

Jobbágy, E.G., Jackson, R.B., 2001. The distribution of soil nutrients with depth: Global patterns and the imprint of plants. Biogeochemistry 53, 51–77. https://doi.org/10.1023/A:1010760720215

Kohler, A., Kuo, A., Nagy, L.G., Morin, E., Barry, K.W., Buscot, F., Canbäck, B., Choi, C., Cichocki, N., Clum, A., Colpaert, J., Copeland, A., Costa, M.D., Doré, J., Floudas, D., Gay, G., Girlanda, M., Henrissat, B., Herrmann, S., Hess, J., Högberg, N., Johansson, T., Khouja, H.-R., LaButti, K., Lahrmann, U., Levasseur, A., Lindquist, E.A., Lipzen, A., Marmeisse, R., Martino, E., Murat, C., Ngan, C.Y., Nehls, U., Plett, J.M., Pringle, A., Ohm, R.A., Perotto, S., Peter, M., Riley, R., Rineau, F., Ruytinx, J., Salamov, A., Shah, F., Sun, H., Tarkka, M., Tritt, A., Veneault-Fourrey, C., Zuccaro, A., Tunlid, A., Grigoriev, I.V., Hibbett, D.S., Martin, F., 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nature Genetics 47, 410–415. https://doi.org/10.1038/ng.3223

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22, 5271–5277. https://doi.org/10.1111/mec.12481

Kraft, N.J.B., Adler, P.B., Godoy, O., James, E.C., Fuller, S., Levine, J.M., 2015. Community assembly, coexistence and the environmental filtering metaphor. Functional Ecology 29, 592–599. https://doi.org/10.1111/1365-2435.12345

Kranabetter, J.M., Durall, D.M., MacKenzie, W.H., 2009. Diversity and species distribution of ectomycorrhizal fungi along productivity gradients of a southern boreal forest. Mycorrhiza 19, 99–111. https://doi.org/10.1007/s00572-008-0208-z

Lang, C., Seven, J., Polle, A., 2011. Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. Mycorrhiza 21, 297–308. https://doi.org/10.1007/s00572-010-0338-y

Lazarević, J., Stojičić, D., Keča, N., 2016. Effects of temperature, pH and carbon and nitrogen sources on growth of in vitro cultures of ectomycorrhizal isolates from Pinus heldreichii forest. Forest Systems 25, 048. https://doi.org/10.5424/fs/2016251-07036

Leberecht, M., Tu, J., Polle, A., 2016. Acid and calcareous soils affect nitrogen nutrition and organic nitrogen uptake by beech seedlings (Fagus sylvatica L.) under drought, and their ectomycorrhizal community structure. Plant Soil 409, 143–157. https://doi.org/10.1007/s11104-016-2956-4

Lilleskov, E.A., Fahey, T.J., Horton, T.R., Lovett, G.M., 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83, 104–115. https://doi.org/10.1890/0012-9658(2002)083[0104:BEFCCO]2.0.CO;2

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., Finlay, R.D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173, 611–620. https://doi.org/10.1111/j.1469-8137.2006.01936.x

lmamura, A., 2001. Report on Laccaria amethystina, newly confirmed as an ammonia fungus. Mycoscience 42, 623–625. https://doi.org/10.1007/BF02460961

Marx, D.H., Bryan, W.C., Davey, C.B., 1970. Influence of temperature on aseptic synthesis of ectomycorrhizae by Thelephora terrestris and Pisolithus tinctorius on loblolly pine. Forest Science 16, 424–431. https://doi.org/10.1093/forestscience/16.4.424

Martin, F., Aerts, A., Ahrén, D., Brun, A., Danchin, E.G.J., Duchaussoy, F., Gibon, J., Kohler, A., Lindquist, E., Pereda, V., Salamov, A., Shapiro, H.J., Wuyts, J., Blaudez, D., Buée, M., Brokstein, P., Canbäck, B., Cohen, D., Courty, P.E., Coutinho, P.M., Delaruelle, C., Detter, J.C., Deveau, A., DiFazio, S., Duplessis, S., Fraissinet-Tachet, L., Lucic, E., Frey-Klett, P., Fourrey, C., Feussner, I., Gay, G., Grimwood, J., Hoegger, P.J., Jain, P., Kilaru, S., Labbé, J., Lin, Y.C., Legué, V., Le Tacon, F., Marmeisse, R., Melayah, D., Montanini, B., Muratet, M., Nehls, U., Niculita-Hirzel, H., Secq, M.P.O.-L., Peter, M., Quesneville, H., Rajashekar, B., Reich, M., Rouhier, N., Schmutz, J., Yin, T., Chalot, M., Henrissat, B., Kües, U., Lucas, S., Van de Peer, Y., Podila, G.K., Polle, A., Pukkila, P.J., Richardson, P.M., Rouzé, P., Sanders, I.R., Stajich, J.E., Tunlid, A., Tuskan, G., Grigoriev, I.V., 2008. The genome of Laccaria bicolor provides insights into mycorrhizal symbiosis. Nature 452, 88–92. https://doi.org/10.1038/nature06556

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMB net. journal 17, 10–12. https://doi.org/10.14806/ej.17.1.200

Mason, L.M., Eagar, A., Patel, P., Blackwood, C.B., DeForest, J.L., 2021. Potential microbial bioindicators of phosphorus mining in a temperate deciduous forest. Journal of Applied Microbiology 130, 109–122. https://doi.org/10.1111/jam.14761

McDonald, D., Clemente, J.C., Kuczynski, J., Rideout, J.R., Stombaugh, J., Wendel, D., Wilke, A., Huse, S., Hufnagle, J., Meyer, F., Knight, R., Caporaso, J.G., 2012. The biological observation matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome. GigaScience 1. https://doi.org/10.1186/2047-217X-1-7

Mrak, T., Hukić, E., Štraus, I., Unuk Nahberger, T., Kraigher, H., 2020. Ectomycorrhizal community composition of organic and mineral soil horizons in silver fir (Abies alba Mill.) stands. Mycorrhiza 30, 541–553. https://doi.org/10.1007/s00572-020-00970-y

Mucha, J., Peay, K.G., Smith, D.P., Reich, P.B., Stefański, A., Hobbie, S.E., 2018. Effect of simulated climate warming on the ectomycorrhizal fungal community of boreal and temperate host species growing near their shared ecotonal range limits. Microb Ecol 75, 348–363. https://doi.org/10.1007/s00248-017-1044-5

Mueller, G.M., History, F.M. of N., 1992. Systematics of Laccaria (Agaricales) in the continental United States and Canada, with discussions on extralimital taxa and descriptions of extant types. Field Museum of Natural History, Chicago, Ill. : https://doi.org/10.5962/bhl.title.2598

Nagy, L.G., Riley, R., Tritt, A., Adam, C., Daum, C., Floudas, D., Sun, H., Yadav, J.S., Pangilinan, J., Larsson, K.-H., Matsuura, K., Barry, K., Labutti, K., Kuo, R., Ohm, R.A., Bhattacharya, S.S., Shirouzu, T., Yoshinaga, Y., Martin, F.M., Grigoriev, I.V., Hibbett, D.S., 2016. Comparative genomics of early-diverging mushroom-forming fungi provides insights into the origins of lignocellulose decay capabilities. Molecular Biology and Evolution 33, 959–970. https://doi.org/10.1093/molbev/msv337

Nguyen, D.Q., Schneider, D., Brinkmann, N., Song, B., Janz, D., Schöning, I., Daniel, R., Pena, R., Polle, A., 2020. Soil and root nutrient chemistry structure root-associated fungal assemblages in temperate forests. Environmental Microbiology 22, 3081–3095. https://doi.org/10.1111/1462-2920.15037

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nicolás, C., Almeida, J.P., Ellström, M., Bahr, A., Bone, S.E., Rosenstock, N.P., Bargar, J.R., Tunlid, A., Persson, P., Wallander, H., 2017. Chemical changes in organic matter after fungal colonization in a nitrogen fertilized and unfertilized Norway spruce forest. Plant Soil 419, 113–126. https://doi.org/10.1007/s11104-017-3324-8

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2013. vegan: Community Ecology Package.

Orwin, K.H., Kirschbaum, M.U.F., John, M.G.S., Dickie, I.A., 2011. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. Ecology Letters 14, 493–502. https://doi.org/10.1111/j.1461-0248.2011.01611.x

Pausas, J.G., Verdú, M., 2010. The jungle of methods for evaluating phenotypic and phylogenetic structure of communities. BioScience 60, 614–625. https://doi.org/10.1525/bio.2010.60.8.7

Pena, R., Lang, C., Lohaus, G., Boch, S., Schall, P., Schöning, I., Ammer, C., Fischer, M., Polle, A., 2017. Phylogenetic and functional traits of ectomycorrhizal assemblages in top soil from different biogeographic regions and forest types. Mycorrhiza 27, 233–245. https://doi.org/10.1007/s00572-016-0742-z

Peršoh, D., Stolle, N., Brachmann, A., Begerow, D., Rambold, G., 2018. Fungal guilds are evenly distributed along a vertical spruce forest soil profile while individual fungi show pronounced niche partitioning. Mycol Progress 17, 925–939. https://doi.org/10.1007/s11557-018-1405-6

R development Core Team., 2020. R: The R project for statistical computing: R foundation for statistical computing: Vienna, Austria, 2020. https://www.r-project.org/ (accessed 4.13.21).

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi.org/10.7717/peerj.2584

Rosling, A., Landeweert, R., Lindahl, B.D., Larsson, K.-H., Kuyper, T.W., Taylor, A.F.S., Finlay, R.D., 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. New Phytologist 159, 775–783. https://doi.org/10.1046/j.1469-8137.2003.00829.x

Santalahti, M., Sun, H., Jumpponen, A., Pennanen, T., Heinonsalo, J., 2016. Vertical and seasonal dynamics of fungal communities in boreal Scots pine forest soil. FEMS Microbiology Ecology 92. https://doi.org/10.1093/femsec/fiw170

Sánchez, F., Honrubia, M., Torres, P., 2001. Effects of pH, water stress and temperature on in vitro cultures of ectomycorrhizal fungi from Mediterranean forests. Cryptogamie Mycologie 22, 243–258. https://doi.org/S0181158401010764

Schlatter, D.C., Kahl, K., Carlson, B., Huggins, D.R., Paulitz, T., 2018. Fungal community composition and diversity vary with soil depth and landscape position in a no-till wheat-based cropping system. FEMS Microbiology Ecology 94. https://doi.org/10.1093/femsec/fiy098

Schröter, K., Wemheuer, B., Pena, R., Schöning, I., Ehbrecht, M., Schall, P., Ammer, C., Daniel, R., Polle, A., 2019. Assembly processes of trophic guilds in the root mycobiome of temperate forests. Molecular Ecology 28, 348–364. https://doi.org/10.1111/mec.14887

Seidel, D., Annighöfer, P., Ehbrecht, M., Magdon, P., Wöllauer, S., Ammer, C., 2020. Deriving stand structural complexity from airborne laser scanning data—What does it tell us about a forest? Remote Sensing 12, 1854. https://doi.org/10.3390/rs12111854

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42, 391–404. https://doi.org/10.1016/j.soilbio.2009.10.014

Solly, E.F., Schöning, I., Boch, S., Kandeler, E., Marhan, S., Michalzik, B., Müller, J., Zscheischler, J., Trumbore, S.E., Schrumpf, M., 2014. Factors controlling decomposition rates of fine root litter in temperate forests and grasslands. Plant Soil 382, 203–218. https://doi.org/10.1007/s11104-014-2151-4

Taniguchi, T., Kitajima, K., Douhan, G.W., Yamanaka, N., Allen, M.F., 2018. A pulse of summer precipitation after the dry season triggers changes in ectomycorrhizal formation, diversity, and community composition in a Mediterranean forest in California, USA. Mycorrhiza 28, 665–677. https://doi.org/10.1007/s00572-018-0859-3

Taylor, D.L., Bruns, T.D., 1999. Community structure of ectomycorrhizal fungi in a Pinus muricata forest: minimal overlap between the mature forest and resistant propagule communities. Molecular Ecology 8, 1837–1850. https://doi.org/10.1046/j.1365-294x.1999.00773.x

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., Kesel, A.D., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science 346. https://doi.org/10.1126/science.1256688

Tedersoo, L., Kõljalg, U., Hallenberg, N., Larsson, K.-H., 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytologist 159, 153–165. https://doi.org/10.1046/j.1469-8137.2003.00792.x

Toju, H., Kishida, O., Katayama, N., Takagi, K., 2016. Networks depicting the fine-scale co-occurrences of fungi in soil horizons. PLOS ONE 18.

Toju, H., Tanabe, A.S., Yamamoto, S., Sato, H., 2012. High-coverage ITS primers for the DNA-based identification of Ascomycetes and Basidiomycetes in environmental samples. PLoS ONE 7, e40863. https://doi.org/10.1371/journal.pone.0040863

Treseder, K.K., Lennon, J.T., 2015. Fungal traits that drive ecosystem dynamics on land. Microbiol. Mol. Biol. Rev. 79, 243–262. https://doi.org/10.1128/MMBR.00001-15

van der Linde, S., Suz, L.M., Orme, C.D.L., Cox, F., Andreae, H., Asi, E., Atkinson, B., Benham, S., Carroll, C., Cools, N., De Vos, B., Dietrich, H.-P., Eichhorn, J., Gehrmann, J., Grebenc, T., Gweon, H.S., Hansen, K., Jacob, F., Kristöfel, F., Lech, P., Manninger, M., Martin, J., Meesenburg, H., Merilä, P., Nicolas, M., Pavlenda, P., Rautio, P., Schaub, M., Schröck, H.-W., Seidling, W., Šrámek, V., Thimonier, A., Thomsen, I.M., Titeux, H., Vanguelova, E., Verstraeten, A., Vesterdal, L., Waldner, P., Wijk, S., Zhang, Y., Žlindra, D., Bidartondo, M.I., 2018. Environment and host as large-scale controls of ectomycorrhizal fungi. Nature 558, 243–248. https://doi.org/10.1038/s41586-018-0189-9

Vandenkoornhuyse, P., Baldauf, S.L., Leyval, C., Straczek, J., Young, J.P.W., 2002. Extensive fungal diversity in plant roots. Science 295, 2051–2051. https://doi.org/10.1126/science.295.5562.2051

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Van, A.L., Dufresne, A., 2015. The importance of the microbiome of the plant holobiont. New Phytologist 206, 1196–1206. https://doi.org/10.1111/nph.13312

von Hoyningen-Huene, A.J.E., Schneider, D., Fussmann, D., Reimer, A., Arp, G., Daniel, R., 2019. Bacterial succession along a sediment porewater gradient at Lake Neusiedl in Austria. Scientific Data 6, 163. https://doi.org/10.1038/s41597-019-0172-9

Voříšková, J., Brabcová, V., Cajthaml, T., Baldrian, P., 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. New Phytologist 201, 269–278. https://doi.org/10.1111/nph.12481

Wang, M., Shi, S., Lin, F., Jiang, P., 2014. Response of the soil fungal community to multi-factor environmental changes in a temperate forest. Applied Soil Ecology 81, 45–56. https://doi.org/10.1016/j.apsoil.2014.04.008

White, T. j., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR protocols; Academic Press, Inc.: Cambridge, MA, USA, 1990; pp. 315–322. ISBN 978-0-12-372180-8. [WWW Document].

Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis, Use R! Springer-Verlag, New York. https://doi.org/10.1007/978-0-387-98141-3

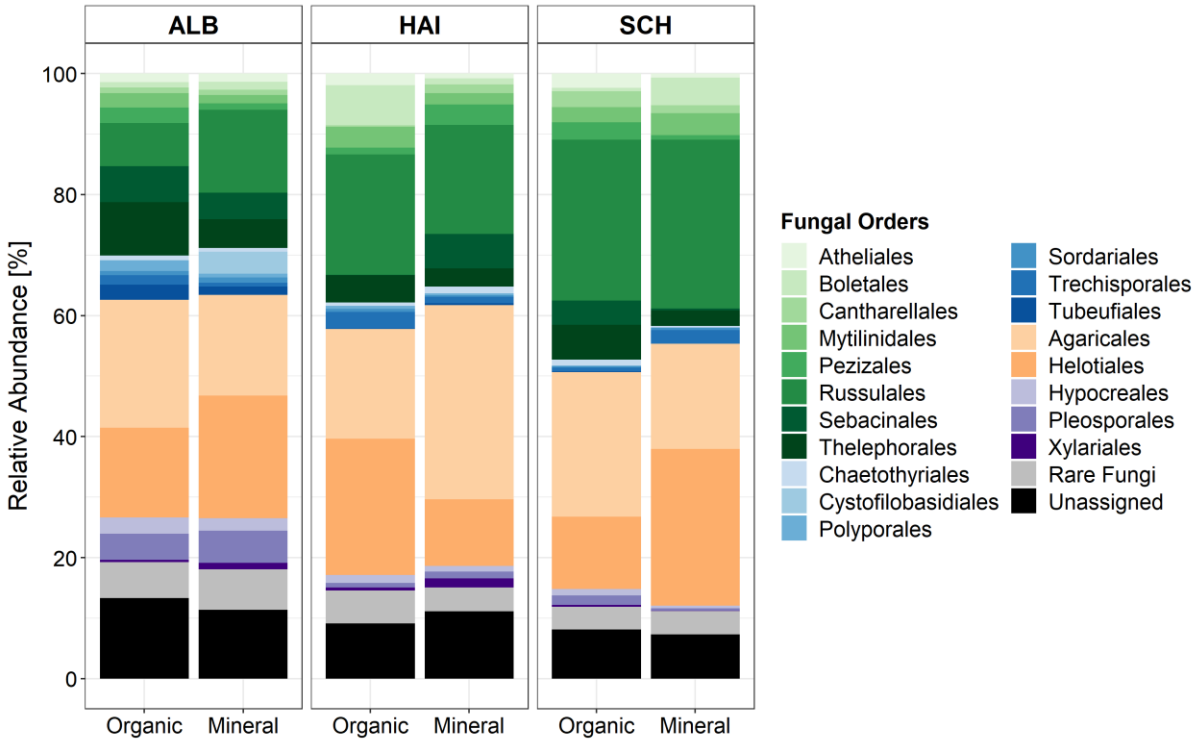
Wubet, T., Christ, S., Schöning, I., Boch, S., Gawlich, M., Schnabel, B., Fischer, M., Buscot, F., 2012. Differences in soil fungal communities between European beech (Fagus sylvatica L.) dominated forests are related to soil and understory vegetation. PLOS ONE 7, e47500. https://doi.org/10.1371/journal.pone.0047500

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30, 614–620. https://doi.org/10.1093/bioinformatics/btt593

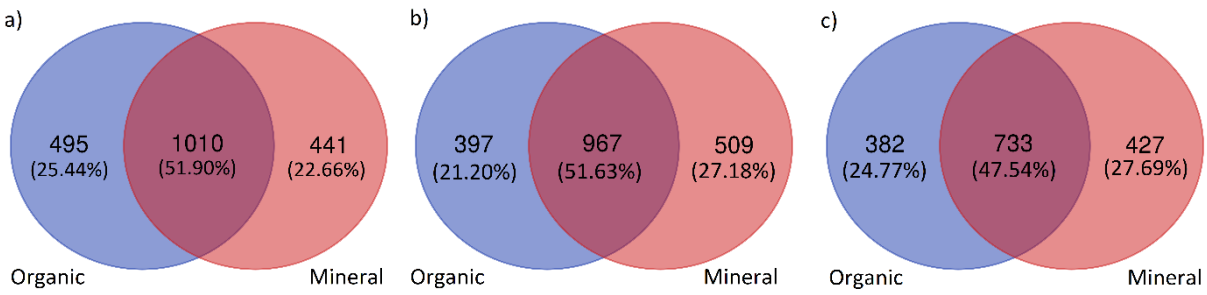
Žifčáková, L., Větrovský, T., Howe, A., Baldrian, P., 2016. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environmental Microbiology 18, 288–301. https://doi.org/10.1111/1462-2920.13026

2.9 Supplementary materials – chapter 2

Supplement Figure S2.1: Relative abundance of root-associated fungi at the rank of order-level separated by different soil layers and regions. ALB = Schwäbische Alb, HAI = Hainich-Dün and SCH = Schorfheide-Chorin. "Rare Fungi", fungal orders < 0.5% of the total abundance were summarized. Unassigned fungi that were not possible to identify at order level were summarized. Fungal orders coloured in greens refer to symbiotrophic (SYM); blues to saprotrophic (SAP); oranges to (SYM + SAP); purples to (SAP + PAT); grey to Rare Fungi and black to Unassigned fungi.



Supplement Figure S2.2: Number of exclusive and shared root-associated fungal OTUs in the organic layer and mineral soil in three regions. Three regions are: Schwäbische Alb (a), Hainich-Dün (b) and Schorfheide-Chorin (c).



Supplement Table S2.1: Key site characteristics of the three biogeographic regions in Biodiversity Exploratories. Schwäbische Alb = ALB, Hainich-Dün = HAI and Schorfheide-Chorin = SCH. Data were compiled and modified from (Fischer et al., 2010; Gan et al., 2020; Seidel et al., 2020; Nguyen et al., 2020; Solly et al., 2014).

Parameters	**ALB**	**HAI**	**SCH**
Location	South-west Germany	Central Germany	Northeast Germany
Size (km^2)	422	1300	1300
Geology	Calcareous bedrock with karst phenomena	Calcareous bedrock	Young glacial landscape
Altitude a.s.l (m)	460-860	285-550	3-140
Longitudes east to west (decimal degree)	9.58024-9.02362	10.77917-10.17332	14.14796-13.39094
Latitude north to south (decimal degree)	48.53435-48.34996	51.37872-50.93735	53.22390-52.79023
Mean annual temperature (°C)	6.0-7.0	6.5-8.0	8.0-8.5
Mean annual precipitation (mm)	700-1000	500-800	500-600
Plot size (m)	100 x 100	100 x 100	100 x 100
Soil types	Cambisol (eutric) and Leptosol	Luvisol	Cambisol (dystric)
Mean soil pH	5.23 ± 0.10	4.80 ± 0.12	3.55 ± 0.02
Mean sand ($g\ kg^{-1}$)	59.60 ± 9.84	58.00 ± 9.84	871 ± 9.84
Mean silt ($g\ kg^{-1}$)	444 ± 15.70	642 ± 15.70	84.80 ± 15.70
Mean clay ($g\ kg^{-1}$)	496 ± 14.40	301 ± 14.00	44.8 ± 14.40
Main tree species	Beech (*Fagus sylvatica*)	Beech (*Fagus sylvatica*)	Beech (*Fagus sylvatica*)
	Spruce (*Picea abies)*	Spruce (*Picea abies*)	Pine (*Pinus sylvstris*)
			Oak (*Quercus sp.*)

Supplement Table S2.2: Root-associated fungal observed species richness, estimated richness (Chao1), Shannon diversity (H′) and Evenness (E_H) in different soil layers across three biogeographic regions. Symbiotrophic = SYM, saprotrophic = SAP, pathotrophic = PAT, ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin. Data indicate means ± SE (n = 50). Linear models were used to compare the means of the Chao1, Shannon and evenness between the regions. Generalized linear models were used to compare the means of the species richness between the regions. Significant differences of the means are shown in bold. Different letters denote significant differences between soil layers and within the regions.

	ALB		HAI		SCH		p (regions)	
	Organic	**Mineral**	**Organic**	**Mineral**	**Organic**	**Mineral**	**Organic**	**Mineral**
All Fungi								
OTU Richness	188.36 ± 8.70 (e)	146.26 ± 5.24 (b)	164.22 ± 8.16 (d)	156.84 ± 5.92 (c)	158.24 ± 4.61 (cd)	106.08 ± 5.90 (a)	< **0.001**	< **0.001**
Chao1	285.77 ± 13.07 (c)	223.99 ± 7.94 (b)	258.97 ± 12.29 (bc)	237.56 ± 8.67 (b)	243.76 ± 7.40 (b)	160.48 ± 9.17 (a)	**0.030**	< **0.001**
Shannon (*H′*)	2.93 ± 0.11 (b)	2.83 ± 0.08 (ab)	2.79 ± 0.11 (ab)	2.95 ± 0.07 (b)	2.98 ± 0.07 (b)	2.56 ± 0.08 (a)	0.360	**0.002**
Evenness (E_H)	0.12 ± 0.01 (a)	0.13 ± 0.01 (a)	0.12 ± 0.01 (a)	0.13 ± 0.01 (a)	0.13 ± 0.01 (a)	0.14 ± 0.01 (a)	0.141	0.175
SYM								
OTU Richness	24.90 ± 1.70 (b)	44.22 ± 2.11 (c)	22.54 ± 1.54 (b)	46.30 ± 2.35 (c)	19.34 ± 0.87 (a)	24.92 ± 1.90 (b)	< **0.001**	< **0.001**
Chao1	39.30 ± 3.52 (b)	61.13 ± 3.05 (c)	34.25 ± 2.78 (ab)	63.95 ± 3.34 (c)	24.72 ± 1.24 (a)	32.04 ± 2.50 (ab)	< **0.001**	< **0.001**
Shannon (*H′*)	1.64 ± 0.08 (ab)	2.17 ± 0.08 (c)	1.45 ± 0.10 (a)	1.53 ± 0.07 (bc)	1.92 ± 0.09 (a)	1.57 ± 0.08 (a)	0.280	< **0.001**
Evenness (E_H)	0.26 ± 0.02 (b)	0.24 ± 0.02 (b)	0.25 ± 0.02 (b)	0.17 ± 0.01 (a)	0.27 ± 0.01 (b)	0.24 ± 0.01 (ab)	0.751	< **0.001**
SAP								
OTU Richness	47.80 ± 2.36 (d)	29.96 ± 1.45 (b)	40.68 ± 2.12 (c)	32.06 ± 1.41 (b)	42.42 ± 1.55 (c)	24.12 ± 1.38 (a)	< **0.001**	< **0.001**
Chao1	70.40 ± 4.08 (b)	46.85 ± 2.67 (a)	65.21 ± 3.89 (b)	47.92 ± 2.30 (a)	64.96 ± 2.89 (b)	38.38 ± 2.70 (a)	0.495	**0.017**
Shannon (*H′*)	2.30 ± 0.10 (a)	1.95 ± 0.08 (a)	2.15 ± 0.12 (a)	2.20 ± 0.10 (a)	2.28 ± 0.10 (a)	1.95 ± 0.08 (a)	0.596	**0.070**
Evenness (E_H)	0.26 ± 0.02 (a)	0.28 ± 0.02 (a)	0.31 ± 0.03 (a)	0.36 ± 0.03 (a)	0.29 ± 0.02 (a)	0.36 ± 0.03 (a)	0.521	**0.019**
PAT								
OTU Richness	10.20 ± 0.64 (d)	4.56 ± 0.32 (b)	7.92 ± 0.51 (c)	5.30 ± 0.32 (b)	8.24 ± 0.59 (c)	3.18 ± 0.27 (a)	< **0.001**	< **0.001**
Chao1	14.71 ± 1.01 (c)	6.50 ± 0.60 (a)	13.33 ± 1.09 (bc)	7.37 ± 0.65 (a)	11.30 ± 0.89 (b)	4.52 ± 0.52 (a)	**0.056**	**0.003**
Shannon (*H′*)	1.56 ± 0.07 (d)	1.05 ± 0.07 (b)	1.45 ± 0.08 (cd)	1.21 ± 0.07 (bc)	1.61 ± 0.08 (d)	0.74 ± 0.08 (a)	0.334	< **0.001**
Evenness (E_H)	0.58 ± 0.03 (a)	0.76 ± 0.04 (b)	0.67 ± 0.04 (ab)	0.72 ± 0.03 (b)	0.72 ± 0.03 (b)	0.80 ± 0.04 (b)	**0.006**	0.263

Supplement Table S2.3: β-diversity of root-associated fungal community assemblages of all fungi (OTUs), symbiotrophic (SYM), and saprotrophic (SAP) between organic layer and mineral soil. Linear models were used to determine the extent of the fungal turnover (β_{SIM}), nestedness (β_{SNE}) and total β-diversity (β_{SOR}) among the regions. Data indicates n = 50. Significant differences of the means are shown in bold.

Fungal groups	Diversity groups	Sum sq	DF	F value	p value (regions)
All fungi	Turnover	0.755	2	33.221	< **0.001**
	Nestedness	0.117	2	13.373	< **0.001**
	β-diversity	0.279	2	20.482	< **0.001**
SYM	Turnover	0.764	2	13.989	< **0.001**
	Nestedness	0.159	2	6.557	**0.002**
	β-diversity	1.608	2	41.20	< **0.001**
SAP	Turnover	0.509	2	14.122	< **0.001**
	Nestedness	0.175	2	9.977	< **0.001**
	β-diversity	0.101	2	5.636	**0.004**

Supplement Table S2.4: Relative abundance of the root-associated fungal orders and their sequence reads abundance that was used for the analyses presented in this study. Fungal order at least and higher than 0.5% of the total relative abundance were used in the analysis.

Fungal order	Taxa	Seq reads	Relative abundance
Agaricales	300	251005	21.57
Russulales	294	219690	18.88
Helotiales	300	206307	17.73
Thelephorales	283	57089	4.91
Sebacinales	224	39445	3.39
Mytilinidales	243	29840	2.56
Boletales	257	28914	2.48
Pleosporales	287	25927	2.23
Pezizales	268	22713	1.95
Trechisporales	241	17202	1.48
Atheliales	191	16855	1.45
Hypocreales	300	16702	1.44
Cantharellales	232	14290	1.23
Tubeufiales	172	8461	0.73
Chaetothyriales	290	8412	0.72
Xylariales	197	7327	0.63
Cystofilobasidiales	45	7050	0.61
Polyporales	135	6308	0.54
Sordariales	265	5847	0.50
unidentified	300	116894	10.04
Others	228	6202	0.53
Mortierellales	292	5187	0.45
Hymenochaetales	126	5009	0.43
Auriculariales	138	4330	0.37
Capnodiales	263	4208	0.36
Glomerales	138	4121	0.35
Chaetosphaeriales	219	3359	0.29
Archaeorhizomycetales	86	3153	0.27
Diaporthales	93	2763	0.24
Rhytismatales	110	2716	0.23
Thelebolales	158	2097	0.18
Eurotiales	224	1829	0.16
Venturiales	149	1696	0.15
Tremellales	244	1555	0.13
Trichosporonales	193	1353	0.12
Hysterangiales	12	1050	0.09
Minutisphaerales	26	723	0.06
Glomerellales	156	593	0.05
Sporidiobolales	90	531	0.05
Geoglossales	23	511	0.04
Gomphales	11	475	0.04

Supplement Table S2.4 (continued)

Fungal order	Taxa	Seq reads	Relative abundance
Leucosporidiales	75	352	0.03
Phallales	27	350	0.03
Onygenales	53	291	0.03
Filobasidiales	59	289	0.02
Orbiliales	107	272	0.02
Ostropales	45	260	0.02
Dothideales	92	256	0.02
Mucorales	69	232	0.02
Botryosphaeriales	60	195	0.02
Holtermanniales	32	193	0.02
Myrmecridiales	41	181	0.02
Lecanorales	65	160	0.01
Saccharomycetales	45	149	0.01
Coniochaetales	62	148	0.01
Microascales	50	134	0.01
Acarosporales	5	109	0.01
Tremellodendropsidales	28	108	0.01
Verrucariales	1	76	0.01
Phacidiales	28	48	0.00
Diversisporales	14	46	0.00
Zoopagales	32	42	0.00
Olpidiales	9	36	0.00
Atractiellales	13	35	0.00
Archaeosporales	14	23	0.00
Umbelopsidales	15	18	0.00
Magnaporthales	2	16	0.00
Kriegeriales	10	15	0.00
Taphrinales	14	15	0.00
Exobasidiales	8	11	0.00
Calosphaeriales	1	10	0.00
Spizellomycetales	8	10	0.00
Myriangiales	6	9	0.00
Caliciales	2	8	0.00
Cystobasidiales	4	6	0.00
Urocystidales	4	6	0.00
Annulatascales	3	5	0.00
Boliniales	1	5	0.00
Dacrymycetales	4	5	0.00
Teloschistales	1	5	0.00
Malasseziales	3	3	0.00
Patellariales	1	3	0.00
Agaricostilbales	1	2	0.00
Entorrhizales	1	1	0.00
Ophiostomatales	1	1	0.00
Phaeomoniellales	1	1	0.00

Supplement Table S2.5: Classification of the top ninety (> 85% of the total relative abundance) root-associated fungal orders according to their functional groups. SYM = symbiotrophic, SAP = saprotrophic and PAT = pathotrophic. Only SYM fungal orders are coloured in grey and SAP in yellow.

Fungal order	Classification	Trophic mode	Taxa	Seq reads	Relative abundance
Agaricales	SYM+SAP	PAT	9	18	0.01
Agaricales		SAP	780	121047	44.31
Agaricales		SYM	840	152130	55.69
Russulales	SYM	SAP	81	705	0.11
Russulales		SYM	867	657039	99.89
Helotiales	SYM+SAP	PAT	498	10107	4.80
Helotiales		SAP	900	136800	64.99
Helotiales		SYM	606	63576	30.20
Thelephorales	SYM	SAP	15	144	0.28
Thelephorales		SYM	768	51342	99.72
Sebacinales	SYM	SYM	663	117075	100.00
Mytilinidales	SYM	SYM	711	89424	99.93
Mytilinidiales	SYM	SAP	45	66	0.07
Boletales		SAP	45	618	1.05
Boletales		SYM	720	57990	98.95
Pleosporales	SAP+PAT	PAT	360	11271	35.74
Pleosporales		SAP	702	20241	64.18
Pleosporales		SYM	24	24	0.08
Pezizales	SYM	SAP	117	1479	2.52
Pezizales		SYM	720	57261	97.48
Trechisporales	SAP	SAP	516	38856	100.00
Atheliales	SYM	SAP	135	2127	4.25
Atheliales		SYM	528	47898	95.75
Hypocreales	SAP+PAT	PAT	846	10035	23.92
Hypocreales		SAP	894	31911	76.08
Cantharellales	SYM	PAT	9	36	0.11
Cantharellales		SAP	129	300	0.92
Cantharellales		SYM	297	32151	98.97
Tubeufiales	SAP	SAP	510	24876	100.00
Chaetothyriales	SAP	SAP	837	11373	97.16
Chaetothyriales		SYM	114	333	2.84
Xylariales	SAP+PAT	PAT	360	5382	90.02
Xylariales		SAP	255	597	9.98
Cystofilobasidiales	SAP	PAT	21	36	0.17
Cystofilobasidiales		SAP	117	21057	99.83
Polyporales	SAP	SAP	177	18108	100.00
Sordariales	SAP	SAP	750	14013	100.00

Supplement Table S2.6: Indicator root-associated fungal taxa and their functional group in organic layer and mineral soil in three biogeographic regions, ALB = Schwäbische Alb, HAI = Hainich-Dün and SCH = Schorfheide-Chorin. Only the significant fungi species of symbiotrophic (SYM) and saprotrophic (SAP) groups are shown in the table. Indicator root-associated fungal taxa from multiple trophic modes and unassigned groups are not shown in the table. Indicator fungi species are coloured with orange for the organic layer and blue for mineral topsoil.

Region	ASV ID	Orders	Genus	Species	Trophic mode	Habitat preference	Stat	P value
ALB	ASV_000007	Russulales	Lactarius	Lactarius_rubrocinctus	SYM	Mineral	0.70	**0.02**
HAI	ASV_000013	Russulales	unidentified	Russulaceae_sp	SYM	Mineral	0.77	**0.03**
HAI	ASV_000031	Sebacinales	Sebacina	Sebacina_sp	SYM	Mineral	0.57	**0.02**
SCH	ASV_000035	Mytilinidales	Cenococcum	Cenococcum_sp	SYM	Mineral	0.69	**0.02**
ALB	ASV_000040	Agaricales	Laccaria	Laccaria_amethystina	SYM	Organic	0.63	**0.04**
HAI	ASV_000040	Agaricales	Laccaria	Laccaria_amethystina	SYM	Organic	0.71	**0.04**
SCH	ASV_000040	Agaricales	Laccaria	Laccaria_amethystina	SYM	Organic	0.69	**0.03**
HAI	ASV_000044	Agaricales	Hygrophorus	Hygrophorus_eburneus	SYM	Mineral	0.66	**0.01**
SCH	ASV_000054	Russulales	Russula	Russula_ionochlora	SYM	Mineral	0.71	**0.01**
HAI	ASV_000056	Boletales	Melanogaster	Melanogaster_sp	SYM	Organic	0.64	**0.01**
SCH	ASV_000056	Boletales	Melanogaster	Melanogaster_sp	SYM	Organic	0.67	**0.01**
ALB	ASV_000062	Agaricales	Hygrophorus	Hygrophorus_unicolor	SYM	Mineral	0.60	**0.01**
HAI	ASV_000062	Agaricales	Hygrophorus	Hygrophorus_unicolor	SYM	Mineral	0.63	**0.01**
ALB	ASV_000086	Cantharellales	Membranomyces	Membranomyces_sp	SYM	Mineral	0.47	**0.04**
HAI	ASV_000089	Helotiales	Phialocephala	Phialocephala_sp	SYM	Organic	0.52	**0.03**
HAI	ASV_000106	Atheliales	Piloderma	Piloderma_sp	SYM	Mineral	0.81	**0.01**
ALB	ASV_000112	Pezizales	Tuber	Tuber_sp	SYM	Mineral	0.61	**0.01**
HAI	ASV_000112	Pezizales	Tuber	Tuber_sp	SYM	Mineral	0.48	**0.02**
ALB	ASV_000131	Russulales	Russulales	Russulaceae_sp	SYM	Mineral	0.68	**0.01**
HAI	ASV_000131	Russulales	Russulales	Russulaceae_sp	SYM	Mineral	0.80	**0.01**
SCH	ASV_000131	Russulales	Russulales	Russulaceae_sp	SYM	Mineral	0.50	**0.02**
SCH	ASV_000133	Helotiales	Acephala	Acephala_macrosclerotiorum	SYM	Organic	0.55	**0.04**
ALB	ASV_000149	Russulales	Lactarius	Lactarius_pallidus	SYM	Mineral	0.50	**0.02**

Supplement Table S2.6 (continued)

Region	ASV ID	Orders	Genus	Species	Trophic mode	Habitat preference	Stat	P value
HAI	ASV_000149	Russulales	Lactarius	Lactarius_pallidus	SYM	Mineral	0.52	**0.04**
ALB	ASV_000189	Russulales	Russula	Russula_sp	SYM	Mineral	0.51	**0.01**
ALB	ASV_000237	Sebacinales	Sebacina	Sebacina_sp	SYM	Mineral	0.53	**0.01**
HAI	ASV_000243	Russulales	Lactarius	Lactarius_sp	SYM	Mineral	0.72	**0.01**
ALB	ASV_000246	Agaricales	Hymenogaster	Hymenogaster_sp	SYM	Mineral	0.53	**0.01**
ALB	ASV_000258	Agaricales	Inocybe	Inocybe_fibrosoides	SYM	Mineral	0.62	**0.01**
SCH	ASV_000261	Thelephorales	Tomentella	Tomentella_sp	SYM	Mineral	0.37	**0.02**
HAI	ASV_000271	Agaricales	Amanita	Amanita_sp	SYM	Mineral	0.40	**0.04**
SCH	ASV_000271	Agaricales	Amanita	Amanita_sp	SYM	Mineral	0.54	**0.01**
ALB	ASV_000272	Agaricales	Hygrophorus	Hygrophorus_discoxanthus	SYM	Mineral	0.67	**0.01**
ALB	ASV_000306	Russulales	Russula	Russula_foetens	SYM	Mineral	0.53	**0.01**
HAI	ASV_000306	Russulales	Russula	Russula_foetens	SYM	Mineral	0.35	**0.03**
ALB	ASV_000308	Pezizales	Otidea	Otidea_alutacea	SYM	Mineral	0.54	**0.04**
HAI	ASV_000314	Cantharellales	Clavulina	Clavulina_sp	SYM	Mineral	0.57	**0.04**
HAI	ASV_000318	Mytilinidales	Cenococcum	Cenococcum_geophilum	SYM	Mineral	0.64	**0.01**
SCH	ASV_000341	Russulales	Lactarius	Lactarius_vellereus	SYM	Mineral	0.46	**0.03**
HAI	ASV_000352	Sebacinales	Sebacina	Sebacina_sp	SYM	Mineral	0.67	**0.01**
HAI	ASV_000362	Thelephorales	Thelephora	Thelephora_sp	SYM	Mineral	0.60	**0.01**
HAI	ASV_000369	Agaricales	Hymenogaster	Hymenogaster_sp	SYM	Mineral	0.51	**0.01**
HAI	ASV_000398	Russulales	Russula	Russula_sp	SYM	Mineral	0.37	**0.01**
SCH	ASV_000398	Russulales	Russula	Russula_sp	SYM	Mineral	0.64	**0.01**
ALB	ASV_000405	Sebacinales	Sebacina	Sebacina_sp	SYM	Mineral	0.56	**0.01**
HAI	ASV_000458	Agaricales	Hygrophorus	Hygrophorus_sp	SYM	Mineral	0.45	**0.01**
ALB	ASV_000486	Pezizales	Tarzetta	Tarzetta_sp	SYM	Mineral	0.62	**0.03**
ALB	ASV_000559	Thelephorales	Tomentella	Tomentella_sublilacina	SYM	Organic	0.40	**0.01**
SCH	ASV_000559	Thelephorales	Tomentella	Tomentella_sublilacina	SYM	Organic	0.66	**0.01**
ALB	ASV_000675	Russulales	Russula	Russula_acrifolia	SYM	Mineral	0.58	**0.01**

Supplement Table S2.6 (continued)

Region	ASV ID	Orders	Genus	Species	Trophic mode	Habitat preference	Stat	P value
ALB	ASV_000741	Agaricales	Hymenogaster	Hymenogaster_sp	SYM	Mineral	0.60	**0.03**
HAI	ASV_000811	Agaricales	Inocybe	Inocybe_asterospora	SYM	Mineral	0.62	**0.01**
ALB	ASV_000820	Pezizales	Tuber	Tuber_sp	SYM	Mineral	0.73	**0.01**
SCH	ASV_000823	Pezizales	Hydnotrya	Hydnotrya_sp	SYM	Mineral	0.81	**0.01**
ALB	ASV_000870	Thelephorales	Thelephora	Thelephora_anthocephala	SYM	Mineral	0.54	**0.01**
ALB	ASV_000914	Glomerales	unidentified	Glomeraceae_sp	SYM	Mineral	0.63	**0.01**
HAI	ASV_000914	Glomerales	unidentified	Glomeraceae_sp	SYM	Mineral	0.64	**0.01**
HAI	ASV_001095	Russulales	Russula	Russula_olivacea	SYM	Mineral	0.40	**0.01**
HAI	ASV_002257	Sebacinales	Sebacina	Sebacina_sp	SYM	Mineral	0.69	**0.01**
HAI	ASV_006579	Russulales	unidentified	Russulaceae_sp	SYM	Mineral	0.74	**0.01**
HAI	ASV_000041	Agaricales	Megacollybia	Megacollybia_platyphylla	SAP	Organic	0.61	**0.01**
SCH	ASV_000041	Agaricales	Megacollybia	Megacollybia_platyphylla	SAP	Organic	0.51	**0.01**
HAI	ASV_000067	Cystofilobasidiales	Mrakia	Mrakia_aquatica	SAP	Organic	0.66	**0.01**
ALB	ASV_000097	Helotiales	Lachnum	Lachnum_sp	SAP	Mineral	0.51	**0.01**
SCH	ASV_000097	Helotiales	Lachnum	Lachnum_sp	SAP	Mineral	0.55	**0.04**
ALB	ASV_000109	Agaricales	Hydropus	Hydropus_moserianus	SAP	Mineral	0.60	**0.01**
HAI	ASV_000109	Agaricales	Hydropus	Hydropus_moserianus	SAP	Mineral	0.35	**0.03**
HAI	ASV_000110	Helotiales	unidentified	Hyaloscyphaceae_sp	SAP	Organic	0.61	**0.01**
ALB	ASV_000122	Tubeufiales	Titaea	Titaea_maxilliformis	SAP	Organic	0.88	**0.01**
HAI	ASV_000122	Tubeufiales	Titaea	Titaea_maxilliformis	SAP	Organic	0.85	**0.01**
ALB	ASV_000162	Agaricales	Agrocybe	Agrocybe_erebia	SAP	Mineral	0.62	**0.01**
HAI	ASV_000162	Agaricales	Agrocybe	Agrocybe_erebia	SAP	Mineral	0.66	**0.01**
HAI	ASV_000165	Trechisporales	Luellia	Luellia_recondita	SAP	Organic	0.48	**0.01**
SCH	ASV_000165	Trechisporales	Luellia	Luellia_recondita	SAP	Organic	0.73	**0.04**
ALB	ASV_000174	Helotiales	Calycellina	Calycellina_fagina	SAP	Organic	0.92	**0.01**
HAI	ASV_000174	Helotiales	Calycellina	Calycellina_fagina	SAP	Organic	0.90	**0.01**
SCH	ASV_000174	Helotiales	Calycellina	Calycellina_fagina	SAP	Organic	0.88	**0.01**

Supplement Table S2.6 (continued)

Region	ASV ID	Orders	Genus	Species	Trophic mode	Habitat preference	Stat	P value
ALB	ASV_000281	Helotiales	Encoelia	Encoelia_sp	SAP	Organic	0.65	**0.01**
ALB	ASV_000303	Trechisporales	Trechispora	Trechispora_sp	SAP	Organic	0.37	**0.01**
ALB	ASV_000384	Sordariales	Apodus	Apodus_sp	SAP	Organic	0.80	**0.01**
HAI	ASV_000384	Sordariales	Apodus	Apodus_sp	SAP	Organic	0.68	**0.05**
HAI	ASV_000401	Pleosporales	Paraconiothyrium	Paraconiothyrium_sp	SAP	Organic	0.51	**0.01**
SCH	ASV_000416	Trichosporonales	Apiotrichum	Apiotrichum_porosum	SAP	Organic	0.86	**0.01**
SCH	ASV_000589	Hypocreales	unidentified	Bionectriaceae_sp	SAP	Organic	0.80	**0.02**
HAI	ASV_000599	Helotiales	Alatospora	Alatospora_acuminata	SAP	Organic	0.79	**0.01**
ALB	ASV_000615	Hypocreales	Ijuhya	Ijuhya_sp	SAP	Organic	0.40	**0.01**
HAI	ASV_000661	Helotiales	Varicosporium	Varicosporium_elodeae	SAP	Mineral	0.66	**0.02**
ALB	ASV_000663	Hypocreales	Trichoderma	Trichoderma_polysporum	SAP	Organic	0.79	**0.02**
SCH	ASV_000677	Helotiales	unidentified	Hyaloscyphaceae_sp	SAP	Mineral	0.69	**0.02**
ALB	ASV_000772	Chaetothyriales	Cladophialophora	Cladophialophora_sp	SAP	Mineral	0.86	**0.01**
HAI	ASV_000774	Agaricales	Pseudoclitocybe	Pseudoclitocybe_cyathiformis	SAP	Organic	0.37	**0.03**
SCH	ASV_000850	Sordariales	Cephalotheca	Cephalotheca_sp	SAP	Organic	0.66	**0.03**
HAI	ASV_000955	Agaricales	Lycoperdon	Lycoperdon_nigrescens	SAP	Organic	0.52	**0.02**
SCH	ASV_001094	Helotiales	Cryptosporiopsis	Cryptosporiopsis_sp	SAP	Organic	0.64	**0.01**
SCH	ASV_001166	Helotiales	unidentified	Hyaloscyphaceae_sp	SAP	Organic	0.73	**0.01**
ALB	ASV_002043	Chaetothyriales	Cladophialophora	Cladophialophora_sp	SAP	Organic	0.78	**0.01**
HAI	ASV_002043	Chaetothyriales	Cladophialophora	Cladophialophora_sp	SAP	Organic	0.79	**0.01**
SCH	ASV_002658	Archaeorhizomycetales	Archaeorhizomyces	Archaeorhizomyces_sp	SAP	Organic	0.57	**0.02**
HAI	ASV_003876	Agaricales	Agrocybe	Agrocybe_erebia	SAP	Mineral	0.49	**0.01**
ALB	ASV_003921	Pleosporales	Subplenodomus	Subplenodomus_galicola	SAP	Organic	0.58	**0.01**
ALB	ASV_004119	Atheliales	Athelopsis	Athelopsis_lembospora	SAP	Mineral	0.66	**0.01**
ALB	ASV_007068	Agaricales	Cyclocybe	Cyclocybe_sp	SAP	Mineral	0.53	**0.01**

CHAPTER 3

Root mycobiome assembly in temperate forests is massively affected by soil layer

3.1 Introduction

Forest ecosystems harbour about two-thirds of terrestrial plants and offer multiple ecosystem services (Fichtner and Härdtle, 2021). In the past decade, understanding the relationship between trees and below-ground microbial communities have received considerable attention due to the well-recognized role of these interactions for ecosystem functioning (Gamfeldt et al., 2013; Wardle et al., 2004). Tree species differ in their physiological and functional traits; for example, they exhibit different root chemical properties (Lang and Polle, 2011). Thus, tree species can modify soil chemical properties (Gamfeldt et al., 2013; Langenbruch et al., 2012; Reich et al., 2005) and influence the microclimate (Augusto et al., 2002; Aussenac, 2000). As a consequence, variation of tree species richness may affect the richness of below-ground microbial communities (Gao et al., 2017; Tedersoo et al., 2016) by providing a higher variation in resource availability and provision of ecological niches (Hooper et al., 2005; Liu et al., 2015; Wardle, 2006). Moreover, the presence of certain tree species may particularly attract biotrophic fungi through a greater likelihood to enable associations when the tree species increase (Tedersoo et al., 2016).

In forest ecosystems, fungi are highly diverse below-ground key components and perform multiple ecological functions (Baldrian, 2017; Blackwell, 2011; Heijden et al., 2008). Fungi colonize tree roots in diverse ways according to their lifestyle ranges as plant symbionts (symbiotrophs), pathogens (pathotrophs) and decomposer (saprotrophs) (Vandenkoornhuyse et al., 2015). These fungal lifestyles respond divergently to various biotic and abiotic factors because of specific nutrient acquisition strategies of distinct taxa (Kernaghan, 2013; Schappe et al., 2020). Therefore, differences in richness of symbiotrophic (SYM) and saprotrophic (SAP) fungi in response to tree species richness is expected, especially for SYM fungi that directly interact and SAP fungi that interact indirectly with the roots of the host (Ishida et al., 2007; Purahong et al., 2018; Roy-Bolduc et al., 2016; Urbanová et al., 2015). Support for differences in the fungal trophic groups was shown in several previous studies, where SYM richness was strongly affected by tree species richness (TSR) than SAP fungal richness. For example, increasing TSR from 1 to 16 different tree species positively affected fungal richness in a young subtropical Chinese forest (Weißbecker et al., 2019). A stronger positive influence of TSR on SYM fungal richness than SAP richness was also found in temperate forests (Tedersoo et al., 2016), in the western Amazonian rainforest (Peay et al., 2013), and at the global scale (Tedersoo et al., 2014). Although SAP fungal richness showed a weaker relationship with TSR than SYM richness (Peay et al., 2013; Tedersoo et al., 2016), these free-living organisms may benefit from different sources of organic substrates as TSR increase.

However, Tedersoo et al. (2014) reported that the influence of tree diversity on fungal richness largely depends on the context. For example, they found tree diversity was a significant factor for the richness of ectomycorrhizal fungi in the study in Finland and for SAP fungi in Estonia, indicating that both SYM and SAP fungi may benefit from higher tree diversity in particular conditions. At the global scale, the richness of overall soil fungi and their functional groups were unrelated to tree diversity with the exception of ectomycorrhizal fungi (Tedersoo et al., 2014), indicating a strong symbiotic relationship of ectomycorrhizal fungi with the roots of the plants. The positive effect of the TSR on fungal species richness can be further attributed to tree identity effects due to the strong fungal host preference (Ishida et al., 2007; Lang et al., 2011; Tedersoo et al., 2010, 2008). Tedersoo et al. (2016) also found that the diversity of soil fungi is influenced positively by individual tree species more than by tree diversity per se at the local scale.

Roots are important habitat for root-associated fungi (RAF) (Vandenkoornhuyse et al., 2002). RAF richness is vertically stratified among different soil layers with a tendency to decrease with increasing soil depth in temperate (Carteron et al., 2020; Voříšková et al., 2014) and in boreal forests (Rosling et al., 2003; Tedersoo et al., 2003). While SAP fungi dominate in the litter or uppermost organic soil layer, SYM make up most of the fungal community assemblages in the mineral soil (Baldrian et al., 2012; Dickie et al., 2002; Rosling et al., 2003; Lindahl et al., 2007; Clemmensen et al., 2013; Carteron et al., 2020, this thesis, chapter 2). Previous research in temperate and boreal forests suggested that multiple edaphic factors (e.g. soil C, N, C/N ratio, P and cations) structured these fungal trophic groups in vertical soil layers (Dickie et al., 2002; Rosling et al., 2003; Lindahl et al., 2007; Clemmensen et al., 2013; Carteron et al., 2020; this thesis, chapter 2). SYM and SAP are also well known to mediate tree diversity, productivity and nutrient uptake (Baxter and Dighton, 2005; Heijden et al., 2006; Wagg et al., 2011). Despite the important role of RAF in ecosystem functioning, the majority of the previous studies related the effects of the tree species to soil fungi (Gao et al., 2017; Peay et al., 2013; Tedersoo et al., 2016; Weißbecker et al., 2019) or investigated the host preference of root-colonized ectomycorrhizal fungi using several tree species (Ishida et al., 2007; Lang et al., 2011; Tedersoo et al., 2008), and surprisingly ignored the relationship of RAF richness with tree species and nutrient resources in different soil layers.

In this study, we used the large-scale and long-term Biodiversity Exploratories (Fischer et al., 2010) to investigate the importance of tree species identity, richness and root and soil chemistry for RAF communities whose richness was either dominated by SAP (organic layer) or SYM

(mineral topsoil) (this thesis, chapter 2). We investigated roots in 150 forest plots across three biogeographic regions of Germany. The regions differ in environmental conditions (Fischer et al., 2010), root and soil nutrient concentrations (Schröter et al., 2019; Nguyen et al., 2020; this thesis, chapter 2) and tree species composition (Seidel et al., 2020). The main dominant tree species in these regional forest stands is beech (*Fagus sylvatica*) (Seidel et al., 2020). The stands contain various other deciduous tree species such as ash *(Fraxinus excelsior),* lime *(Tilia sp.),* and oak *(Quercus rubur)* (Seidel et al., 2020). Numerous stands are further composed of coniferous tree species including pine *(Pine sylvestris),* and spruce *(Picea abies)* (Seidel et al., 2020). We expect that TSR, tree identity, root, and soil nutrient resources drive the richness of RAF as well as of, SYM and SAP in different soil layers. We tested the following hypotheses in the organic layer and mineral topsoil separately: i) tree species identity influences SYM fungal richness due to fungal host preferences but not that of SAP fungal richness; therefore, SYM fungal richness increases with TSR, while SAP fungi remain unaffected; ii) SYM fungal richness increases with increasing root nutrient resources, while SAP fungal richness increases with soil nutrient resources increase.

3.2 Materials and methods

3.2.1 Study regions characteristics and sampling procedures

This study was conducted in the Biodiversity Exploratories project framework across three biogeographic regions of Germany (http://www.biodiversity-exploratories.de) (Fischer et al., 2010). The research regions are located in Schorfheide-Chorin (SCH) in north-eastern, Hainich-Dün (HAI) in central and Schwäbische Alb (ALB) in south-western Germany (Fischer et al., 2010). The regions differ in soil characteristics, tree species composition and climatic conditions (Fischer et al., 2010; Seidel et al., 2020; Solly et al., 2014). The soil in ALB is typically shallow on bedrock and clay-rich; the parent material is calcareous bedrock, and the soil has a clayey-loamy texture in HAI; the soil is predominantly sandy in SCH (Solly et al., 2014). The forest stands are composed of broadleaved deciduous trees, mainly beech and oak or of coniferous trees, mainly pine or spruce (Seidel et al., 2020). The details site characteristics of these regions have been described elsewhere (Supplement Table S2.1, this thesis, chapter 2).

In early May 2017, root and soil samples were collected from the organic layer and mineral topsoil in 150 forest plots (50 plots in each region) across all three regions as described previously (this thesis, chapter 2). Briefly, aliquots of 1 g of fine roots (< 2 mm in diameter) were collected, washed with tap water, immediately frozen in liquid nitrogen and stored at -80 ^{o}C. All the remaining collected roots were stored at +4 ^{o}C.

3.2.2 Determination of main tree species on the plot

The root samples that had been stored at +4 °C were used to identify the tree species under dissecting microscope (Leica DFC 420 C, Wetzlar, Germany) based on their distinct morphological characteristics as described by Hölscher et al. (2002) (Supplement Figure S3.1). Tree species were defined as others only when it was not possible to identify them based on their root morphological characteristics. We further defined the main tree species at the plot level, whose fine roots were contributed at least and more than 40% of the total fine root mass. Tree species richness (TSR) was determined based on the count number of the various distinct tree species presence on each plot. The relative weight of the fine roots of the distinct tree species at plot level was calculated as:

$$\begin{aligned}\text{The relative weight of the fine roots of the distinct tree species} \\ = \text{absolute weight of the fine roots of that tree species} \\ + \text{relative abundance of that respective fine roots}\end{aligned}$$

3.2.3 Determination of nutrient elements in the soil and roots

Details for the measurement of nutrient elements have been described earlier (this thesis, chapter 2). Briefly, aliquots of dry soil and roots were milled and used for carbon (C) and nitrogen (N) analyses in a CN analyzer "Vario Max" (Elementar Analysensysteme GmbH, Hanau, Germany). Potentially plant-available phosphorous (P_{sol}) was extracted from soil with Bray I extraction solution (Bray and Kurtz, 1945) and total extract for other elements were prepared by pressure extraction in 65% nitric acid (Heinrichs et al., 1986). Measurements were performed by inductively coupled plasma–optical emission mass spectroscopy (ICP-OES) (iCAP 7000 Series ICP–OES, Thermo Fisher Scientific, Dreieich, Germany).

To determine non-structural carbohydrates, fine frozen roots were milled with a ball mill (Type MM400, Retsch GmbH, Haan, Germany) to a fine homogenous powder at a frequency of 30 s^{-1} for 3 min in liquid nitrogen. Subsamples (75 mg) of the fine roots powder were immediately extracted in 1.5 ml extraction medium (dimethyl sulfoxide: HCl of 80:20 v/v). The mixture was placed in a thermoblock (S133-6101, Liebisch, Bielefeld, Germany) for 30 min at 60°C, then cooled on ice, and centrifuged for 5 min at 4 °C at 3075 g force (5417 R, Eppendorf, Hamburg, Germany). Two hundred µl of supernatant was mixed with 1200 µl of 0.2 M cold citrate buffer (pH 10.6), centrifuged for 5 min at 4 °C at 3075 g force and used for carbohydrate measurements as described by (Clausing et al., 2021). The obtained absorbance was used to calculate the concentrations of the carbohydrates in fine roots using the following formula:

$$\mathrm{C} = \frac{\mathrm{V} * \mathrm{MG}}{\varepsilon * d * v * 1000}\ \Delta\mathrm{E}$$

Here, C = concentration of the carbohydrate (mg ml^{-1}), V = test volume (ml), MG = Molecular weight of the carbohydrate (180.16 g mol^{-1}), ε = extinction coefficient of NADPH = 6.3 (l * $mmol^{-1}$ * cm^{-1}), d = cuvette thickness (1 cm), v = sample volume (ml), ΔE = the difference of the absorbance between the blank and the sample measurements (g^{-l}).

3.2.4 Determination of the root and soil resource indices

The following root nutrient elements were used to calculate a root resource index (RRI) for each sample: N, K, Ca, Mg, Mn, Fe, S, P_{sol}, glucose, fructose and starch. For roots in the organic layer (RRI_{org}) and for roots in the mineral soil (RRI_{min}) was calculated as:

$$RRI_{org\_i} = \frac{N_{org\_i}}{N_R} + \frac{K_{org\_i}}{K_R} + \frac{Ca_{org\_i}}{Ca_R} + \frac{Mg_{org\_i}}{Mg_R} + \frac{Mn_{org\_i}}{Mn_R} + \frac{Fe_{org\_i}}{Fe_R} + \frac{S_{org\_i}}{S_R} + \frac{Psol_{org\_i}}{Psol_R} + \frac{Glucose_{org\_i}}{Glucose_R} + \frac{Fructose_{org\_i}}{Fructose_R} + \frac{Starch_{org\_i}}{Starch_R}$$

$$RRI_{min\_i} = \frac{N_{min\_i}}{N_R} + \frac{K_{min\_i}}{K_R} + \frac{Ca_{min\_i}}{Ca_R} + \frac{Mg_{min\_i}}{Mg_R} + \frac{Mn_{min\_i}}{Mn_R} + \frac{Fe_{min\_i}}{Fe_R} + \frac{S_{min\_i}}{S_R} + \frac{Psol_{min\_i}}{Psol_R} + \frac{Glucose_{min\_i}}{Glucose_R} + \frac{Fructose_{min\_i}}{Fructose_R} + \frac{Starch_{min\_i}}{Starch_R}$$

Where i refers to plot i. N_{org_i} and N_{min}_i refer to the concentration of N in roots from the organic layer in plot i (mg g^{-1} DW) and to N in roots from the mineral soil in plot i (mg g^{-1} DW), respectively. A corresponding denomination was used for all other elements and carbohydrates. N_R represents the mean value of N for all plots in all regions and both soil layers. By this normalization, differences of elements between the organic layer and mineral soil were maintained.

Corresponding calculations were conducted for the soil resource indices in the organic layer (SRI_{org_i}) and in the mineral soil (SRI_{min_i}):

$$SRI_{org\_i} = \frac{N_{org\_i}}{N_R} + \frac{K_{org\_i}}{K_R} + \frac{Ca_{org\_i}}{Ca_R} + \frac{Mg_{org\_i}}{Mg_R} + \frac{Mn_{org\_i}}{Mn_R} + \frac{Fe_{org\_i}}{Fe_R} + \frac{S_{org\_i}}{S_R} + \frac{Psol_{org\_i}}{Psol_R}$$

$$SRI_{min\_i} = \frac{N_{min\_i}}{N_R} + \frac{K_{min\_i}}{K_R} + \frac{Ca_{min\_i}}{Ca_R} + \frac{Mg_{min\_i}}{Mg_R} + \frac{Mn_{min\_i}}{Mn_R} + \frac{Fe_{min\_i}}{Fe_R} + \frac{S_{min\_i}}{S_R} + \frac{Psol_{min\_i}}{Psol_R}$$

3.2.5 DNA extraction and bioinformatic analysis

Approximately 180 mg of frozen homogenized root powder were used for the genomic DNA extraction with the innuPREP Plant DNA Kit (Analytik Jena AG, Jena, Germany), according to the instructions of the manufacturer. To amplify the fungal ribosomal internal transcribed spacer 2 (ITS2) region in roots, we used ITS3KYO2 (5′-GATGAAGAACGYAGYRAA-3′) as the forward primer (Toju et al., 2012) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as the reverse primer (White et al., 1990). The polymerase chain reaction (PCR), determination of the PCR product size, purification, quantification and bioinformatics pipeline has been described

elsewhere (this thesis, chapter 2). Sequencing was conducted on the Illumina MiSeq platform using the MiSeq Reagent Kit v3 (Illumina Inc., San Diego, USA) at the Göttingen Genomics Laboratory (G2L), Germany. Amplicon sequence variants were clustered at a 97% threshold and referred to as operational taxonomic units (OTUs). For this analyses, the count table of Fungal OTUs (Dataset ID: 30973 and 30974, this thesis, chapter 2) was used and functionally annotated as symbiotroph (SYM), saprotroph (SAP) and pathotroph (PAT) using the annotation tool 'FunGuild' (Nguyen et al., 2016).

3.2.6 Statistical analysis

All the statistical analyses were conducted in R v4.0.3 (R Development Core Team., 2020). Data were visualized using the 'ggplot2′ package (Wickham, 2009) in R. Data normal distribution and homogeneity of the variances were investigated visually using histograms and residual plots. Data were further logarithmically transformed to meet the criteria of the normal distribution when necessary. The generalized linear model with Poisson distribution was used with the function *glm()* from 'lme4' package to compare parameters for which count data were available such as fungal and tree species richness (Bates et al., 2015). Linear models were used with the function *lm()* from 'lme4' package to compare parameters for which continuous data were available such as Shannon diversity, evenness, RRI, SRI and relative weight of the fine roots (Bates et al., 2015). Fungal species richness, Chao1, Shannon diversity and evenness were calculated using the 'vegan' package (Oksanen et al., 2013). The significance level was tested using the *Anova()* function from the 'car' package (Fox and Wisberg, 2021). Pairwise comparisons were conducted with a *post hoc* test (HSD Tukey's honestly significant difference) using the function *glht()* from the 'multcomp' package (Hothorn et al., 2008).

To investigate the effects of TSR, RRI and SRI on root fungal richness, the Pearson correlation coefficients were calculated using the function *cor.test()*from the 'stats' package. To further distinguish the influence of tree species identity (TSI) on root fungal richness, we used the function *glmnet()* from the 'glmnet' package (Friedman et al., 2010) and conducted the Least Absolute Shrinkage and Selection Operator (LASSO) regression. The LASSO regression was used to estimate the influence of each predictor against the response variables (Warton et al., 2015). The LASSO model further provides regression coefficients for all predictors to measure their stability scores (Warton et al., 2015). Predictors with zero coefficients are considered non-significant, and coefficients above zero are considered to represent influential predictors of the response variables (Meinshausen and Bühlmann, 2006). In this study, we used RAF richness (all fungi) and richness of SYM and SAP fungi as response variables and the relative weight of

the fine roots of different tree species as predictors to fit the LASSO regression model. Further, generalized linear models were used in the final LASSO predicted model to test the level of significance among the predicted influential tree species on richness of RAF, SYM and SAP.

To partition the variance of our all predictors (e.g. RRI, SRI, TSR and main tree species on the plot) on root fungal richness, we further used redundancy analysis (RDA) with the function *varpart()* from 'vegan' package (Oksanen et al., 2013), including the richness of RAF, SYM and SAP as a response variable and RRI, SRI, TSR and main tree on the plot as the explanatory predictors. The significance level of each predictor on fungal richness was tested using the function *anova.rda()* with 999 permutation steps.

3.3. Results

3.3.1 Effects of the tree species richness and tree identity on the root-associated fungal richness

We identified the following seven tree species on the basis of their morphological root characteristics in the organic layer and mineral topsoil: beech (*Fagus sylvatica)*, spruce (*Picea abies*), pine (*Pinus sylvestris*), oak (*Quercus robur*), ash (*Fraxinus excelsior*), maple (*Acer pseudoplatanus)*, and lime (*Tilia*) (Figure 3.1, Supplement Figure S3.1). We defined a tree species whose fine roots contributed at least and more than 40% to total fine root mass as the main tree species on that plot (Figure 3.1).

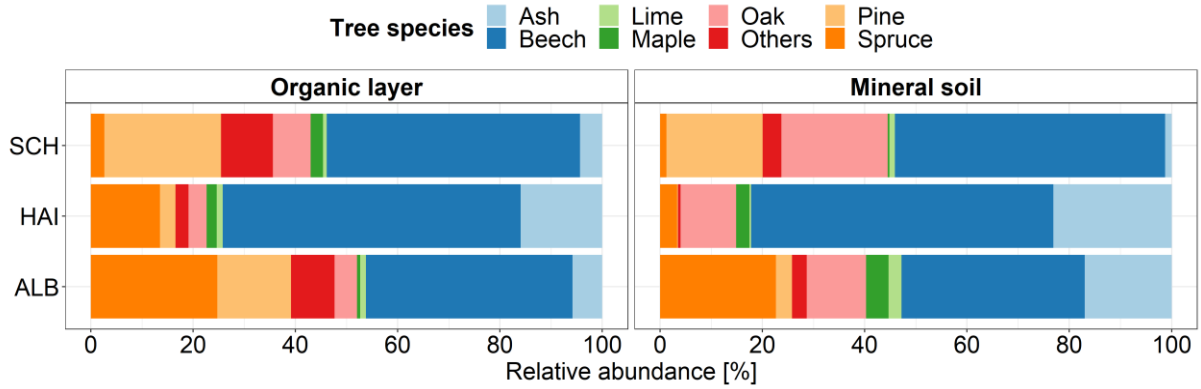


Figure 3.1: Relative abundance of the fine roots mass of the different tree species in different soil layers and in three biogeographic regions. ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide Chorin. Each colour of stack bars refer to distinct tree species: Ash (light blue), Beech (blue), Lime (light green), Maple (green), Oak (light red), Others (red), Pine (light orange) and Spruce (Orange).

Lime was the only tree species, which was rare and thus, did not occur as the main tree species on any single plot (Figure 3.1). Beech was the most dominant tree species in both soil layers in all three regions contributing about 40 to 60% of the relative root mass per Exploratory,

followed by spruce, ash, pine and oak in ALB; ash, spruce, oak and pine in HAI; and pine, oak and ash in SCH (each region: organic, $p < 0.001$, mineral, $p < 0.001$; Figure 3.1).

The relative weight of the fine roots of beech differed significantly between soil layers in ALB and HAI (ALB, $F = 40.17$, $p < 0.001$; HAI, $F = 99.84$, $p < 0.001$) and showed higher abundance in mineral soil than the organic layer (Supplement Table S3.1). In comparison to ALB and HAI, the relative weight of the fine roots of beech was equally distributed between the soil layers in SCH ($F = 0.00$, $p = 0.95$, Supplement Table S3.1). The relative weight of the fine roots of ash and maple were varied significantly between the different soil layers in all three regions and showed higher abundance in mineral soil than in the organic layer in ALB and HAI but not in SCH (F and p values in Supplement Table S3.1). In contrast to ash and maple, the relative weight of the fine roots of oak was also varied significantly between soil layers and always showed higher relative abundance in mineral soil than in the organic layer (F and p values in Supplement Table S3.1). The relative weight of the fine roots of the others (unidentified) tree species did not show any significant differences between the soil layers in all regions (F and p values in Supplement Table S3.1). In addition to the relative abundance of tree species, TSR also varied significantly between the soil layers in all three regions ($p < 0.001$, Supplement Table S3.2) and showed higher richness in mineral soil than the organic layer in ALB and HAI but not in SCH (Supplement Table S3.2).

To investigate the relationship between TSR and fungal richness, we plotted TSR against the richness of RAF, SYM and SAP. Pearson correlation revealed a significant positive relationship between TSR and richness of RAF, SYM and SAP in the mineral soil but not in the organic layer (Figure 3.2). We further compared the regression line of SYM with SAP richness that resulted the slopes did not differ significantly ($t = 1.20$, $p = 0.231$, Figure 3.2 b,c, Supplement Table S3.3), indicating the TSR had similar effects on both SYM and SAP richness in mineral soil.

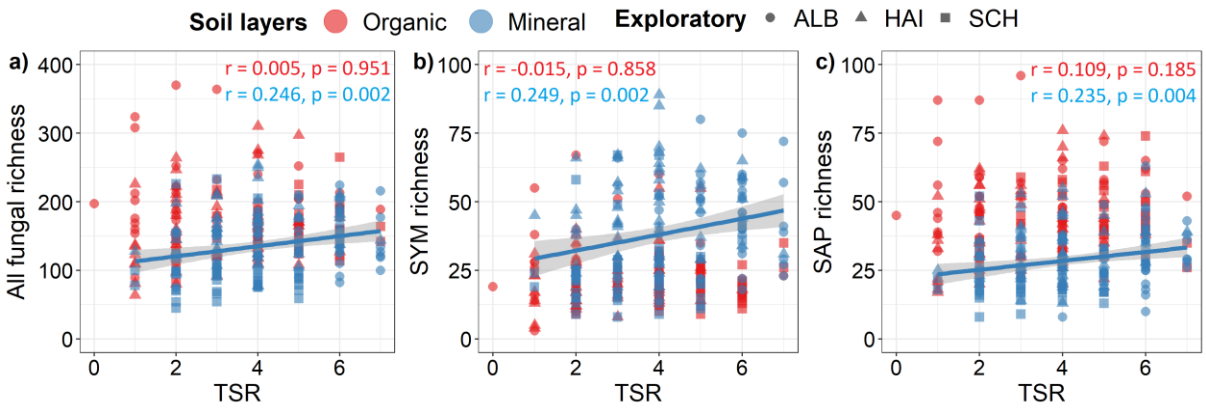


Figure 3.2: Relationship between tree species richness (TSR) and root-associated fungi richness in the organic layer (red colour) and mineral soil (blue colour). Pearson correlation test was used to determine the relationship between TSR and root-associated fungal richness. The richness of (a) all fungi, (b) symbiotrophic (SYM) and (c) saprotrophic (SAP) fungi were annotated on the basis of operational taxonomic units (OTUs). ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide Chorin. Round shape refers to ALB, triangle to HAI and squared to SCH region. Only the significance relationships are shown in regression lines with standard errors in light grey colour.

In the next step, we tested the impact of individual tree species on fungal richness using the LASSO regression model. We found that none of the tree species was a potential predictor for RAF and SYM richness in the organic layer (Figure 3.3 a,b). According to the dashed line in Figure 3.3, beech and lime were be potential predictors for SAP richness in the organic layer (Figure 3.3c). However, the LASSO coefficients were negative for beech and consequently, the statistical analysis did not support that beech has a significant effect on SAP richness in the organic layer (Supplement Table S3.4). In contrast to beech, SAP richness was significantly affected by lime in organic layer (Figure 3.3c, Supplement Table S3.4). In the mineral soil, RAF richness was affected by beech and ash, while the effects of lime, oak and others were not significant (Figure 3.3d, Supplement Table S3.4). When only the richness of the SYM group of fungi was tested, we uncovered significant effects of beech, ash, spruce and oak (Figure 3.3e, Supplement Table S3.4). We found ash and lime were the potential predictors for SAP richness in mineral soil (Figure 3.3f), and only ash had a significant effect on SAP richness (Supplement Table S3.4). Overall, the effect of tree species identity was more pronounced on SYM richness in mineral soil (Supplement Table S3.4).

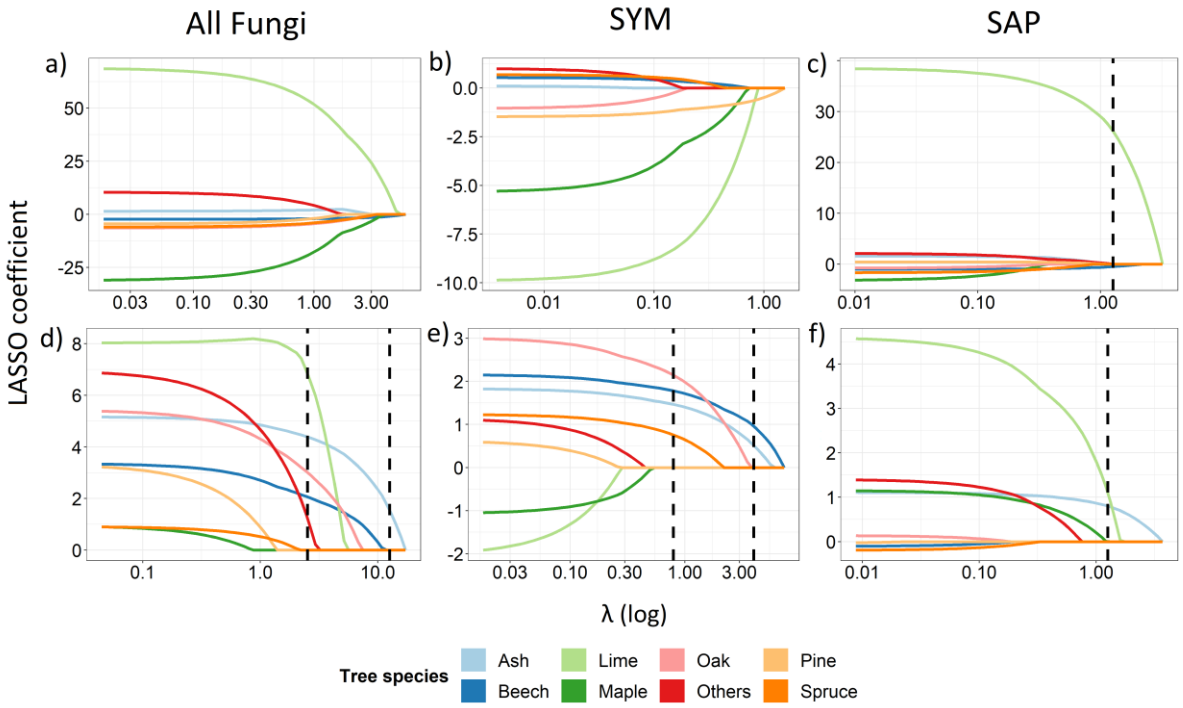


Figure 3.3: Impact of the individual tree species on root-associated fungal richness in organic layer (a- c) and in mineral topsoil (d-f). Least absolute shrinkage and selection operator (LASSO) logistic regression models were used to uncover the impact of the individual tree species on root fungal richness. The richness of all fungi, symbiotrophic (SYM) and saprotrophic (SAP) fungi were annotated based on the operational taxonomic units (OTUs). The vertical line indicates the influencial tree species which may contribute to fungal richness. Each colour of the line refer to distinct tree species: Ash (light blue), Beech (blue), Lime (light green), Maple (green), Oak (light red), Others (red), Pine (light orange) and Spruce (Orange). Statistical details are shown in Supplement Table S3.4.

3.3.2 Effects of the root and soil nutrient resources on the root-associated fungal richness

TSR showed significant relationships with RRI and SRI in both mineral soil and the organic layer (Supplement Table S3.5), but surprisingly, the relationship of TSR with SRI or RRI were negative in the organic layer. To find out whether the resources influenced the richness of RAF, we evaluated linear models for the richness of fungal groups on the roots and soil or root resources. In the mineral soil, the richness of RAF, SYM and SAP showed significant positive relationships with RRI (Figure 3.4 a-c, Supplement Table S3.6) as well as with SRI (Figure 3.4 d-f, Supplement Table S3.6). In the organic layer, the richness of RAF, SYM and SAP were unrelated to RRI (Figure 3.4 a-c, Supplement Table S3.6). However, RAF and SYM richness in the organic layer were positively related to SRI (Figure 3.4 d,e, Supplement Table S3.6), whereas SAP richness was unaffected by SRI (Figure 3.4f, Supplement Table S3.6). We further compared the regression lines of SRI for the richness of RAF and SYM, which resulted a

significant difference in the slopes between the soil layers for SYM richness but not for the RAF richness (RAF: t = 0.54, p = 0.59; SYM: t = 3.54, p < 0.001; Figure 3.4 d, e).

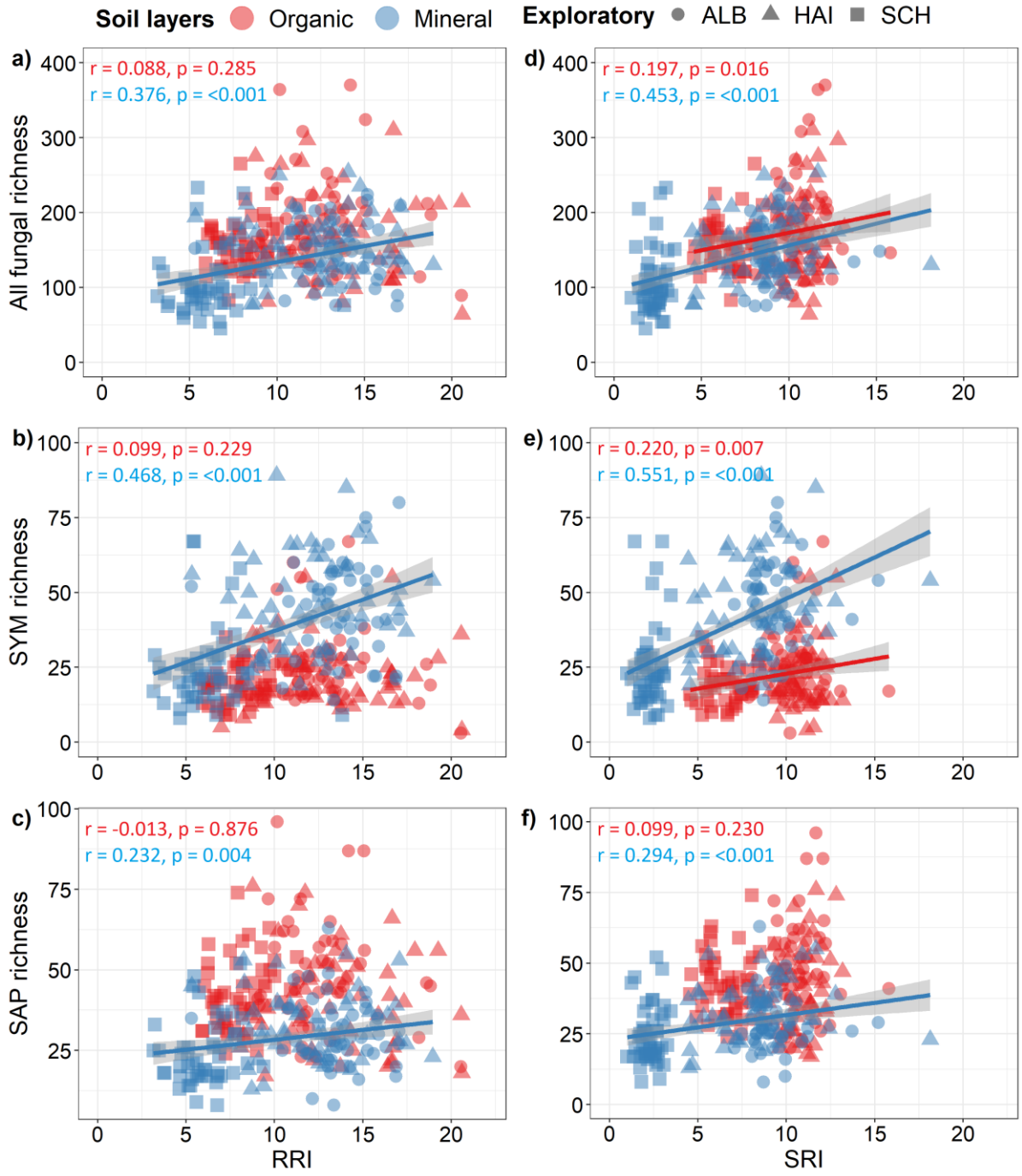


Figure 3.4: Dependence of the root-associated fungal richness on root resource index (RRI) and soil resource index (SRI) in the organic layer and mineral soil. The richness of all fungi, symbiotrophic (SYM) and saprotrophic (SAP) fungi were annotated on the basis of operational taxonomic units (OTUs). Dependence were determined by the Pearson correlation analysis. Only the significance effects of RRI (a-c) and SRI (d-f) on root fungal richness are shown in regression lines with standard errors in light grey colour. Red colour refer to organic layer and blue to mineral soil. ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide Chorin. Round shape refer to ALB, triangle to HAI and squared to SCH region.

Moreover, the regression slopes further suggested that influence of SRI was more pronounced on SYM richness in mineral soil than in the organic layer (organic, slope = 0.99; mineral, slope = 2.76, Figure 3.4e, Supplement Table S3.6). Overall, this result suggests that RRI drives root fungal richness only in the mineral soil, while SRI drives the richness of RAF and SYM in both soil layers (Figure 3.4 a-f).

In the final step, we investigated the contribution of RRI, SRI, main tree species on the plot and TSR on RAF, SYM and SAP richness in the organic layer and in mineral soil. Variation partitioning revealed that the total effects of RRI, SRI, main tree species and TSR explained only 3.3 % of the variation in RAF richness, 4.6 % variation in SYM and 2.3% variation in SAP richness in the organic layer (Figure 3.5). The factor, which explained most of this variation was SRI (RAF: 3.2%, F = 5.98, p = 0.02, Table 3.1), SYM (4.2%, F = 7.57, p = 0.01, Table 3.1) and SAP richness (0.03%, F = 1.46, p = 0.22, Table 3.1). In mineral soil, the total effects of RRI, SRI, main tree species and TSR explained 46.9% of the variation in RAF richness, 67.5% variation in SYM and 23.5% variation in SAP richness (Figure 3.5). Here, most of the variation in RAF, SYM and SAP richness was explained by significant effects of all these predictors, i.e. RRI and SRI, main tree species on the plot and TSR in mineral soil (explained variation, F and p values in Table 3.1). This finding indicates that RAF richness in the mineral topsoil is driven by the vegetation and their nutrient resources, while RAF in the organic soil layer is marginally driven only by soil resources.

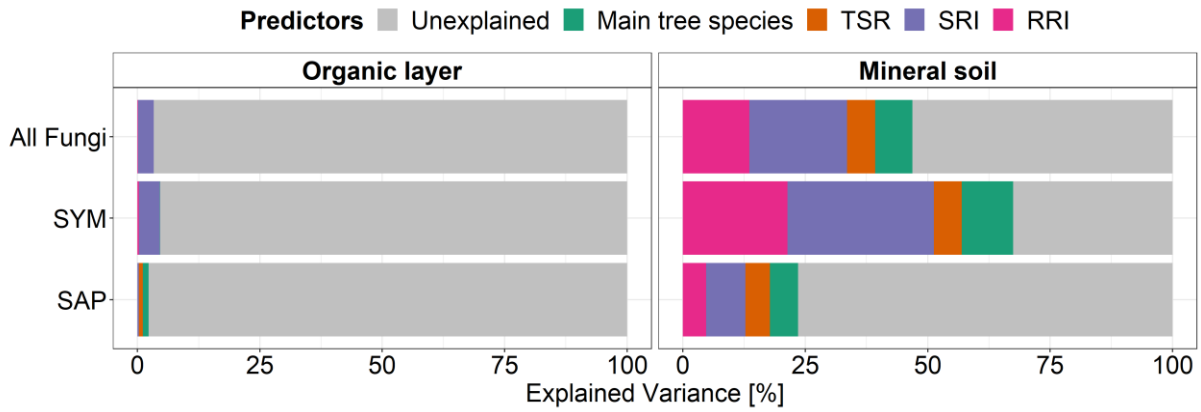


Figure 3.5: The variation on the root-associated fungal species richness explained by the following predictors: root resource index (RRI), soil resource index (SRI), main tree species on the plot (main tree species), tree species richness (TSR). Further statistical informations are shown in table 3.1. The richness of all fungi, symbiotrophic (SYM) and saprotrophic (SAP) fungi were annotated on the basis of operational taxonomic units (OTUs). Pink colour of the stack bars refer to RRI, violet to SRI, orange to TSR, green to main tree species and grey to unexplained variations.

Table 3.1: The variation on the root-associated fungal species richness explained by the following predictors: root resource index (RRI), soil resource index (SRI), main tree species on the plot (main tree), tree species richness (TSR). The richness of all fungi, symbiotrophic (SYM) and saprotrophic (SAP) fungi were annotated based on operational taxonomic units (OTUs). Significant predictors at $p \leq 0.05$ are indicated with bold letters.

	Predictors	Full model				Adj. r^2	F	p
		Adj. r^2	Residual	F	P			
Organic layer								
All Fungi	RRI					0.001	1.15	0.251
	SRI					0.032	5.98	**0.017**
	Main tree	0.033	0.97	1.77	0.08	-0.022	0.37	0.857
	TSR					-0.006	0.10	0.757
SYM	RRI					0.003	1.47	0.212
	SRI					0.042	7.57	**0.009**
	Main tree	0.046	0.95	1.68	0.12	0.001	1.03	0.344
	TSR					-0.007	0.00	0.998
SAP	RRI					-0.007	0.03	0.889
	SRI					0.003	1.46	0.224
	Main tree	0.023	0.98	2.03	**0.05**	0.012	1.36	0.230
	TSR					0.008	2.19	0.142
Mineral soil								
All Fungi	RRI					0.136	24.41	**<0.001**
	SRI					0.199	37.97	**<0.001**
	Main tree	0.469	0.53	9.11	**<0.001**	0.076	4.07	**0.003**
	TSR					0.058	10.11	**0.002**
SYM	RRI					0.213	41.43	**<0.001**
	SRI					0.299	63.91	**<0.001**
	Main tree	0.675	0.33	12.68	**<0.001**	0.105	5.38	**0.001**
	TSR					0.057	9.95	**0.001**
SAP	RRI					0.048	8.47	**0.003**
	SRI					0.080	13.95	**0.004**
	Main tree	0.235	0.77	5.41	**<0.001**	0.058	3.28	**0.019**
	TSR					0.050	8.81	**0.006**

3.4 Discussion

Tree species richness, tree diversity, and the presence of certain tree species may mediate the ecosystem stability, ecosystem functioning and thereby further provide multiple ecosystem services. However, little is known about the effects of the TSR and tree identity on RAF richness in different soil layers. Here, we investigate the influence of tree species, root and soil chemistry on RAF richness in different soil layers in temperate forests.

Different tree species vary in their physiological and functional traits (Augusto et al., 2002; Aussenac, 2000; Gamfeldt et al., 2013; Lang and Polle, 2011; Langenbruch et al., 2012; Reich et al., 2005). Therefore, increasing TSR may influence fungal richness through a higher variation of resource availability. Consequently, fungal species richness associated with a certain tree species can benefit from neighbouring tree communities in forest ecosystem when

TSR increases (Bogar and Kennedy, 2013; Hubert and Gehring, 2008; Zhang et al., 2017). For example, the community composition and diversity of ectomycorrhizal fungi of *Alnus rhombifolia* showed significant changes in community structure and diversity by neighbour trees *Betula occidentalis* in a temperate forests in USA when they were planted together than alone (Bogar and Kennedy, 2013). Likewise, Buée et al., (2009) found higher number of soil fungal OTUs in a mixture of oak, spruce and pine-dominated forest than only in beech stands. Moreover, higher TSR usually results in higher soil C storage than monospecific stands (Gamfeldt et al., 2013; Huang et al., 2018) that may also affect fungal richness. The influence of the tree species might be greater for RAF than soil fungi since SYM fungi are direct interacts with the roots of the trees and depend on energy and carbohydrate from their host, whereas these factors indirectly influence SAP fungi. Previously, it has been shown that TSR strongly affect soil SYM fungal richness compared to SAP in temperate forest (Tedersoo et al., 2016), in northern Baltic region (Tedersoo et al., 2020), in western Amazonian rainforest (Peay et al., 2013) and at the global scale (Tedersoo et al., 2014). However, our study is different from these previous studies because all these studies have been investigated soil fungal richness either only from the mineral topsoil (about 5 cm depth) (Peay et al., 2013; Tedersoo et al., 2020, 2016) or mixture of both organic layer and mineral soil (Tedersoo et al., 2014). Therefore, it is still not known about the effect of the tree species on fungal richness in organic layer and mineral soil separately. Here, we investigated the effect of the tree species on RAF richness in organic layer and mineral soil. We found a positive relationship between TSR and the richness of RAF, SYM and SAP in the mineral topsoil, but not in the organic layer. This result suggests that forest floor may overrules the effects of TSR on RAF richness.

The relationship between the TSR and fungal richness can be attributed to fungal host preference (Dickie, 2007). Evidence corroborating this hypothesis has been obtained, analysing ectomycorrhizal fungi on roots of conifer-broadleaf forests in Japan (Ishida et al., 2007), in Tasmanian wet sclerophyll forest (Tedersoo et al., 2008), in western Amazonia forest (Tedersoo et al., 2010), in temperate forest in Europe (Lang et al., 2011) and in Oak-dominated cool temperate forest in Japan (Toju et al., 2013), but not in the studies in tropical forests (Smith et al., 2011; Tedersoo et al., 2011). Our result indicates that tree identity effects are more pronounced on SYM fungal richness compared to SAP, but the effects varied in different soil layers. For examples, we found beech, ash, spruce, and oak strongly influence SYM fungal richness in mineral soil, but it was not the same case in organic layer. In contrast to SYM, only ash significantly influence SAP richness in mineral soil and lime in the organic layer. It should be noted that the relative weight of the fine roots of lime was not very abundant and not the

among main tree species at the plot level. Overall, these results clearly indicate fungal host preferences for certain tree species. However, many of the tree species did not show significant influence when overall RAF richness was counted. Only beech and ash significantly influence RAF richness in mineral soil but no tree identity effects were found in the organic layer. Since RAF are composed of SYM and SAP and many fungi from these trophic groups could assign to a function that has contrasting behaviour, and therefore, may lead to the loss of the signatures by a distinct tree species on total RAF richness. Pine was among the abundant tree species in two regions but did not show any influence on root fungal richness. Pine is known to produce highly acidic litter on the forest floor which may negatively affect fungal richness (Tedersoo et al., 2014; Urbanová et al., 2015b). Therefore, we expected a strong influence of pine in RAF richness. However, previous study reported both positive and negative influence of pine in soil fungal richness. For example, pine strongly suppressed the richness of total soil fungal richness in a study in Estonia, while it had a marginal positive influence on total soil fungal richness in a study in Finland (Tedersoo et al., 2016). Roots of the several deciduous and coniferous tree species such as beech, lime, pine, and spruce are highly colonized with ectomycorrhizal fungi (Crahay et al., 2013; Lang et al., 2011; Pena et al., 2017; Rudawska et al., 2011), while those of maple and ash form arbuscular mycorrhizal fungal associations (Lang et al., 2011).

The nutrient concentrations of the roots and their surrounding soil chemical properties varied among the different tree species (Lang and Polle, 2011; Langenbruch et al., 2012; Schmidt et al., 2015). For example, higher concentrations of K, Mg and S were observed in roots of ash compared to beech and lime (Lang and Polle, 2011). High pH and Mg and low Al nutrient concentration in soil with ash compared to beech forest stands (Langenbruch et al., 2012). Therefore, variation of the tree species may offer a wider range of resource availability, thus leading to higher fungal richness on roots with mixed tree species than of monospecific forest stands, as mentioned earlier. We found TSR were positively correlated with RRI and SRI in mineral soil and but opposite trends in organic layer. This result was surprising as we expected that nutrient resources increase with the increase of TSR. Several previous studies reported that TSR is usually declined with increasing soil fertility gradient (Dickson and Foster, 2011; Liu et al., 2015; Reich, 2009). Here, the organic layer was characterized by high nutrient-rich soil conditions than mineral topsoil (this thesis, chapter 2), which might explain the lower relative weight of the fine roots of several tree species in the organic layer than mineral soil. We further tested the hypothesis that richness of the different functional components of RAF is related to resource availability, i.e. SAP fungi are less affected by RRI than SRI, whereas SYM fungi are strongly affected by RRI and less by SRI. We observed the dependency of RAF, SYM and SAP

fungal richness on RRI and SRI varied in different soil layers. The dependency of RAF richness on nutrient resources was mainly found in the mineral soil, except for the marginal effects of SRI on RAF and SYM richness in organic layer. These results do not meet our expectation, at least for the SYM fungi because SYM fungi directly interact with the roots of the tree. Therefore, we expect the dependency of SYM fungal richness to RRI in both soil layers. Previously, it has been reported that decomposition of litter usually results in a high proportion of basic cations, such as calcium and magnesium, which thereby alters the soil pH and nutrient availability in forest floor (Lladó et al., 2018; Nordén, 1994; Prescott & Grayston, 2013) and consequently, may affect fungal community structure (Tedersoo et al., 2014). This might explain the lack of dependency of fungal richness on nutrient resources in the organic layer.

We further demonstrated that about 95 to 96% of the variation of richness of RAF, SYM and SAP in the organic layer was unexplained by the measured environmental variables (RRI, SRI, TSR and main tree species on the plot). Only SRI was the statistically significant driver of RAF and SYM richness but the biological relevance is hard to assess because only less than 5% of the variance was explained by this factor. In contrast to organic layer, about 25 to 70% of the variation of richness of RAF, SYM and SAP were explained by the measured variables listed above. Although all these variables were the statistically significant driver of fungal richness in mineral soil, but their contribution to SYM fungal richness (67.4%) was way higher than SAP (23.6%). The unexplained variance could be attributed to numerous unmeasured variables that may also influence the fungal richness in different soil layers. For examples, soil pH, precipitation and temperature show a strong influence on soil fungal communities at the global scale (Tedersoo et al., 2014). However, there were a large fraction of the measured variables unexplained the variation of fungal richness in the organic layers. Here, we can only speculate few possible reasons that might indirectly overrule the effects of vegetation, root and soil chemistry in the organic layer. For example, plant litter chemistry on the forest floor may create a relatively homogenous environment that shows rather a vertical (age of the decay, decomposition rate) than a horizontal differentiation (Prescott & Grayston, 2013). Furthermore, the forest floor usually has a high nutrient availability, less variable and it might be more directly affected by temperature and precipitation than the deeper mineral soil (Prescott & Grayston, 2013). In addition, according to the ecological concept, microbial species richness and their assemblages are determined by stochastic and deterministic processes (Cline and Zak, 2014). Several studies of soil localized fungi (Peay et al., 2016), root-associated fungi (Schröter et al., 2019), both soil and root fungi (Beck et al., 2015) has supported this ecological theory. For example, Beck et al. (2015) observed a stronger convergence of soil localized fungi than

root-associated fungi in the same geographic region, indicating different mechanism may influence in shaping soil and root-associated fungal community structure, i.e. stochastic or neutral processes for root-associated fungal assembly and deterministic or niche-based processes for soil fungi. Here, our study may suggest that the richness of fungi in the organic layer is shaped by neutral processes, whereas the richness of fungi in mineral soil is governed by deterministic processes.

3.5 Conclusion

We demonstrated that the richness of root-associated fungi is driven by tree species richness, tree identity, root and soil chemistry in temperate forests. One of the significant findings of this study is that the richness of the root-associated fungi in these forests ecosystem is massively affected by soil layers. Forest floor strongly overrules the effects of the vegetations and nutrient resources on the richness of root-associated mycobiome. Our study further shows the importance of higher diversity of tree species for symbiotic and saprotrophic fungal richness that are prevalent in many boreal and temperate forest ecosystems. Such interaction between these below-ground functional components and the above-ground tree species are important for better ecosystem functioning. We further distinguish the significant influence of the individual tree species for symbiotic fungi; this will help us to select the tree species in future forest management practice related project. Overall this study suggests that the variation of the tree species is essential to maintain the higher belowground microbial diversity in forest ecosystem.

3.6 Declaration

I contributed to the joined soil sampling 2017, organized by soil and microbe core project of the Biodiversity Exploratories. I analysed the roots according to tree species in the organic layer and mineral soil across all the Exploratories, conducted the root and soil chemistry measurements (C and N, root carbohydrates), provided the materials for ICP and Illumina sequencing. I analysed and synthesised the data using advanced statistical methods. Illumina amplicon sequencing, DNA sequence processing and annotation were conducted by Dominik Schneider and Rolf Daniel at the Department of Genomic and Applied Microbiology, University of Göttingen.

3.7 Data availability

All data have been deposited in the BExIS database (https://www.bexis.uni-jena.de) under the following accession numbers (data owner): soil chemistry - 26228 and 26229 (Polle), root chemistry, root dynamics and tree species - 31048 and 31049 (Polle) and root-associated fungal taxonomy - 30973 and 30974 (Polle).

3.8 References

Augusto, L., Ranger, J., Binkley, D., Rothe, A., 2002. Impact of several common tree species of European temperate forests on soil fertility. Ann. For. Sci. 59, 233–253. https://doi.org/10.1051/forest:2002020

Aussenac, G., 2000. Interactions between forest stands and microclimate: Ecophysiological aspects and consequences for silviculture. Ann. For. Sci. 57, 287–301. https://doi.org/10.1051/forest:2000119

Baldrian, P., 2017. Microbial activity and the dynamics of ecosystem processes in forest soils. Current Opinion in Microbiology, Environmental microbiology 9 37, 128–134. https://doi.org/10.1016/j.mib.2017.06.008

Baldrian, P., Kolařík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. The ISME Journal 6, 248–258. https://doi.org/10.1038/ismej.2011.95

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Baxter, J.W., Dighton, J., 2005. Phosphorus source alters host plant response to ectomycorrhizal diversity. Mycorrhiza 15, 513–523. https://doi.org/10.1007/s00572-005-0359-0

Blackwell, M., 2011. The Fungi: 1, 2, 3 … 5.1 million species? American Journal of Botany 98, 426–438. https://doi.org/10.3732/ajb.1000298

Bogar, L.M., Kennedy, P.G., 2013. New wrinkles in an old paradigm: neighborhood effects can modify the structure and specificity of Alnus-associated ectomycorrhizal fungal communities. FEMS Microbiology Ecology 83, 767–777. https://doi.org/10.1111/1574-6941.12032

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Science 59, 39–46. https://doi.org/10.1097/00010694-194501000-00006

Carteron, A., Beigas, M., Joly, S., Turner, B.L., Laliberté, E., 2020. Temperate forests dominated by arbuscular or ectomycorrhizal fungi are characterized by strong shifts from saprotrophic to mycorrhizal fungi with increasing soil depth. Microb Ecol. https://doi.org/10.1007/s00248-020-01540-7

Clausing, S., Pena, R., Song, B., Müller, K., Mayer-Gruner, P., Marhan, S., Grafe, M., Schulz, S., Krüger, J., Lang, F., Schloter, M., Kandeler, E., Polle, A., 2021. Carbohydrate depletion in roots impedes phosphorus nutrition in young forest trees. New Phytologist 229, 2611–2624. https://doi.org/10.1111/nph.17058

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and associated

fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615–1618. https://doi.org/10.1126/science.1231923

Cline, L.C., Zak, D.R., 2014. Dispersal limitation structures fungal community assembly in a long-term glacial chronosequence. Environmental Microbiology 16, 1538–1548. https://doi.org/10.1111/1462-2920.12281

Crahay, C., Wevers, J., Munaut, F., Colpaert, J.V., Declerck, S., 2013. Cryopreservation of ectomycorrhizal fungi has minor effects on root colonization of Pinus sylvestris plantlets and their subsequent nutrient uptake capacity. Mycorrhiza 23, 463–471. https://doi.org/10.1007/s00572-013-0489-8

Dickie, I.A., 2007. Host preference, niches and fungal diversity. New Phytologist 174, 230–233. https://doi.org/10.1111/j.1469-8137.2007.02055.x

Dickie, I.A., Xu, B., Koide, R.T., 2002. Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. New Phytologist 156, 527–535. https://doi.org/10.1046/j.1469-8137.2002.00535.x

Dickson, T.L., Foster, B.L., 2011. Fertilization decreases plant biodiversity even when light is not limiting. Ecology Letters 14, 380–388. https://doi.org/10.1111/j.1461-0248.2011.01599.x

Fichtner, A., Härdtle, W., 2021. Forest ecosystems: A functional and biodiversity perspective, Environmental Challenges and Solutions. Springer International Publishing, Cham, pp. 383–405. https://doi.org/10.1007/978-3-030-57710-0_16

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.-D., Weisser, W.W., 2010. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic and Applied Ecology 11, 473–485. https://doi.org/10.1016/j.baae.2010.07.009

Fox, J., Wisberg, S., 2021. An R companion to applied regression. SAGE Publications Inc.

Friedman, J.H., Hastie, T., Tibshirani, R., 2010. Regularization paths for generalized linear models via coordinate descent. Journal of Statistical Software 33, 1–22. https://doi.org/10.18637/jss.v033.i01

Gamfeldt, L., Snäll, T., Bagchi, R., Jonsson, M., Gustafsson, L., Kjellander, P., Ruiz-Jaen, M.C., Fröberg, M., Stendahl, J., Philipson, C.D., Mikusiński, G., Andersson, E., Westerlund, B., Andrén, H., Moberg, F., Moen, J., Bengtsson, J., 2013. Higher levels of multiple ecosystem services are found in forests with more tree species. Nature Communications 4, 1340. https://doi.org/10.1038/ncomms2328

Gao, C., Shi, N.-N., Chen, L., Ji, N.-N., Wu, B.-W., Wang, Y.-L., Xu, Y., Zheng, Y., Mi, X.-C., Ma, K.-P., Guo, L.-D., 2017. Relationships between soil fungal and woody plant assemblages differ between ridge and valley habitats in a subtropical mountain forest. New Phytologist 213, 1874–1885. https://doi.org/10.1111/nph.14287

Heijden, M.G.A.V.D., Bardgett, R.D., Straalen, N.M.V., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Heijden, M.G.A.V.D., Streitwolf-Engel, R., Riedl, R., Siegrist, S., Neudecker, A., Ineichen, K., Boller, T., Wiemken, A., Sanders, I.R., 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. New Phytologist 172, 739–752. https://doi.org/10.1111/j.1469-8137.2006.01862.x

Heinrichs, H., Brumsack, H.-J., Loftfield, N., König, N., 1986. Verbessertes druckaufschlusssystem für biologische und anorganische materialien. Zeitschrift für Pflanzenernährung und Bodenkunde 149, 350–353. https://doi.org/10.1002/jpln.19861490313

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., Wardle, D.A., 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75, 3–35. https://doi.org/10.1890/04-0922

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biometrical Journal 50, 346–363. https://doi.org/10.1002/bimj.200810425

Hölscher, D., Hertel, D., Leuschner, C., Hottkowitz, M., 2002. Tree species diversity and soil patchiness in a temperate broad-leaved forest with limited rooting space. Flora - Morphology, Distribution, Functional Ecology of Plants 197, 118–125. https://doi.org/10.1078/0367-2530-00021

Huang, Y., Chen, Y., Castro-Izaguirre, N., Baruffol, M., Brezzi, M., Lang, A., Li, Y., Härdtle, W., Oheimb, G. von, Yang, X., Liu, X., Pei, K., Both, S., Yang, B., Eichenberg, D., Assmann, T., Bauhus, J., Behrens, T., Buscot, F., Chen, X.-Y., Chesters, D., Ding, B.-Y., Durka, W., Erfmeier, A., Fang, J., Fischer, M., Guo, L.-D., Guo, D., Gutknecht, J.L.M., He, J.-S., He, C.-L., Hector, A., Hönig, L., Hu, R.-Y., Klein, A.-M., Kühn, P., Liang, Y., Li, S., Michalski, S., Scherer-Lorenzen, M., Schmidt, K., Scholten, T., Schuldt, A., Shi, X., Tan, M.-Z., Tang, Z., Trogisch, S., Wang, Z., Welk, E., Wirth, C., Wubet, T., Xiang, W., Yu, M., Yu, X.-D., Zhang, J., Zhang, S., Zhang, N., Zhou, H.-Z., Zhu, C.-D., Zhu, L., Bruelheide, H., Ma, K., Niklaus, P.A., Schmid, B., 2018. Impacts of species richness on productivity in a large-scale subtropical forest experiment. Science 362, 80–83. https://doi.org/10.1126/science.aat6405

Hubert, N.A., Gehring, C.A., 2008. Neighboring trees affect ectomycorrhizal fungal community composition in a woodland-forest ecotone. Mycorrhiza 18, 363–374. https://doi.org/10.1007/s00572-008-0185-2

Ishida, T.A., Nara, K., Hogetsu, T., 2007. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer–broadleaf forests. New Phytologist 174, 430–440. https://doi.org/10.1111/j.1469-8137.2007.02016.x

Kernaghan, G., 2013. Functional diversity and resource partitioning in fungi associated with the fine feeder roots of forest trees. Symbiosis 61, 113–123. https://doi.org/10.1007/s13199-013-0265-8

Lang, C., Polle, A., 2011. Ectomycorrhizal fungal diversity, tree diversity and root nutrient relations in a mixed Central European forest. Tree Physiology 31, 531–538. https://doi.org/10.1093/treephys/tpr042

Lang, C., Seven, J., Polle, A., 2011. Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. Mycorrhiza 21, 297–308. https://doi.org/10.1007/s00572-010-0338-y

Langenbruch, C., Helfrich, M., Flessa, H., 2012. Effects of beech (Fagus sylvatica), ash (Fraxinus excelsior) and lime (Tilia spec.) on soil chemical properties in a mixed deciduous forest. Plant Soil 352, 389–403. https://doi.org/10.1007/s11104-011-1004-7

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., Finlay, R.D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173, 611–620. https://doi.org/10.1111/j.1469-8137.2006.01936.x

Liu, Y., Mao, L., Li, J., Shi, G., Jiang, S., Ma, X., An, L., Du, G., Feng, H., 2015. Resource availability differentially drives community assemblages of plants and their root-associated arbuscular mycorrhizal fungi. Plant Soil 386, 341–355. https://doi.org/10.1007/s11104-014-2261-z

Lladó, S., López-Mondéjar, R., Baldrian, P., 2018. Drivers of microbial community structure in forest soils. Appl Microbiol Biotechnol 102, 4331–4338. https://doi.org/10.1007/s00253-018-8950-4

Meinshausen, N., Bühlmann, P., 2006. High-dimensional graphs and variable selection with the Lasso. The Annals of Statistics 34, 1436–1462. https://doi.org/10.1214/009053606000000281

Nguyen, D.Q., Schneider, D., Brinkmann, N., Song, B., Janz, D., Schöning, I., Daniel, R., Pena, R., Polle, A., 2020. Soil and root nutrient chemistry structure root-associated fungal assemblages in temperate forests. Environmental Microbiology 22, 3081–3095. https://doi.org/10.1111/1462-2920.15037

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nordén, U., 1994. Influence of tree species on acidification and mineral pools in deciduous forest soils of South Sweden. Water Air Soil Pollut 76, 363–381. https://doi.org/10.1007/BF00482713

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2013. vegan: Community ecology package.

Peay, K.G., Baraloto, C., Fine, P.V., 2013. Strong coupling of plant and fungal community structure across western Amazonian rainforests. The ISME Journal 7, 1852–1861. https://doi.org/10.1038/ismej.2013.66

Peay, K.G., Kennedy, P.G., Talbot, J.M., 2016. Dimensions of biodiversity in the Earth mycobiome. Nature Reviews Microbiology 14, 434–447. https://doi.org/10.1038/nrmicro.2016.59

Pena, R., Lang, C., Lohaus, G., Boch, S., Schall, P., Schöning, I., Ammer, C., Fischer, M., Polle, A., 2017. Phylogenetic and functional traits of ectomycorrhizal assemblages in top soil from different biogeographic regions and forest types. Mycorrhiza 27, 233–245. https://doi.org/10.1007/s00572-016-0742-z

Purahong, W., Wubet, T., Krüger, D., Buscot, F., 2018. Molecular evidence strongly supports deadwood-inhabiting fungi exhibiting unexpected tree species preferences in temperate forests. The ISME Journal 12, 289–295. https://doi.org/10.1038/ismej.2017.177

R development Core Team., 2020. R: The R project for statistical computing: R foundation for statistical computing: Vienna, Austria, 2020.

Reich, P.B., 2009. Elevated CO_2 reduces losses of plant diversity caused by nitrogen deposition. Science 326, 1399–1402. https://doi.org/10.1126/science.1178820

Reich, P.B., Oleksyn, J., Modrzynski, J., Mrozinski, P., Hobbie, S.E., Eissenstat, D.M., Chorover, J., Chadwick, O.A., Hale, C.M., Tjoelker, M.G., 2005. Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. Ecology Letters 8, 811–818. https://doi.org/10.1111/j.1461-0248.2005.00779.x

Rosling, A., Landeweert, R., Lindahl, B.D., Larsson, K.-H., Kuyper, T.W., Taylor, A.F.S., Finlay, R.D., 2003. Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. New Phytologist 159, 775–783. https://doi.org/10.1046/j.1469-8137.2003.00829.x

Roy-Bolduc, A., Laliberté, E., Boudreau, S., Hijri, M., 2016. Strong linkage between plant and soil fungal communities along a successional coastal dune system. FEMS Microbiology Ecology 92. https://doi.org/10.1093/femsec/fiw156

Rudawska, M., Leski, T., Stasińska, M., 2011. Species and functional diversity of ectomycorrhizal fungal communities on Scots pine (Pinus sylvestris L.) trees on three different sites. Annals of Forest Science 68, 5–15. https://doi.org/10.1007/s13595-010-0002-x

Schappe, T., Albornoz, F.E., Turner, B.L., Jones, F.A., 2020. Co-occurring fungal functional groups respond differently to tree neighborhoods and soil properties across three tropical rainforests in Panama. Microb Ecol 79, 675–685. https://doi.org/10.1007/s00248-019-01446-z

Schmidt, M., Veldkamp, E., Corre, M.D., 2015. Tree species diversity effects on productivity, soil nutrient availability and nutrient response efficiency in a temperate deciduous forest. Forest Ecology and Management 338, 114–123. https://doi.org/10.1016/j.foreco.2014.11.021

Schröter, K., Wemheuer, B., Pena, R., Schöning, I., Ehbrecht, M., Schall, P., Ammer, C., Daniel, R., Polle, A., 2019. Assembly processes of trophic guilds in the root mycobiome of temperate forests. Molecular Ecology 28, 348–364. https://doi.org/10.1111/mec.14887

Seidel, D., Annighöfer, P., Ehbrecht, M., Magdon, P., Wöllauer, S., Ammer, C., 2020. Deriving stand structural complexity from airborne laser scanning data—what does it tell us about a forest? Remote Sensing 12, 1854. https://doi.org/10.3390/rs12111854

Smith, M.E., Henkel, T.W., Aime, M.C., Fremier, A.K., Vilgalys, R., 2011. Ectomycorrhizal fungal diversity and community structure on three co-occurring leguminous canopy tree species in a Neotropical rainforest. New Phytologist 192, 699–712. https://doi.org/10.1111/j.1469-8137.2011.03844.x

Solly, E.F., Schöning, I., Boch, S., Kandeler, E., Marhan, S., Michalzik, B., Müller, J., Zscheischler, J., Trumbore, S.E., Schrumpf, M., 2014. Factors controlling decomposition rates of fine root litter in temperate forests and grasslands. Plant Soil 382, 203–218. https://doi.org/10.1007/s11104-014-2151-4

Tedersoo, L., Anslan, S., Bahram, M., Drenkhan, R., Pritsch, K., Buegger, F., Padari, A., Hagh-Doust, N., Mikryukov, V., Gohar, D., Amiri, R., Hiiesalu, I., Lutter, R., Rosenvald, R., Rähn, E., Adamson, K., Drenkhan, T., Tullus, H., Jürimaa, K., Sibul, I., Otsing, E., Põlme, S., Metslaid, M., Loit, K., Agan, A., Puusepp, R., Varik, I., Kõljalg, U., Abarenkov, K., 2020. Regional-scale in-depth analysis of soil fungal diversity reveals strong pH and plant species effects in northern Europe. Front. Microbiol. 11. https://doi.org/10.3389/fmicb.2020.01953

Tedersoo, L., Bahram, M., Cajthaml, T., Põlme, S., Hiiesalu, I., Anslan, S., Harend, H., Buegger, F., Pritsch, K., Koricheva, J., Abarenkov, K., 2016. Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. The ISME Journal 10, 346–362. https://doi.org/10.1038/ismej.2015.116

Tedersoo, L., Bahram, M., Jairus, T., Bechem, E., Chinoya, S., Mpumba, R., Leal, M., Randrianjohany, E., Razafimandimbison, S., Sadam, A., Naadel, T., Kõljalg, U., 2011. Spatial structure and the effects of host and soil environments on communities of ectomycorrhizal fungi in wooded savannas and rain forests of Continental Africa and Madagascar. Molecular Ecology 20, 3071–3080. https://doi.org/10.1111/j.1365-294X.2011.05145.x

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., Kesel, A.D., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science 346. https://doi.org/10.1126/science.1256688

Tedersoo, L., Jairus, T., Horton, B.M., Abarenkov, K., Suvi, T., Saar, I., Kõljalg, U., 2008. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytologist 180, 479–490. https://doi.org/10.1111/j.1469-8137.2008.02561.x

Tedersoo, L., Kõljalg, U., Hallenberg, N., Larsson, K.-H., 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytologist 159, 153–165. https://doi.org/10.1046/j.1469-8137.2003.00792.x

Tedersoo, L., Sadam, A., Zambrano, M., Valencia, R., Bahram, M., 2010. Low diversity and high host preference of ectomycorrhizal fungi in Western Amazonia, a neotropical biodiversity hotspot. The ISME Journal 4, 465–471. https://doi.org/10.1038/ismej.2009.131

Toju, H., Tanabe, A.S., Yamamoto, S., Sato, H., 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLOS ONE 7, e40863. https://doi.org/10.1371/journal.pone.0040863

Toju, H., Yamamoto, S., Sato, H., Tanabe, A.S., 2013. Sharing of diverse mycorrhizal and root-endophytic fungi among plant species in an oak-dominated cool–temperate forest. PLOS ONE 8, e78248. https://doi.org/10.1371/journal.pone.0078248

Urbanová, M., Šnajdr, J., Baldrian, P., 2015a. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biology and Biochemistry 84, 53–64. https://doi.org/10.1016/j.soilbio.2015.02.011

Urbanová, M., Šnajdr, J., Baldrian, P., 2015b. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biology and Biochemistry 84, 53–64. https://doi.org/10.1016/j.soilbio.2015.02.011

Vandenkoornhuyse, P., Baldauf, S.L., Leyval, C., Straczek, J., Young, J.P.W., 2002. Extensive fungal diversity in plant roots. Science 295, 2051–2051. https://doi.org/10.1126/science.295.5562.2051

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Van, A.L., Dufresne, A., 2015. The importance of the microbiome of the plant holobiont. New Phytologist 206, 1196–1206. https://doi.org/10.1111/nph.13312

Voříšková, J., Brabcová, V., Cajthaml, T., Baldrian, P., 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. New Phytologist 201, 269–278. https://doi.org/10.1111/nph.12481

Wagg, C., Jansa, J., Schmid, B., Heijden, M.G.A. van der, 2011. Belowground biodiversity effects of plant symbionts support aboveground productivity. Ecology Letters 14, 1001–1009. https://doi.org/10.1111/j.1461-0248.2011.01666.x

Wardle, D.A., 2006. The influence of biotic interactions on soil biodiversity. Ecology Letters 9, 870–886. https://doi.org/10.1111/j.1461-0248.2006.00931.x

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Putten, W.H. van der, Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633. https://doi.org/10.1126/science.1094875

Warton, D.I., Shipley, B., Hastie, T., 2015. CATS regression – a model-based approach to studying trait-based community assembly. Methods in Ecology and Evolution 6, 389–398. https://doi.org/10.1111/2041-210X.12280

Weißbecker, C., Heintz-Buschart, A., Bruelheide, H., Buscot, F., Wubet, T., 2019. Linking Soil fungal generality to tree richness in young subtropical Chinese forests. Microorganisms 7, 547. https://doi.org/10.3390/microorganisms7110547

White, T. j., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR protocols; Academic Press, Inc.: Cambridge, MA, USA, 1990; pp. 315–322. ISBN 978-0-12-372180-8.

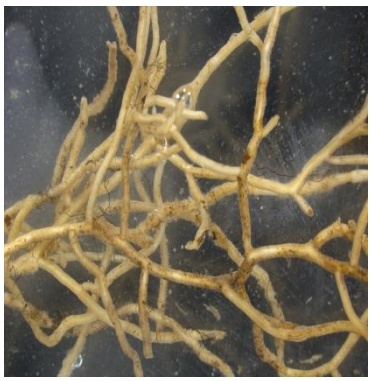
Wickham, H., 2009. ggplot2: Elegant graphics for data analysis, Springer-Verlag, New York. https://doi.org/10.1007/978-0-387-98141-3

Zhang, Y., Ni, J., Tang, F., Jiang, L., Guo, T., Pei, K., Sun, L., Liang, Y., 2017. Diversity of root-associated fungi of Vaccinium mandarinorum along a human disturbance gradient in subtropical forests, China. Journal of Plant Ecology 10, 56–66. https://doi.org/10.1093/jpe/rtw022

3.9 Supplementary materials – Chapter 3

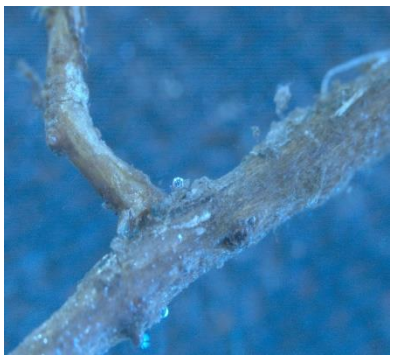
Supplement Figure S3.1: Morphological characteristics of seven tree species in temperate forests. Tree species were determined based on morphological features of their fine roots, such as colours and structures as described by Hölscher et al., (2002).

Beech (*Fagus sylvatica*)



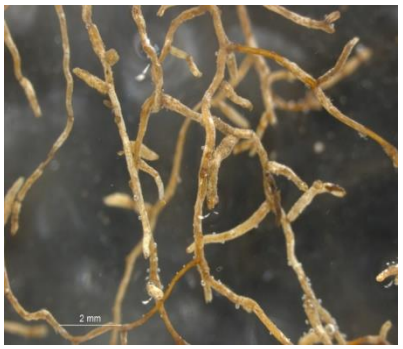
Ash (*Fraxinus excelsior*)

Pine (*Pinus sylvestris*)

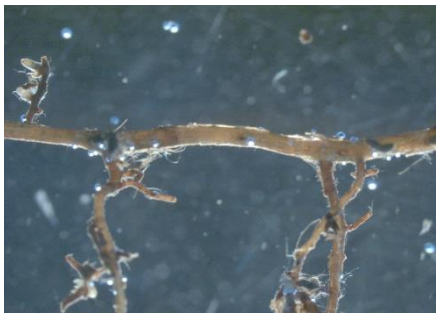


Spruce (*Picea abies*)

Lime (*Tilia*)

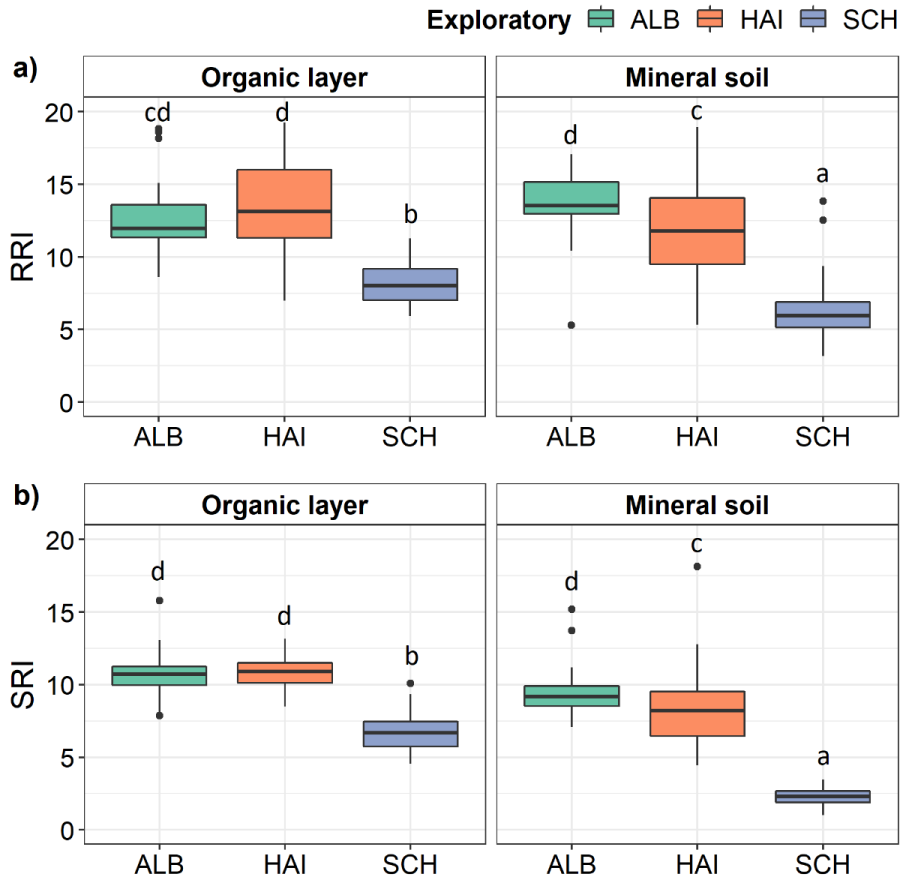


Maple (*Acer pseudoplatanus*)



Oak (*Quercus robur*)

Supplement Figure S3.2: Root resource index (RRI) (a) and soil resource index (SRI) (b) in different soil layers and in three different regions. ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide Chorin. Pairwise differences between different soil layers and regions were compared by a post hoc test (HSD Tukey's honestly significant difference). Different letters are indicated the significant differences at $p \leq 0.05$.



Supplement Table S3.1: Calculated relative weight of the fine roots of different tree species in the organic layer (OL) and mineral soil (MS) in three biogeographic regions. ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin. Data indicate means ± SE (n = 50). Pairwise differences of fine root mass in between soil layers and regions were compared by a post hoc test (HSD Tukey's honestly significant difference). Different letters are indicated the significant differences of the means at $p \leq 0.05$. Linear models were used to test the significant difference of the fine root mass of the tree species between the soil layers in each region. Significant differences of the fine root mass of tree species at $p \leq 0.05$ are indicated with bold letters.

	ALB		**HAI**		**SCH**		**ALB (OL-MS)**		**HAI (OL-MS)**		**SCH (OL-MS)**	
	OL	MS	OL	MS	OL	MS	F	P	F	p	F	p
Beech	1.21 ± 0.17 (ab)	4.79 ± 0.54 (c)	0.87 ± 0.08 (a)	4.87 ± 0.38 (c)	2.29 ± 0.33 (b)	2.26 ± 0.33 (ab)	40.17	< **0.001**	99.84	< **0.001**	0.00	0.945
Ash	0.19 ± 0.05 (a)	2.25 ± 0.52 (b)	0.20 ± 0.05 (a)	1.82 ± 0.47 (b)	0.23 ± 0.06 (a)	0.07 ± 0.03 (a)	15.35	< **0.001**	10.99	**0.001**	5.42	**0.022**
Pine	0.31 ± 0.10 (a)	0.42 ± 0.15 (ab)	0.07 ± 0.02 (a)	0.02 ± 0.01 (a)	1.02 ± 0.24 (bc)	0.94 ± 0.15 (c)	0.43	0.514	6.26	**0.014**	0.09	0.762
Spruce	0.51 ± 0.14 (a)	3.05 ± 0.56 (b)	0.11 ± 0.05 (a)	0.28 ± 0.11 (a)	0.12 ± 0.02 (a)	0.06 ± 0.01 (a)	19.58	< **0.001**	1.83	0.179	6.85	**0.010**
Maple	0.02 ± 0.01 (a)	0.59 ± 0.14 (b)	0.04 ± 0.01 (a)	0.21 ± 0.06 (a)	0.12 ± 0.05 (a)	0.01 ± 0.01 (a)	15.27	< **0.001**	7.17	**0.009**	4.96	**0.028**
Lime	0.03 ± 0.01 (a)	0.34 ± 0.06 (b)	0.02 ± 0.01 (a)	0.03 ± 0.01 (a)	0.04 ± 0.01 (a)	0.05 ± 0.02 (a)	27.99	< **0.001**	0.08	0.776	0.23	0.632
Oak	0.10 ± 0.03 (a)	1.55 ± 0.23 (c)	0.04 ± 0.02 (a)	0.91 ± 0.11 (b)	0.38 ± 0.06 (b)	0.93 ± 0.16 (a)	38.54	< **0.001**	61.05	< **0.001**	10.52	**0.001**
Others	0.22 ± 0.06 (ab)	0.39 ± 0.09 (b)	0.03 ± 0.01 (a)	0.04 ± 0.02 (a)	0.19 ± 0.08 (b)	0.46 ± 0.13 (ab)	2.32	0.131	0.18	0.672	3.31	0.073

Supplement Table S3.2: Root-associated fungal observed species richness, estimated richness (Chao1), Shannon diversity (H′), Evenness (E_H) and tree species richness in different soil layers in three biogeographic regions. SYM = symbiotrophic, SAP = saprotrophic, ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin. Data indicate means ± SE (n = 50). Pairwise differences between different regions were compared by a post hoc test (HSD Tukey's honestly significant difference). Different letters are indicated the significant differences at $p \leq 0.05$.

	ALB		HAI		SCH	
	Organic layer	**Mineral soil**	**Organic layer**	**Mineral soil**	**Organic layer**	**Mineral soil**
All fungi						
OTU Richness	188.36 ± 8.70 (e)	146.26 ± 5.24 (b)	164.22 ± 8.16 (d)	156.84 ± 5.92 (c)	158.24 ± 4.61 (cd)	106.08 ± 5.90 (a)
Chao1	285.77 ± 13.07 (c)	223.99 ± 7.94 (b)	258.97 ± 12.29 (bc)	237.56 ± 8.67 (b)	243.76 ± 7.40 (b)	160.48 ± 9.17 (a)
Shannon (*H′*)	2.93 ± 0.11 (b)	2.83 ± 0.08 (ab)	2.79 ± 0.11 (ab)	2.95 ± 0.07 (b)	2.98 ± 0.07 (b)	2.56 ± 0.08 (a)
Evenness (E_H)	0.12 ± 0.01 (a)	0.13 ± 0.01 (a)	0.12 ± 0.01 (a)	0.13 ± 0.01 (a)	0.13 ± 0.01 (a)	0.14 ± 0.01 (a)
SYM						
OTU Richness	24.90 ± 1.70 (b)	44.22 ± 2.11 (c)	22.54 ± 1.54 (b)	46.30 ± 2.35 (c)	19.34 ± 0.87 (a)	24.92 ± 1.90 (b)
Chao1	39.30 ± 3.52 (b)	61.13 ± 3.05 (c)	34.25 ± 2.78 (ab)	63.95 ± 3.34 (c)	24.72 ± 1.24 (a)	32.04 ± 2.50 (ab)
Shannon (*H′*)	1.64 ± 0.08 (ab)	2.17 ± 0.08 (c)	1.45 ± 0.10 (a)	1.53 ± 0.07 (bc)	1.92 ± 0.09 (a)	1.57 ± 0.08 (a)
Evenness (E_H)	0.26 ± 0.02 (b)	0.24 ± 0.02 (b)	0.25 ± 0.02 (b)	0.17 ± 0.01 (a)	0.27 ± 0.01 (b)	0.24 ± 0.01 (ab)
SAP						
OTU Richness	47.80 ± 2.36 (d)	29.96 ± 1.45 (b)	40.68 ± 2.12 (c)	32.06 ± 1.41 (b)	42.42 ± 1.55 (c)	24.12 ± 1.38 (a)
Chao1	70.40 ± 4.08 (b)	46.85 ± 2.67 (a)	65.21 ± 3.89 (b)	47.92 ± 2.30 (a)	64.96 ± 2.89 (b)	38.38 ± 2.70 (a)
Shannon (*H′*)	2.30 ± 0.10 (a)	1.95 ± 0.08 (a)	2.15 ± 0.12 (a)	2.20 ± 0.10 (a)	2.28 ± 0.10 (a)	1.95 ± 0.08 (a)
Evenness (E_H)	0.26 ± 0.02 (a)	0.28 ± 0.02 (a)	0.31 ± 0.03 (a)	0.36 ± 0.03 (a)	0.29 ± 0.02 (a)	0.36 ± 0.03 (a)
Tree Species						
Richness	3.00 ± 0.23 (ab)	5.34 ± 0.18 (d)	2.68 ± 0.21 (a)	3.76 ± 0.18 (bc)	4.42 ± 0.20 (cd)	3.16 ± 0.17 (ab)

Supplement Table S3.3: Relationship of tree species richness (TSR) with the richness of all fungi, symbitrophic (SYM), saprotrophic (SAP) in organic layer and mineral topsoil. The relationship was determined by the Pearson correlation analysis. Significant relationships at $p \leq 0.05$ are indicated in bold letters.

	Soil layer	All fungi			SYM			SAP		
		Equation	r	p	Equation	r	p	Equation	r	p
TSR	Organic	y = 0.1665x + 169.81	0.005	0.951	y = -0.0927x + 22.6	-0.015	0.858	y = 0.9808x + 40.208	0.109	0.185
TSR	Mineral	y = 7.3711x + 105.83	0.246	**0.002**	y = 2.9162x + 26.387	0.249	**0.002**	y = 1.6193x + 21.998	0.235	**0.004**

Supplement Table S3.4: Influence of the individual tree species on root-associated fungal richness in organic layer and in mineral topsoil. The richness of all fungi, symbiotrophic (SYM) and saprotrophic (SAP) fungi were annotated based on the operational taxonomic units (OTUs). Potential tree species were identified using least absolute shrinkage and selection operator (LASSO) logistic regression models. Further generalized linear models were used in the final LASSO model to test the significance influence of the individual tree species on root fungal richness at $p \leq 0.05$. Significantly influential tree species on root fungal richness at $p \leq 0.05$ are indicated with bold letters.

Groups	Predicted tree species	LASSO coefficient	Estimate std.	Error	t value	p value
Organic layer						
SAP	Beech	-0.47	-1.15	0.71	-1.62	0.108
	Lime	26.22	42.28	16.75	2.53	**0.012**
Mineral topsoil						
All fungi	Beech	2.14	2.81	1.11	2.54	**0.012**
	Ash	4.39	5.03	1.15	4.37	**< 0.001**
	Lime	6.85	11.91	12.01	0.99	0.323
	Oak	3.02	4.69	2.79	1.68	0.095
	Others	1.24	7.24	6.69	1.08	0.281
SYM	Beech	1.78	2.03	0.41	5.01	**< 0.001**
	Ash	1.47	1.72	0.42	4.12	**< 0.001**
	Spruce	0.76	1.19	0.48	2.51	**0.013**
	Oak	2.15	2.77	0.99	2.78	**0.006**
SAP	Ash	0.81	1.18	0.27	4.39	**< 0.001**
	Lime	1.11	4.96	2.78	1.79	0.076

Supplement Table S3.5: Relationship of tree species richness (TSR) with root resource index (RRI) and soil resource index (SRI) in the organic layer and mineral topsoil. The relationship was determined by Pearson correlation analysis. Significant relationships of RRI and SRI with TSR at $p \leq 0.05$ are indicated in bold letters.

		RRI			SRI		
		Equation	r	p	Equation	r	p
TSR	Organic	y = -0.8527x + 14.327	-0.408	**<0.001**	y = -0.5463x + 11.319	-0.394	**<0.001**
TSR	Mineral	y = 1.154x + 5.8403	0.442	**<0.001**	y = 1.0219x + 2.3518	0.438	**<0.001**

Supplement Table S3.6: Relationship of root resource index (RRI) and soil resource index (SRI) with the richness of all fungi, symbitrophic (SYM), saprotrophic (SAP) in organic layer and mineral topsoil. The relationship was determined by Pearson correlation analysis. Significant relationships of RRI and SRI with fungal richness at $p \leq 0.05$ are indicated in bold letters.

	Soil layer	All fungi			SYM			SAP		
		Equation	r	p	Equation	r	p	Equation	r	p
RRI	Organic	y = 1.3713x + 154.68	0.008	0.285	y = 0.2947x + 18.915	0.099	0.229	y = -0.0551x + 44.26	-0.013	0.876
RRI	Mineral	y = 4.3207x + 90.483	0.376	**<0.001**	y = 2.0934x + 16.236	0.468	**<0.001**	y = 0.6145x + 22.184	0.232	**0.004**
SRI	Organic	y = 4.6639x + 126.38	0.197	**0.016**	y = 0.9955x + 12.898	0.220	**0.007**	y = 0.639x + 37.619	0.099	0.230
SRI	Mineral	y = 5.813x + 98.09	0.453	**<0.001**	y = 2.76x + 20.294	0.551	**<0.001**	y = 0.8675x + 22.997	0.294	**<0.001**

CHAPTER 4

Ectomycorrhizal fungal diversity drives beech (*Fagus sylvatica*) N uptake

4.1 Introduction

There is growing interest in the linkage between below-ground microbial diversity and ecosystem functioning because the latter is threatened by human-induced climate change and a high rate of biodiversity loss (Cavicchioli et al., 2019; Hooper et al., 2012). Experiments carried out at the global scale suggest that communities with higher microbial diversity perform better than those with low diversity (Delgado-Baquerizo et al., 2016; Jing et al., 2015). Despite the widely accepted pivotal role of biodiversity in ecosystem functioning and multifunctionality, majority of the previous studies have focused on the relationship between aboveground biodiversity and plant productivity (Marquard et al., 2009; Tilman et al., 2014), and largely ignored the role of belowground microbial diversity in ecosystem functioning (Delgado-Baquerizo et al., 2016; Jing et al., 2015).

Ectomycorrhizal fungi (EMF) are a highly diverse group of below-ground microorganisms in forest ecosystems (Heijden et al., 2015) that colonize root tips of trees (Lang et al., 2011; Pena et al., 2017). In general, EMF species richness and diversity increase with plant community development as a function of soil organic matters (Dickie et al., 2013; Hawkins et al., 2015; Jumpponen et al., 2012) but often decrease with increasing nitrogen (N) availability in soil (de Witte et al., 2017; Lilleskov et al., 2002; van der Linde et al., 2018). N availability is usually low under natural conditions and present at elevated levels associated with anthropogenic activities such as N deposition and irregular summer drought; these variations may influence the EMF assemblages (de Witte et al., 2017; Lilleskov et al., 2019; Lilleskov et al., 2002; van der Linde et al., 2018). Phosphorous (P) deficiency can also induce alterations of EMF community structures and, thereby negatively affect tree growth, development and survival (Braun et al., 2010). Several studies have been shown that species diversity of EMF is further linked to functional diversity. For example, high diversity of EMF mediates plant P uptake efficiency, increases plant growth and development, increases whole plant P and N concentrations, benefits inorganic N uptake under drought and reduces below-ground resource competition of plant species (Baxter and Dighton, 2001; Köhler et al., 2018; Pena et al., 2013b, 2010; Pena and Polle, 2014; Perry et al., 1989). Moreover, high EMF diversity also increases the likelihood of mutualistic associations of EMF species with host roots. Thereby, some critical EMF species may survive in an ecosystem and thus counteract the loss of EMF taxa in temporal and spatial varying conditions, so that ecosystem functioning is maintained (Loreau, 2004; Wagg et al., 2019). However, there is still a uncertainty if all these EMF functional beneficial effects are linked to spatial, temporal, or functional complementarity effects in resource exploitation and transfer to the host, as proposed by the niche complementary hypothesis

(Tilman et al., 1997) or whether these functions are just a random sampling effect (Jonsson et al., 2001; Kipfer et al., 2012).

In addition to taxonomic diversity, EMF species are morphologically heterogeneous at intra– and interspecific levels (Lilleskov et al., 2002; Pena et al., 2013b). Melin and Nilsson (1953) suggested that different EMF species may differ in their ability to provide nutrients resources to their host (Melin and Nilsson, 1953). Each of these EMF species responds differently with regard to nutrient acquisition. For example, stable isotope studies revealed that EMF species significantly differ in uptake of various N forms due to their physiological heterogeneity (Lilleskov et al., 2002; Pena et al., 2013b; Pena and Polle, 2014). Therefore, assessment of individual EMF taxa in their community assemblages would only unravel the information of functional trait-based effects on host N uptake (Johnson et al., 2012). In laboratories conditions, most EMF species preferred ammonium (NH_4^+) over nitrate (NO_3^-) uptake, while few exhibited little preference (Finlay et al., 1992; Gobert and Plassard, 2008). This preference in uptake patterns either to NH_4^+ or NO_3^- by EMF clearly suggest that different inorganic N forms and their availability might be a defining element of the EMF species niche.

Plant N availability is often a growth-limiting factor in temperate forest ecosystem (Rennenberg et al., 2009). EMF can enhance plant N acquisition from the soil solution in exchange for photosynthetically derived sugar (Heijden et al., 2015; Martin et al., 2017; Tedersoo and Bahram, 2019). In forest soil, NH_4^+ and NO_3^- are the main inorganic N sources that are immediately accessible to EMF and transferable by EMF to their host (Chalot and Plassard, 2011; Courty et al., 2015, 2010). The concentrations of NH_4^+ and NO_3^- showed strong seasonal and regional fluctuations in forest soil solution (Dannenmann et al., 2009; Nacry et al., 2013). Several isotope experimental have been investigated host N uptake from NH_4^+, NO_3^- or mixture of both $NH_4^+NO_3^-$ as N source by the activities of roots and their associated EMF assemblages (Finlay et al., 1989; Gessler et al., 1998; Geßler et al., 2005; Gruffman et al., 2014; Guo et al., 2013; Jacob and Leuschner, 2015; Leberecht et al., 2016, 2015; Nguyen et al., 2017; Pena et al., 2013b, 2013a; Pena and Polle, 2014; Stoelken et al., 2010; Zeller et al., 2001), but reported divergent behaviour in take up various inorganic N forms (Gessler et al., 1998; Geßler et al., 2005; Jacob and Leuschner, 2015; Leberecht et al., 2016). For example, roots of the young beech trees enriched more $^{15}NH_4^+$ compared to $^{15}NO_3^-$ in ^{15}N labelling experiments under field conditions (Gessler et al., 1998; Geßler et al., 2005), but the opposite results were also reported in few other studies (Jacob and Leuschner, 2015; Leberecht et al., 2016). In a laboratory conditions, Finlay et al. (1989) reported higher ^{15}N enrichment in mycorrhizal beech seedling

after exposure with ammonium than with nitrate. In a tracer study with $^{15}NH_4^+$, Pena and Polle (2014) reported that ^{15}N of EMF assemblages is positively correlated with host ^{15}N uptake which suggesting that EMF taxon-specific ^{15}N enrichment might be an indicator of total ^{15}N enrichment in N pool through EMF community assemblages. However, it is unclear on the amounts of various inorganic N forms taken up by distinct EMF species and transferred by EMF to their host under field conditions. Therefore, it remains unknown how variation in the EMF species composition of natural fungal communities affects root N uptake.

This study aimed to investigate whether functional diversity or species identity of EMF determines beech N uptake under field conditions. To achieve our aim, we offered NH_4NO_3, where either ammonium or nitrate was labelled by ^{15}N to roots of beech trees in temperate forest and investigated ^{15}N enrichment in all individual EMF taxa forming the whole community. We conducted seasonal (spring, summer and autumn) stable isotope labelling experiments in the central (Hainich-Dün) and northeast (Schorfheide-Chorin) regions of Germany to consider both spatial and temporal variations in beech N uptake. We hypothesized that (i) EMF taxon-specific identity drives the beech N uptake; alternatively, EMF diversity plays a significant role in beech N uptake (ii) interspecific ^{15}N enrichment of ectomycorrhizal fungal species shows significant differences for NH_4^+ and NO_3^-.

4.2 Materials and methods

4.2.1 Field site characteristics and experimental setup

The research sites are located in two biogeographic regions in Germany, which have been characterized under the framework of the Biodiversity Exploratories (http://www.biodiversity-exploratories.de/) (Fischer et al., 2010). The Hainich-Dün (HAI) is located at the central and Schorfheide-Chorin (SCH) in the northeast regions of Germany (Fischer et al., 2010). Five subplots (ca. 5m x 5m) were established in each of three pure beech forest stands in HAI and SCH, respectively. Roots of the young beech trees were labelled and harvested during the growing season in spring before the leaves were fully developed (mid-April. 2019), in summer (mid-July, 2018) and autumn (mid-October, 2018). The average height of the beech trees was 5 to 6 m. Key environmental and soil characteristics of the plots have been described in Supplement Table S4.1.

4.2.2 Stable isotope labelling (^{15}N) and harvesting

For root N uptake studies, a single piece of the attached beech root was gently removed from the soil without any damage, washed with water and incubated in an artificial soil solution for 4 h. The artificial soil solution was prepared with some modification as described by Geßler et

al. (2005) and Simon et al. (2011), as follows: 90 µM $CaCl_2 \cdot 2H_2O$, 70 µM $MgCl_2 \cdot 6H_2O$, 50 µM KCl, 24 µM $MnCl_2 \cdot 4H_2O$, 20 µM NaCl, 7 µM $FeSO_4 \cdot 7H_2O$, 6 µM K_2HPO_4 and 2 µM glycine. pH (4.10) was adjusted to that of the prevailing soil conditions of the plots from both sites. The solution contained an equimolecular concentration of NH_4NO_3 in which either ammonium or nitrate was replaced by 1 mM $^{15}NH_4^+$ or 1 mM $^{15}NO_3^-$ (98% ^{15}N, Cambridge Isotope Laboratories, Inc., Andover, MA, USA). Controls were performed with 1mM ^{14}N (NH_4NO_3). ^{15}N represented the only source of inorganic N in the artificial soil solution. The experiments were always conducted between 8 a.m. to 2 p.m. to avoid diurnal variation in beech N uptake.

The selected root was immersed in 50 ml of the ^{15}N or ^{14}N-containing artificial soil solution. After 4 h of exposures, the attached root was cut and separated into two segments: the incubated segment and a non-incubated segment. Then, the incubated root segment was briefly washed with a non-labelled solution to remove ^{15}N from the root surface. The incubated root segment consisted of ectomycorrhizal root tips (EMRT), fine roots (diameter <2 mm), coarse roots (diameter >2 mm). The non-incubated root segment is defined here as the transport segment, which was attached to the beech tree and outside the soil solution. The enrichment of ^{15}N in the transport segment is the actual net N uptake by the beech roots, which reflects how much N actually was transported from the EMF to the host. In total, five replicates, each from different trees, of ^{15}N-labelled samples per N form and five non-labelled samples (^{14}N) were harvested from each plot. On return from the field to the laboratory, samples were transported in an icebox, stored at 4 °C in wrapped moist tissue paper and immediately used for mycorrhizal analyses. In total, 270 beech roots (90 from each treatment group) were harvested from both sites across all seasons.

4.2.3 Ectomycorrhizal morphological analyses

A total number of 122 labelled beech roots were used for ectomycorrhizal morphological assessment. The collected fine roots were inspected under a dissecting microscope (Leica DFC 420 C, Wetzlar, Germany) and used to determine the abundance of the vital EMF, dead and non-mycorrhizal (NM) root tips (RTs) (Winkler et al., 2010). Dead RTs were distinguished from vital RTs by their shrunken and dry appearance. A total of 450 RTs were counted from each ^{15}N labelled root sample. If the available root material was not enough to reach 450 EMRTs per sample, then all the available RTs were counted. The EMRTs were further assigned to a morphotypes (MTs) based on their distinct morphological traits such as mantle colour and structure, type of branching, the abundance of the external hyphae, the shape of unramified ends

and rhizomorphs (Agerer, 2001). All the MTs were photographed using a digital camera (Leica DFC 420 C, Wetzlar, Germany) attached to the microscope at 10x to 40x magnification (Supplement Table S4.2). A range of about 40 to 100 RTs from each distinct MT was cut by using fine forceps. A total of 8 to10 distinct RTs per MT were used for molecular analysis, and the remaining RTs of each distinct MT were stored at -80 °C and used for ^{15}N measurements. Two different sets of forceps were used to cut the labelled and non-labelled RTs to avoid cross-contamination.

4.2.4 DNA extraction and sequence analyses

EMF species were identified by DNA Sanger sequencing of the internal transcribed spacer (ITS) region for each assigned MT. The RTs that were assigned to one MT were ground with a pellet mixer (VWR 431-01009, Darmstadt, Germany). DNA was extracted using the innu PREP Plant DNA isolation Kit following the SLS protocol as recommended by the manufacturer (845-KS-1060250, Analytik Jena, Germany). For EMF identification, the fungal rDNA ITS region was amplified by PCR Tag Polymerase using the primer pair ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns, 1993; White et al., 1990). The PCR mixture in a total volume of 22 µl contained 13.29 µl nuclease-free water, 2.20 µl 10x PCR buffer $(NH_4)_2SO_4$, 1.76 µl 25 mM $MgCl_2$, 0.44 µl 10 mM dNTPs Mix, 0.11 µl polymerase (5U/µl), 1.10 µl (10 µM) of each primer and 2.00 µl of DNA template (diluted 1:20). The PCR program was initiated at 95 ^oC for 2 min (hold and initial denaturation), followed by 25 cycles of 15 s at 94 ^oC (denaturation), 30 s at 57 ^oC (annealing), 60 s at 72 ^oC (extension) and terminated at 72 ^oC for 5 min. The PCR products were separated in a 2% agarose gel to check the positive amplification (Biozym, LE 840004). Ligation of the PCR product was performed using the pGEM-T Vector System (Promega, A3610) according to the protocol of the manufacturer but with only half of the recommended amount of chemicals and transformed into JM109 competent cells (Promega chemical competent, A3610). Positive clones with the target DNA were used as the template for further DNA amplification followed by the same PCR program and controlled in 2% agarose gel (Biozym, LE 840004). PCR products were further cleaned using the innu PREP PCR pure kit (Analytik Jena, 845-KS-5010250). The Sanger sequencing was done by a sequencing service (Microsynth, Göttingen, Germany).

Staden package (v.4.11.2) was used to assemble the sequences. For fungal identification, Basic Local Alignment Search Tools (BLAST) were used to search the sequences against the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and UNITE

(http://unite.ut.ee) open-access databases. A species name was assigned to MT when the ITS sequence had at least 97% or higher similarities to the species identity scores. The sequences were deposited in GenBank under the accession numbers (MN947338 - MN947405).

4.2.5 Determination of ^{15}N, ^{14}N and C

Both labelled and non-labelled beech EMRTs, fine, coarse, and transport root tissues were oven-dried at 60°C for one week and ground to a fine homogenous powder with a ball mill (Retsch, Type MM400, Haan, Germany). To measure ^{15}N enrichment in distinct EMF taxa, each sample of the collected MTs that had been stored at -80 °C was freeze-dried for one week and crushed with a spatula inside the Eppendorf tube to get a homogenous mixture. Then, aliquots of the root tissues and distinct MTs were weighed into 4 mm x 6 mm tin cartouches (IVA Analysentechnik, Meerbusch, Germany) using a super-micro balance (S4, Sartorius, Goettingen, Germany). Approximately 0.7 to 1.0 mg acetanilide was used as the laboratory standard (71.09% C, 10.36% N). The following amount of samples were used for the determination of the ^{15}N, ^{14}N and C: 0.29 to 1.50 mg for individual MT, 1.0 to 1.5 mg for EMRTs, 2 to 5 mg for fine roots, coarse roots and the transport root segments, respectively. The amounts of ^{14}N, ^{15}N and C were measured in the root and mycorrhizal samples at the Centre for Stable Isotope Research and Analysis (KOSI, Georg-August-Universität Göttingen, Germany). Separate devices were used for the measurements of labelled and non-labelled samples (labelled - Isotope mass spectrometer: Delta C, Finnigan MAT, Bremen, Germany; Interface: Conflo III, Thermo Electron Cooperation, Bremen, Germany; Element analyzer: NA1108, Fisons-Instruments, Rodano, Milano, Italy and non-labelled - mass spectrometer: Delta V Plus, Finnigan MAT, Bremen, Germany; Interface: Conflo III, Finnigan MAT, Bremen, Germany; Element analyzer: NA1110, Fisons-Instruments, Rodano, Milano, Italy).

4.2.6 Calculation and Statistical analyses

The relative ^{15}N contents were expressed as the ratio: $^{15}N\ atom\% = \left(\frac{^{15}N}{^{14}N} + {}^{15}N\right) \times 100$...(Eqn. 1)

^{15}N atom % excess (APE) = atom% $^{15}N_{labelled\ tissue}$ - atom% $_{Non\text{-}labelled\ tissue}$ …… (Eqn. 2)

Here, non-labelled tissue refers to tissues of samples collected according to the respective site and season.

^{15}N enrichment in particular tissues (EMRTs, fine, coarse and transport) and in distinct EMF species was calculated using the following equations:

^{15}N enrichment $_{in\ root\ tissue}$ ($\mu g\ g^{-1}$ DW) = APE x 10 x N …… (Eqn. 3)

^{15}N enrichment $_{in\ EMF\ species}$ (µg g^{-1} DW) = APE x 10 x N …….. (Eqn. 4)

Here, N is the concentration of nitrogen of the tissue (mg g^{-1} DW) and 10 the conversion factor between APE and microgram per gram.

Mean ^{15}N enrichment of the whole root was calculated as:

^{15}N pool (µg $root^{-1}$) = ^{15}N enrichment $_{fine\ roots}$ x DW $_{fine\ roots}$ + ^{15}N enrichment $_{coarse\ roots}$ x DW $_{coarse\ roots}$ + ^{15}N enrichment $_{transport}$ x DW $_{transport}$ …… (Eqn. 5)

Where DW refers to the total dry mass of that compartment.

$$^{15}N\ \text{enrichment}_{whole\ root}\ (\mu g\ g^{-1} DW) = \frac{^{15}N\ \text{pool}\ (\mu g\ root^{-1})}{\text{total root dry mass (g)}}$$ ..... (Eqn. 6)

^{15}N efficiencies of EMF was calculated as:

^{15}N efficiencies of each EMF species (e.g. $EMF_{Lactarius\ subdulcis}$, $EMF_{Lactarius\ blennius}$, $EMF_{Tomentella\ stuposa}$ ……. EMF_n) was calculated by using the following equation:

$$^{15}N\ \text{efficiencies of each EMF species}\ (\mu g\ g^{-1} DW) = \frac{\text{Abundance of distinct EMF species x mean } ^{15}N \text{ enrichment of that distinct EMF species}}{\text{Abundance of all EMF species in the respective sample}}$$ ..... (Eqn. 7)

Here, mean ^{15}N enrichment of the distinct EMF refers to the mean of the measured ^{15}N enrichment of that distinct EMF species according to the respective season. Abundance of all EMF species refers to the total number of counted EMRTs from all EMF species in that respective sample.

Then the calculated ^{15}N efficiencies of each EMF species was further used to calculate the total ^{15}N efficiencies of EMF of the respective sample. The following equation was used:

Total ^{15}N efficiencies of EMF assemblages (µg g^{-1} DW) =

$\sum$ (^{15}N efficiencies $_{Lactarius\ subdulcis}$ + ^{15}N efficiencies $_{Lactarius\ blennius}$ + ^{15}N efficiencies $_{Tomentella\ stuposa}$ ……. + ^{15}N efficiencies $_n$) ……..... (Eqn. 8)

$$\text{EMF colonization rate}\ (\%) = \frac{\text{EMRT}}{(\text{EMRT} + \text{NM})} \text{x } 100$$ ……... (Eqn. 9)

$$\text{Root Vitality Index}\ (\%) = \frac{\text{Vital NM RT + Vital mycorrhizal RT}}{\text{Total RT}} \text{ x } 100$$ …… (Eqn. 10)

All the statistical analyses were performed in R version 4.0.2 (R development Core Team., 2020). Data were visualized using 'ggplot2′ package (Wickham, 2009) in R. Data normal distribution and homogeneity of the variance was inspected visually using histograms and

residual plots. Data were further logarithmically transformed to meet the criteria of normal distribution and homogeneity of variance where necessary. Generalized linear mixed effect models (Poisson regression, chi-square test) with the function *glmer()* were used from 'lme4' package to compare means of the count data such as EMF species richness, EMRTs, NM RTs and dead RTs (Bates et al., 2015). Linear mixed-effect model with the function *lmer()* was used from 'lme4' package to compare means of the continuous data such as C, N, C: N ratio, $^{13}C/^{12}C$, ^{15}N enrichment derived from NH_4^+ (^{15}N-NH_4^+) and NO_3^- (^{15}N-NO_3^-) in EMF species, EMRTs and root segments (Bates et al., 2015). Kruskal-Wallis test was used to compare means of the percentage data such as root vitality index and EMF colonization rate using the function *kruskal.test()* from the 'stats' package. To determine the preferable offered inorganic N source (NH_4^+ and NO_3^-) of EMRTs and different root segments in different sites, we fitted linear mixed effect models with season as a random factor. Pairwise differences between the groups (different root segments, different seasons and sites) were compared with a *post hoc* test (HSD Tukey's honestly significant difference) using the function *glht()* from the 'multcomp' package (Hothorn *et al.*, 2008). To distinguish significant difference of ^{15}N enrichment among EMF species in different seasons, we fitted the linear mixed effect models with study sites as a random factor (Bates et al., 2015). The level of the significance was tested using *Anova()* function from the 'car' package. Effects of the study sites and seasons on EMF community composition were tested by analysis of similarity (ANOSIM with 999 permutation steps) using function *anosim()* in 'vegan' package.

The EMF Shannon diversity (H′), species richness and evenness were calculated using 'vegan' package in R (Oksanen et al., 2013). To evaluate the importance of EMF diversity on root N-uptake, we fitted linear models using the *lm()* function from the 'stats' package (Bates et al., 2015). As dependent variables, we used the logarithmized ^{15}N enrichment of transport root segment, and EMF Shannon diversity, species richness, season, total vital RTs, fine root DW and plots as predictors. The *vif()* function from the 'car' package (Fox and Wisberg, 2021) was used to check for collinearity among the predictors. For both treatment groups (^{15}N-NH_4^+ and ^{15}N-NO_3^-), EMF species richness was removed from the models since it had the highest generalized variance-inflation factors above 4 (4.15 for ^{15}N-NH_4^+, 8.61 for ^{15}N-NO_3^-). The models were then subjected to *stepAIC()* function with backward selection from the 'MASS' package (Venables and Ripley, 2002) to determine the best model with the remaining predictors. Further, Pearson correlation between Shannon diversity and ^{15}N enrichment in transport segment was calculated using *cor.test()* function from the 'stats' package. The similar approach was used to evaluate the importance of EMF diversity in the whole root segments.

To further investigate and distinguish the influence of EMF taxon-specific on ^{15}N uptake, we used the function *glmnet()* from the 'glmnet' package (Friedman et al., 2010) and conducted the Least Absolute Shrinkage and Selection Operator (LASSO) regression. The LASSO regression was used to estimate the contribution of each predictor against the dependent variables (Warton et al., 2015). The LASSO approach further provides regression coefficients for all predictors to measure their stability scores. Predictors with zero coefficient are considered non-significant, and a coefficient above 0.6 is usually a significant predictor (Meinshausen and Bühlmann, 2006). Here, we used the log-transformed ^{15}N enrichment in the transport root segment as the dependent variable to fit the LASSO regression model. As independent variables, the ^{15}N efficiencies of all 53 EMF species were used as the species community matrix in the LASSO model. A similar approach was used to investigate the EMF taxon-specific effects on ^{15}N enrichments in the whole root as well.

4.3 Results

4.3.1 Fine root tip dynamics

The morphological assessment of a total number of 63,487 RTs revealed 18,019 dead RTs (28.38%), 108 NM RTs (0.17%) and 45,360 EMRTs (71.45%) across all the seasons and sites. Root vitality index, EMRTs and dead RTs varied significantly across the different seasons in HAI and SCH ($p < 0.001$, Table 4.1), whereas EMF colonization rate and NM RTs did not show significant changes ($p > 0.05$, Table 4.1). EMF colonization rate was more than 99% of the vital RTs, and only 0.6 to 1.1% of RTs were not colonized by EMF. EMF colonization rate, EMRTs and NM RTs did not exhibit any significant differences across sites ($p > 0.05$, Table 4.1), whereas mean root vitality indices and dead RTs significantly affects by the sites ($p < 0.05$, Table 4.1). Overall, mean root vitality indices and EMRTs were highest in spring and mean dead RTs were highest in summer than other seasons (Table 4.1).

Table 4.1: Characteristics of the root tips of young beech roots in different seasons and regions. HAI = Hainich-Dun, SCH = Schorfheide-Chorin, Vitality index (%), Colonization rate (%) = Ectomycorrhizal colonization rate, EMRTs = Ectomycorrhizal root tips, Dead-RTs = Dead root tips, NM-RTs = Non-mycorrhizal root tips. Data are shown as means ± SE (n = 36). Generalized linear mixed effect models with Poisson distribution were used to compare means of the species richness, EMRTs and NM-RTs among the regions with the treatment group ($^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$) as a random factor. Kruskal-Wallis test was used to compare the means of vitality index and colonization rate among the regions. Linear mixed-effect models were used to compare the means of Shannon diversity and evenness among the regions with the treatment group ($^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$) as a random factor. Significant differences of the means among the regions are shown in bold. Different letters denote significant differences of the means among the seasons and regions at $p \leq 0.05$.

	HAI			SCH			p values
	Spring	**Summer**	**Autumn**	**Spring**	**Summer**	**Autumn**	**HAI-SCH**
Vitality index	77.54 ± 1.92 (bc)	63.68 ± 1.69 (a)	68.39 ± 1.57 (a)	83.01 ± 2.02 (c)	71.63 ± 2.14 (ab)	65.99 ± 2.67 (a)	**0.03**
Colonization rate	99.88 ± 0.06 (a)	99.71 ± 0.10 (a)	99.74 ± 0.10 (a)	99.81 ± 0.10 (a)	99.85 ± 0.07 (a)	99.58 ± 0.15 (a)	0.19
EMRTs	414.67 ± 12.38 (c)	319.22 ± 15.45 (a)	369.06 ± 17.72 (b)	422.78 ± 11.28 (c)	363.33 ± 14.59 (b)	311.39 ± 20.38 (a)	0.62
Dead-RTs	121.00 ± 10.92 (b)	181.67 ± 9.81 (e)	168.11 ± 8.86 (d)	89.33 ± 11.45 (a)	143.28 ± 11.02 (c)	158.22 ± 13.41 (d)	< **0.001**
NM-RTs	0.56 ± 0.28 (a)	0.94 ± 0.34 (a)	0.94 ± 0.37 (a)	0.83 ± 0.46 (a)	0.61 ± 0.29 (a)	1.11 ± 0.32 (a)	0.83
Species richness	9.22 ± 0.57 (cd)	4.61 ± 0.47 (a)	6.00 ± 0.49 (ab)	11.33 ± 0.94 (d)	7.50 ± 0.59 (bc)	6.00 ± 0.58 (ab)	**0.00**
Shannon diversity	1.76 ± 0.09 (bc)	1.24 ± 0.07 (a)	1.43 ± 0.07 (ab)	1.87 ± 0.11 (c)	1.57 ± 0.10 (ac)	1.48 ± 0.10 (ab)	**0.04**
Evenness	0.67 ± 0.04 (a)	0.81 ± 0.03 (b)	0.74 ± 0.04 (ab)	0.64 ± 0.04 (a)	0.70 ± 0.03 (ab)	0.81 ± 0.02 (b)	0.32

4.3.2 Ectomycorrhizal fungal community composition

Molecular investigations of the MTs revealed a total of 53 EMF species from all samples. Of these, eight could only be assigned to the order or genus level (*Tomentella sp., Helotiales sp1., Helotiales sp.2, Pezizales sp1., Pezizales sp2., Pezizaceae sp., Byssocorticium sp., Uncultured Thelephoraceae*), and 43 were identified at the species level (Supplement Table S4.2). Two MTs from summer and autumn could not be amplified by PCR, and therefore kept their original MTs numbers, MTSU6 and MTAU37, which had been based on their unique morphological features. The EMF community compositions differed between the different seasons and sites, resulting in significant differences in the community assemblages (ANOSIM, season in HAI, $R = 0.31$, $p < 0.001$ and season in SCH, $R = 0.43$, $p < 0.001$; Sites, $R = 0.04$, $p = 0.01$). In spring, the top most abundant EMF species were *Lactarius subdulcis* (28.28%), followed by *Lactarius blennius* (12.23%), *Tomentella coerulea* (7.29%), *Tomentella bryophila* (5.23%) and *Russula paludosa* (4.31%). In summer, the most abundant EMF species were *Lactarius subdulcis* (32.03%), followed by *Inocybe splendens* (12.82%), *Lactarius blennius* (10.57%), *Tomentella stuposa* (10.17%) and *Pezizales sp. 2* (5.33%). In autumn, the most abundant EMF species were *Lactarius subdulcis* (22.86%), followed by *Cenococcum geophilum* (10.79%), *Lactarius blennius* (10.01%), *Pachyphlodes conglomerata* (8.56%) and *Tomentella coerulea* (7.43%). *Lactarius subdulcis* was the only unique EMF species present across all sites and seasons. A total of three from 53 detected EMF taxa (*Lactarius subdulcis, Lactarius blennius, Tomentella stuposa*) were observed in each of the three seasons studied here.

For the overall comparison of the EMF species richness, Shannon diversity and evenness, we used a subset of the samples (n = 36 instead of 50) to achieve equal numbers of replicates per season (Table 4.1). This reduction in sample size did not result in any loss of EMF species. The overall richness of the EMF species was variable in different seasons and ranged from a total number of 14 to 30 EMF species, with higher numbers in spring and autumn than in summer; these fluctuations were significant between the sites and seasons ($p < 0.001$, Supplement Figure S4.1). Average EMF species richness, Shannon diversity and evenness varied significantly among different seasons ($p < 0.05$, Table 4.1).

4.3.3 Preferred N source by ectomycorrhizas and beech roots

The enrichment of ^{15}N varied among the different root segments (as defined in Eqn. 3) between and within NH_4^+ and NO_3^- treatment groups ($p < 0.001$, Figure 4.1 and Supplement Table S4.3) and significantly higher enrichment after exposure to NH_4^+ than to NO_3^- ($p < 0.001$, Figure 4.1, Supplement Table S4.3). The calculated ^{15}N enrichment of the whole root (as defined in Eqn.

6) also showed similar results that significantly higher enrichment after exposure to NH_4^+ than to NO_3^- ($p < 0.001$, Supplement Table S4.3) but exhibited no significant differences with regard to the sites (^{15}N-NH_4^+, $p = 0.428$; ^{15}N-NO_3^-, $p = 0.763$, Supplement Table S4.3). These findings suggest that both EMRTs and beech roots prefer NH_4^+ as the potential inorganic N source compared to NO_3^-. Among the root segments, EMRTs showed pronounced ^{15}N enrichment than other beech root segments (Figure 4.1). The ^{15}N enrichment also varied significantly among different seasons with the highest enrichment in spring and autumn ($p < 0.001$, Supplement Table S4.3). This result indicates that the temporal variations in net N uptake by the beech roots are highly sensitive to seasonal change.

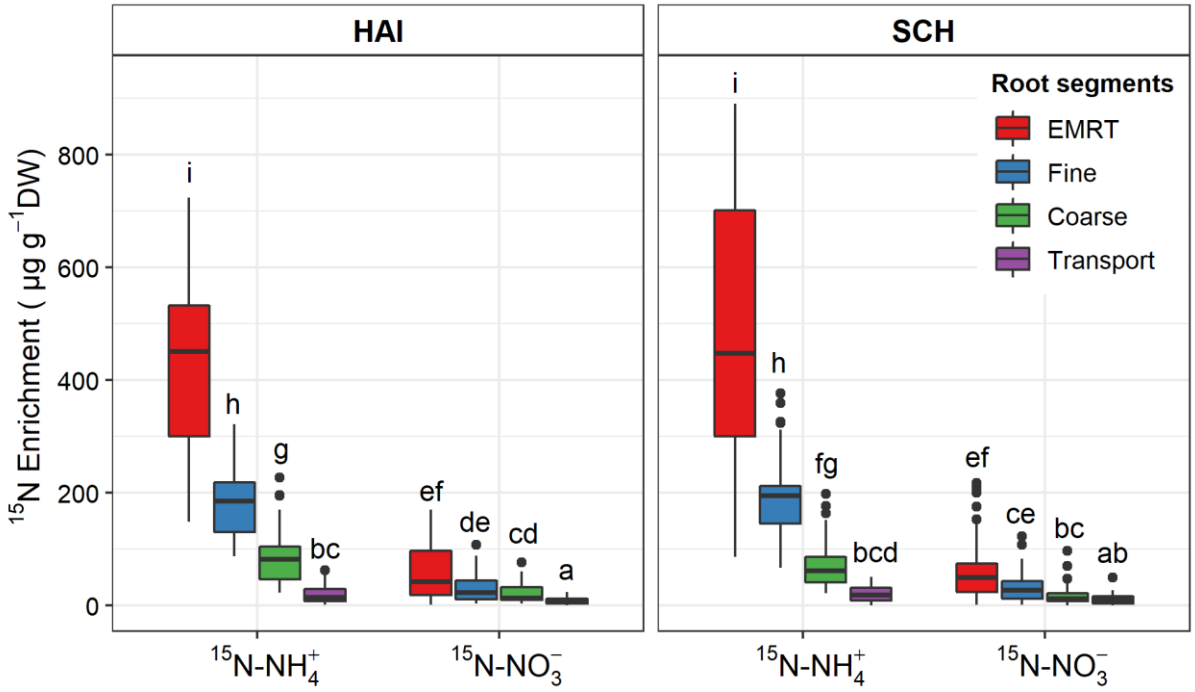


Figure 4.1: ^{15}N enrichment in ectomycorrhizal root tips (EMRT), fine roots (fine), coarse roots (coarse) and transport root segments (transport) after exposure either with NH_4^+ or NO_3^-. Red colour refers to EMRT, blue to fine, green to coarse and violet to transport root segments. Linear mixed-effects models were used to compare the means of ^{15}N enrichment in different root segments with seasons (spring, summer and autumn) as a random factor. Pairwise comparison between different root segments were compared by a *post hoc test* (HSD Tukey's honestly significant difference). Different letters represent the significant differences of the means at $p \leq 0.05$.

4.3.4 Interspecific ^{15}N enrichment of ectomycorrhizas formed with different fungal species

To determine EMF taxon-specific ^{15}N enrichment (as defined in Eqn. 4), we analysed the enrichment of ^{15}N from all 53 EMF species. After 4 h of exposure, ^{15}N enrichment in all EMF

species differed significantly between and within NH_4^+ and NO_3^- in all season ($p < 0.001$, F values in Figure 4.2 and Supplement Table S4.4).

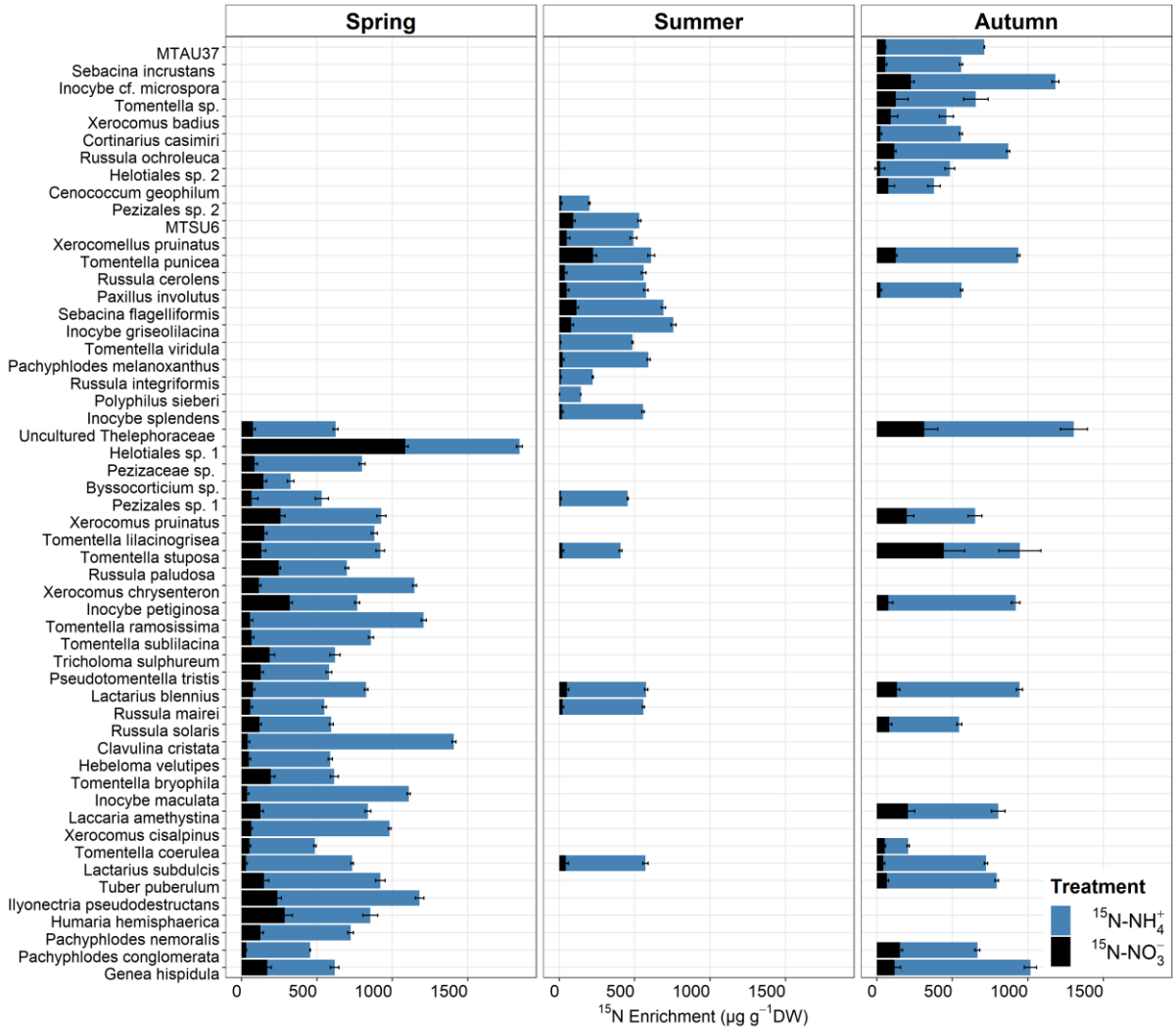


Figure 4.2: ^{15}N enrichment in ectomycorrhizal fungi (EMF) species after exposure either with NH_4^+ or NO_3^-. Blue colours section of the stack bar refers to NH_4^+ and black to NO_3^-. Linear mixed effect model was used to compare the means ^{15}N enrichment in EMF species with sites as a random factor. ANOVA was used to compare the means of the ^{15}N enrichment among EMF species (^{15}N-NH_4^+: spring, $F = 56.49$, $p < 0.001$, summer, $F = 24.02$, $p < 0.001$, autumn, $F = 17.09$, $p < 0.001$; ^{15}N-NO_3^-: spring, $F = 43.65$, $p < 0.001$, summer, $F = 7.84$, $p < 0.001$, autumn, $F = 7.94$, $p < 0.001$).

All EMF species showed higher ^{15}N enrichment from NH_4^+ than from NO_3^- except one *Helotiales sp.1*, which suggests that most EMF species prefer NH_4^+ over NO_3^-. The ^{15}N enrichment of EMF species from NH_4^+ ranged from 1366.20 ± 2.18 to 180.38 ± 0.26 µg g^{-1} DW in spring, 678.78 ± 2.14 to 142.02 ± 0.64 µg g^{-1} DW in summer and 991.34 ± 100.86 to 155.90 ± 23.60 µg g^{-1} DW in autumn (Figure 4.2, Supplement Table S4.4). The ^{15}N enrichment of EMF species from NO_3^- ranged from 1086.04 ± 2.96 to 30.78 ± 0.26 µg g^{-1} DW in spring,

225.24 ± 1.95 to 2.60 ± 0.07 µg g^{-1} DW in summer and 442.32 ± 80.30 to 20.47 ± 18.33 µg g^{-1} DW in autumn (Figure 4.2, Supplement Table S4.4). Across all seasons, EMF species with notably high ^{15}N enrichment from NH_4^+ were *Clavulina cristata, Tomentella ramosissima, Inocybe maculate, Xerocomus chrysenteron, Uncultured Thelephoraceae* and those with low ^{15}N enrichment from NH_4^+ were *Polyphilus sieberi, Tomentella coerulea, Byssocorticium sp., Pezizales sp.2.*, and *Russula integriformis* (Figure 4.2). EMF species with notably high ^{15}N enrichment from NO_3^- were *Helotiales sp.1, Tomentella stuposa, Inocybe petiginosa, Uncultured Thelephoraceae, Humaria hemisphaerica* and those with low ^{15}N enrichment from NO_3^- were *Polyphilus sieberi, Tomentella viridula, Russula integriformis, Pezizales sp.* and *Inocybe splendens* (Figure 4.2). These results indicate clear interspecific differences in EMF ^{15}N enrichment, which might be due to their physiological ability of N acquisition under the current experimental conditions. This finding further suggests that EMF species with much lower ^{15}N enrichment did not actively participate in N acquisition from the whole community assemblages. There were also significant differences in C and N content, C/N ratio, ^{13}C:^{12}C, ^{15}N-NH_4^+:^{15}N-NO_3^- ratio among the EMF species ($p < 0.001$, F values in Supplement Table S4.4 and Supplement Table S4.5).

4.3.5 N uptake by beech root in relation to ectomycorrhizal fungi diversity

To test our hypothesis, whether EMF diversity might influence the amount of the ^{15}N uptake in transport (as defined in Eqn. 3) and whole root (as defined in Eqn. 6) segment, we included the following predictor variables in our models: EMF Shannon diversity, species richness, season, vital RTs, fine root DW and plots and then we chose the best model based on AIC (Table 4.2).

Best models are shown according to *Step-Akaike information criterion (AIC)* with backward selection:

^{15}N-NH_4^+: log (^{15}N_Transport) ~ H′

^{15}N-NH_4^+: log (^{15}N_Whole root) ~ H′ + Season + Vital RTs

^{15}N-NO_3^-: log (^{15}N_Transport) ~ H′ + Season + Fine DW

^{15}N-NO_3^-: log (^{15}N_Whole root) ~ H′ + Season + Vital RTs + Fine DW

Table 4.2: Model selection for beech N uptake in transport and whole root segments. Multiple linear regression models were used for ^{15}N uptake derived from NH_4^+ and NO_3^- in transport and whole root segments, including EMF Shannon diversity (H′), seasons, vital root tips (VRT), fine root dry weight (Fine DW) and plots as potential predictors. Data are n = 62 for ^{15}N-NH_4^+, n = 60 for ^{15}N-NO_3^-. Significant effects at $p \leq 0.05$ are indicated with bold letters.

Treatment	Root segments	Predictors	F value	P value
^{15}N-NH_4^+	Transport	EMF diversity (H′)	14.71	< **0.001**
^{15}N-NH_4^+	Whole root	EMF diversity (H′)	42.38	< **0.001**
		Seasons	18.77	< **0.001**
		VRT	16.60	< **0.001**
^{15}N-NO_3^-	Transport	EMF diversity (H′)	10.64	**0.001**
		Seasons	14.17	< **0.001**
		Fine DW	2.43	0.124
^{15}N-NO_3^-	Whole root	EMF diversity (H′)	49.61	< **0.001**
		Seasons	6.77	**0.002**
		VRT	4.22	**0.045**
		Fine DW	5.57	**0.022**

Multiple linear regression analysis from the chosen models showed that EMF diversity as a significant predictor for ^{15}N uptake in the transport segment from both NH_4^+ and NO_3^- (Table 4.2). In addition to EMF diversity, the model for ^{15}N uptake from NO_3^- in transport segment kept seasons as a significant predictor (Table 4.2). Furthermore, our models showed that EMF diversity was a significant predictor for ^{15}N uptake in the whole root segment from both NH_4^+ and NO_3^- (Table 4.2). In addition to EMF diversity, the model for ^{15}N uptake in the whole root segment kept seasons and vital RTs as significant predictors from NH_4^+ treatment (Table 4.2), and season, vital RTs and fine roots DW from NO_3^- treatment (Table 4.2). According to our chosen models, EMF Shannon diversity was the only predictor presence in all four models (Table 4.2).

A scatter diagram and Pearson correlation were used to visualize the relationship between EMF diversity and ^{15}N uptake in the transport segment (Figure 4.3). Pearson correlation test revealed a significant positive correlation with EMF diversity and ^{15}N uptake in the transport segment for NH_4^+ as well as for NO_3^- as N source (^{15}N-NH_4^+: $R^2 = 0.20$, $p = < 0.001$ and ^{15}N-NO_3^-: $R^2 = 0.15$, $p = 0.002$). We further observed much stronger significant positive correlation between EMF diversity and ^{15}N enrichment in the whole root (^{15}N-NH_4^+: $R^2 = 0.50$, $p = < 0.001$ and ^{15}N-NO_3^-: $R^2 = 0.46$, $p = <0.001$, Supplement Figure S4.2) than in the transport segment (Figure 4.3).

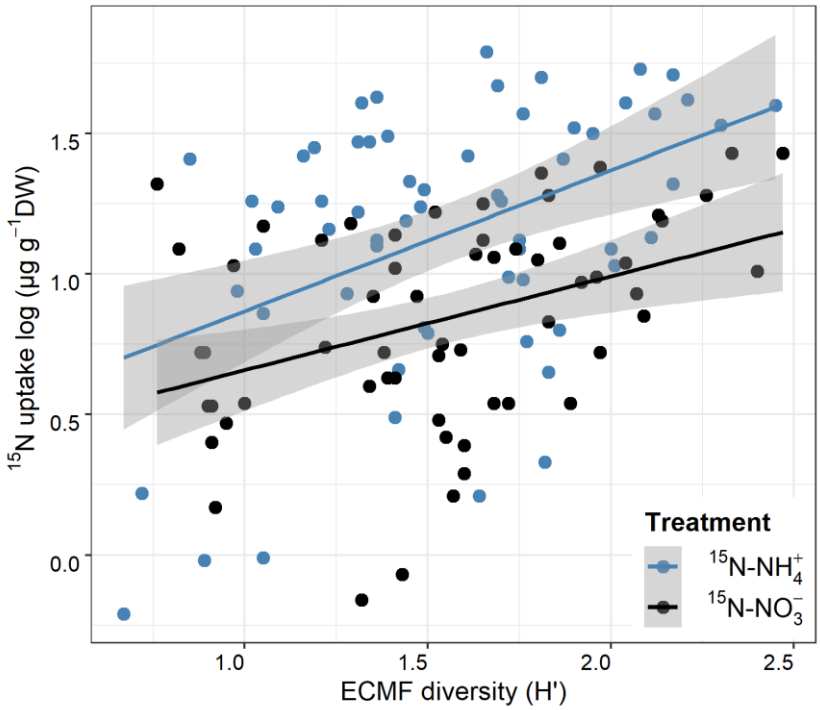


Figure 4.3: Dependence of beech N uptake on ectomycorrhizal fungal (EMF) diversity. Pearson correlation analysis was used to calculate the relationship between EMF Shannon diversity (H′) and ^{15}N uptake in the root transport segment. Blue coloured dots refer to NH_4^+ and black to NO_3^- uptake. Data are n = 62 for ^{15}N-NH_4^+ and n = 60 for ^{15}N-NO_3^-. Statistical information: (^{15}N-NH_4^+: y = 0.50x + 0.37, R^2 = 0.20, p <0.001, and ^{15}N-NO_3: y = 0.34x + 0.32, R^2 = 0.15, p = 0.002; slope: t = 0.99, p = 0.33).

4.3.6 Ectomycorrhizal fungi species identity effects

To disentangle whether increases of the beech root ^{15}N uptake were due to effects of the EMF diversity, we used LASSO regression models to evaluate the importance of distinct EMF taxa on ^{15}N enrichment from NH_4^+ or from NO_3^- uptake in the transport segment (Figure 4.4) and as well as in the whole root segment (Supplement Figure S4.3). Evaluating the LASSO results showed that for each of the four models, high lambda values were always favoured to obtain the optimum model for predicting the influential EMF species in ^{15}N uptake in transport and whole root segments (Figure 4.4; Supplement Figure S4.3). Since lambda constituted the shrinkage penalty in LASSO regression, this meant effectively setting all coefficients to zero in four models, thus only the removal of all EMF species results the best model (Figure 4.4; Supplement Figure S4.3). This finding suggests that no single EMF species appears to have effects and that instead, a high EMF diversity is beneficial for beech N-uptake.

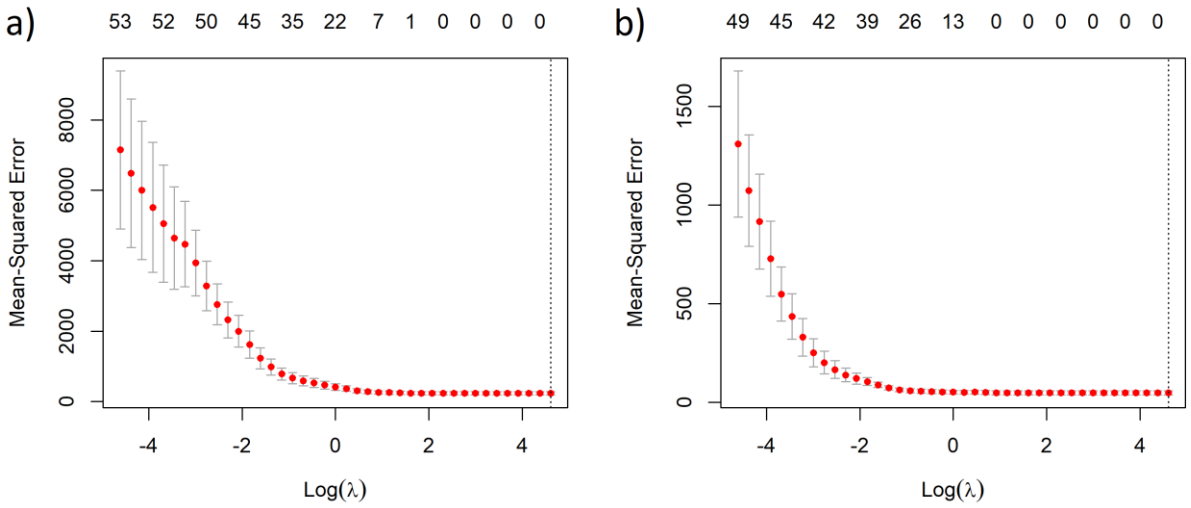


Figure 4.4: Ectomycorrhizal fungal (EMF) taxon-specific influence on N uptake in the root transport segment from NH_4^+ (a) and from NO_3^- (b). Least absolute shrinkage and selection operator (LASSO) logistic regression models were used to uncover the impact of the individual EMF species on root N uptake. A total of 53 EMF species were included in the LASSO model to test EMF taxon-specific influence on root N-uptake in root transport segments. The vertical line indicates the total number of EMF species that potentially contribute to N uptake in the transport root segments.

4.4 Discussion

In temperate forest ecosystems, N availability is often limiting plant growth (Rennenberg and Dannenmann, 2015). EMF are known to play a key role in N acquisition, assimilation and transport to their host plants (Heijden et al., 2015; Näsholm et al., 2013; Rennenberg et al., 2009), yet the functional role of EMF in root N uptake is still scarcely understood under field conditions. This study set out to assess the importance of EMF species in European beech root functioning for N uptake in temperate forests.

The EMF colonization rates and species richness in our study are consistent with previous studies in beech forests where more than 95% of the root tips were colonized by EMF (Lang and Polle, 2011; Leberecht et al., 2016; Pena et al., 2017). Similar to other studies, higher EMF species richness and diversity found in spring and autumn than in summer, indicating strong temporal effects in EMF community structure (Courty et al., 2008; Koide et al., 2007). The beech roots showed higher ^{15}N uptake in spring than summer and autumn, suggesting temporal competitiveness for ^{15}N enrichment as reported in a previous study of beech root N uptake (Li et al., 2016). Further, we observed similar temporal competitiveness for ^{15}N enrichment of EMF species.

4.4.1 Ectomycorrhizal and beech root segments prefer NH_4^+ than NO_3^-

Inorganic N sources that can be mainly used by plants and fungi are NH_4^+ and NO_3^- in soil solution (Chalot and Plassard, 2011). The ^{15}N derived from NH_4^+ more enriched in EMRTs and beech root segments than from NO_3^- indicating both EMRTs and beech root segments do not discriminate between the offered different inorganic N sources. These results also indicate that a proportional fraction of EMF ^{15}N enrichment is transferred to the host, thereby resulting in the same uptake patterns of beech roots and their associated EMF assemblages. This observation further supports the physiological mechanism that NH_4^+ is a readily utilized inorganic N source quickly taken by EMF and beech roots due to lower assimilation costs than those associated with the reduction of NO_3^- to NH_4^+ (Stuart and Plett, 2020). Also, nitrate can be easily lost from the systems by leaching (Chalot and Plassard, 2011; Clemmensen et al., 2008; Courty et al., 2015; Schleppi et al., 2012). However, Leberecht et al. (2016) reported that EMF assemblages and beech plants showed divergent enrichment patterns in N uptake in acidic calcareous forest soil, i.e. highest ^{15}N enrichment derived from NH_4^+ in EMF assemblages and from NO_3^- in beech trees.

4.4.2 Ectomycorrhizal fungi diversity drives beech N uptake

Once N is taken up by EMF, N is metabolized, often stored and eventually transported to the host. This is undoubtedly an accepted process, but the taxon-specific N transport mechanisms are still unknown under field conditions (Nehls and Plassard, 2018). It is generally reasoned that a higher EMF richness and diversity might be profitable to the plants with the chance of greater functional diversity, e.g. greater access to various N forms, P and organic matters (Finlay et al., 1992; Jones et al., 2010; Velmala et al., 2014). Several studies showed that higher fungal richness and diversity are beneficial to plant nutrient acquisition, for example beech N uptake from glutamine increased with increasing EMF richness (Leberecht et al., 2016) and beech P uptake efficiency increased with EMF diversity increase (Köhler et al., 2018), thus, supporting the importance of EMF diversity to N acquisition. Baxter and Dighton (2001) found that biomass and shoot N and P content of grey birch seedlings (*Betula populifolia* Marsh.) were more increased in the presence of four EMF species than the presence of a single EMF species. Likewise, a mixture of the eight EMF species improved the growth rate of European silver birch seedlings (*Betula pendula* Roth.) in low than high fertility soil relative to the mixture of two to four EMF species (Jonsson et al., 2001). Another potential advantage of higher EMF richness is biological insurance for plant nutrient uptake with increasing likelihood of associations with beneficial fungal species in changing environmental conditions (Pena et al., 2010; Pena and Polle, 2014).

In contrast EMF greater functional advantage to host plants, several studies also reported opposite behaviour. For instance, pairs of EMF species increased Scots pine growth compared to eight EMF in high fertility soils (Jonsson et al., 2001). *Suillus granulatus* (L.) Roussel improved the shoot growth of drought-stressed Scots pine seedling alone rather than the mixtures of two to four fungal species (Kipfer et al., 2012). N concentrations of needles of Norway spruce negatively correlated with EMF richness when the plants were colonized with up to five than with a single EMF species (Velmala et al., 2013). However, most of these studies were conducted under controlled conditions. Our study provided evidence that beech N uptake increased with increasing EMF diversity in natural forest ecosystems.

4.4.3 Interspecific variation of ^{15}N enrichment in ectomycorrhizal fungal species and taxon-specific influence on beech N uptake

Fungal species richness and species combinations play an important role in ecosystem functioning (Blackwell, 2011; Heijden et al., 2015), but very few studies have considered the importance of individual taxa within the EMF assemblages (Lilleskov et al., 2002; Pena et al., 2013b; Pena and Polle, 2014). In this study, ^{15}N enrichment of EMF was highlighted at the species level at which most functional trait-based information may be obtained, thus reflecting ecophysiological attributes of EMF species.

Several recent isotope studies revealed that EMF taxa differ in uptake of various N forms due to their physiological differences (Lilleskov et al., 2002; Pena et al., 2013b; Pena and Polle, 2014). In a culture study, Finlay et al. (1992) measured yield (mg DW) of nine different EMF species after 20, 40 and 60 days growth in liquid media containing NH_4^+ and NO_3^- sources. They found that all EMF grow well on NH_4^+ and lower growth on NO_3^- medium. Here, we observed a strong interspecific ^{15}N enrichment variations among EMF taxa with higher ^{15}N enrichment derived from NH_4^+ relative to NO_3^-, suggesting difference in N demands of different EMF species and competitiveness among each other.

Previously, it was shown that EMF species *Paxillus involutus* grew well on NH_4^+ medium compared to several other EMF species in culture studies (Finlay et al., 1992). Their finding may explain our results that the ^{15}N enrichment of *P. involutus* from NH_4^+ was comparatively higher than many other EMF species but it was not the similar consistent patterns from NO_3^-. In N deposition gradient study, Lilleskov et al. (2002) also found the percentage of the root tips of *P. involutus* increase with the increase of N availability in the soil. Furthermore, we found only one EMF species from the genus Helotiales showed higher ^{15}N enrichment from NO_3^- than from NH_4^+. In a culture study, France and Reid (1984) reported that several members of

Helotiales enriched higher NO_3^- than NH_4^+ and grow better on NO_3^- medium than NH_4^+ medium.

Among the EMF species, *Cenococcum geophilum* is one of the most important EMF species in temperate forests because of its capacity to mobilize and utilize organic N (Abuzinadah and Read, 1986; Courty et al., 2005; Diedhiou et al., 2010). However, the ability of *C. geophilum* to accumulate litter-derived inorganic N was not great compared to other EMF using ^{15}N-labeled litter (Pena et al., 2013). Here, we also observed similar patterns that *C. geophilum* is less able to take up free inorganic N compared to other EMF species. *C. geophilum* is also well known as drought-tolerant species, and highly abundant in the dry climate and beneficial to its host plant, especially under adverse environmental condition such as drought stress (Cairney and Chambers, 1999; Coleman et al., 2011; Dickie and Reich, 2005; Pena and Polle, 2014; Trappe, 1964). Here, the abundance of *C. geophilum* was higher in a forest with drier soil (SCH) than a forest with moist soil condition (HAI). In addition, *C. geophilum* was highly abundant in autumn (November) than summer (July), which may be because of their drought-tolerant characteristics supported them to survive over warm summer period than other EMF species. Therefore, *C. geophilum* may provide significant benefits to the trees in nutrient and water supply under water-limiting conditions.

Tomentella ramosissima and *Tomentella stuposa* accumulated the highest amount of ^{15}N from both NH_4^+ and NO_3^- along with *Clavulina cristata and Helotiales sp.1*, which demonstrates that these EMF species were more actively involved in N acquisition than other species. Member of the Tomentella genus (e.g. *Tomentella badia*) also found to be accumulated the highest litter-derived N compared to other EMF species using ^{15}N-labeled litter (Pena et al., 2013b). EMF species from the *Tomentella* genera are known as wood debris colonizer (Tedersoo et al., 2003).

Lilleskov et al. (2002, 2011) reported that EMF species from *Cortinarius* genus prefers organic N than inorganic N source. Here, we observed *Cortinarius casimiri* showed intermediate ^{15}N enrichment patterns derived from both NH_4^+ and NO_3^-, which indicates that this species is less able to take up free inorganic N. *Cortinarius* sp. are typically found in the organic layer, use protein and amino acids as their N sources and are sensitive to increased N deposition (Lilleskov et al., 2011, 2002; Treseder, 2008). Therefore, *Cortinarius* sp. usually decline in abundance when inorganic N availability increases, and then they are replaced by nitrophilic EMF genera such as *Inocybe and Tomentella* (Horton et al., 2013; Jones et al., 2012; Lilleskov et al., 2011, 2002).

Some other studies reported that EMF species from the *Russula* genus show even lower ^{15}N enrichment compared to *Cortinarius* in temperate pine forests (Hobbie et al., 2014) as well as in conifer and boreal forests (Taylor et al., 1997; Trudell et al., 2004). These patterns were also consistent with the results of our study. Most EMF species from the Russula genus showed lower ^{15}N enrichment derived from NH_4^+ than *Cortinarius* sp. but it was not always the case from NO_3^-. Root-colonizing fungi of the order of Russulales were always reported as highly abundant in temperate forest (Pena et al., 2017; Schröter et al., 2019). Here, several EMF species of Russulales genus were also abundant (about 40 to 50%) in the same studied forests in all seasons.

Furthermore, we assumed a significant EMF taxon-specific contribution to beech N uptake. Our study revealed that although EMF species differ in their ability to absorb various inorganic N forms, but not a single EMF species alone has a significant influence on beech N uptake. This finding indicates that EMF species may provide facilitation of N supply to the beech plants as a whole community than retain N. However, EMF species and community assemblages can also retain N instead of transporting to their host when plant-derived C supply to EMF is limited (Corrêa et al., 2008; Näsholm et al., 2013).

4.5 Conclusion

Our study revealed substantial interspecific ^{15}N enrichment variation in different EMF species associated with beech roots. We found that NH_4^+ was the preferred inorganic N source of both ectomycorrhizal root tips and beech root segments compared to NO_3^- under field conditions. One of the significant findings to emerge from this study is that no single EMF species alone appears to have substantial effects on beech N uptake and that instead, high EMF diversity is advantageous. Returning to the hypothesis posed at the beginning of this study, it is now possible to state that beech N transport benefits from having higher EMF diversity than only a single or few EMF species. Since EMF residing in soil types or depth may have different functions for plant nutrient supply (Clausing et al., 2021; Clausing and Polle, 2020), N uptake should also be assessed for EMF taxa in different soil layers. In addition to this, tree species also differ in their ability to take up N and establish EMF colonized root tips (Ishida et al., 2007; Lang et al., 2011; Li et al., 2016; Urbanová et al., 2015). Therefore, it is also necessary to include other ecologically important tree species (e.g. pine, spruce) to assess the function of EMF in nutrient uptake (e.g. N and P). This will enhance our understanding of ecosystem functioning under the threats of future climate change.

4.6 Declaration

I led the stable isotope labelling experiments, organized the field sampling, characterized and identified ectomycorrhizal fungi. I prepared samples for nitrogen isotope measurements, sampled root tips for molecular analyses, analysed the data using advance statistical methods and wrote the chapter. Dennis Janz conducted the initial LASSO regression analysis.

4.7 Data availability

All data have been deposited in the BExIS database (https://www.bexis.uni-jena.de) under the following accession numbers (data owner): Ectomycorrhizal fungal community composition - 30910 (Polle), ^{15}N enrichment in different root segments – 30911 (Polle) and ^{15}N enrichment in different ectomycorrhizal fungi – 30914 (Polle).

4.8 References

Abuzinadah, R.A., Read, D.J., 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. New Phytologist 103, 481–493. https://doi.org/10.1111/j.1469-8137.1986.tb02886.x

Agerer, R., 2001. Exploration types of ectomycorrhizae. Mycorrhiza 11, 107–114. https://doi.org/10.1007/s005720100108

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Baxter, J.W., Dighton, J., 2001. Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (Betula populifolia) seedlings in host–symbiont culture conditions. New Phytologist 152, 139–149. https://doi.org/10.1046/j.0028-646x.2001.00245.x

Blackwell, M., 2011. The Fungi: 1, 2, 3 … 5.1 million species? American Journal of Botany 98, 426–438. https://doi.org/10.3732/ajb.1000298

Braun, S., Thomas, V.F.D., Quiring, R., Flückiger, W., 2010. Does nitrogen deposition increase forest production? The role of phosphorus. Environmental Pollution, Advances of air pollution science: from forest decline to multiple-stress effects on forest ecosystem services 158, 2043–2052. https://doi.org/10.1016/j.envpol.2009.11.030

Cairney, J.W.G., Chambers, S.M. (Eds.), 1999. Ectomycorrhizal fungi: Key genera in profile. Springer-Verlag, Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06827-4

Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W., Classen, A.T., Crowther, T.W., Danovaro, R., Foreman, C.M., Huisman, J., Hutchins, D.A., Jansson, J.K., Karl, D.M., Koskella, B., Mark Welch, D.B., Martiny, J.B.H., Moran, M.A., Orphan, V.J., Reay, D.S., Remais, J.V., Rich, V.I., Singh, B.K., Stein, L.Y., Stewart, F.J., Sullivan, M.B., van Oppen, M.J.H., Weaver, S.C., Webb, E.A., Webster, N.S., 2019. Scientists' warning to humanity: microorganisms and climate change. Nature Reviews Microbiology 17, 569–586. https://doi.org/10.1038/s41579-019-0222-5

Chalot, M., Plassard, C., 2011. Ectomycorrhiza and nitrogen provision to the host tree, John Wiley & Sons, Ltd, pp. 69–94. https://doi.org/10.1002/9780470959404.ch4

Clausing, S., Likulunga, L.E., Janz, D., Feng, H.Y., Schneider, D., Daniel, R., Krüger, J., Lang, F., Polle, A., 2021. Impact of nitrogen and phosphorus addition on resident soil and root mycobiomes in beech forests. bioRxiv 2020.12.29.424645. https://doi.org/10.1101/2020.12.29.424645

Clausing, S., Polle, A., 2020. Mycorrhizal phosphorus efficiencies and microbial competition drive root P uptake. Front. For. Glob. Change 3. https://doi.org/10.3389/ffgc.2020.00054

Clemmensen, K.E., Sorensen, P.L., Michelsen, A., Jonasson, S., Ström, L., 2008. Site-dependent N uptake from N-form mixtures by arctic plants, soil microbes and ectomycorrhizal fungi. Oecologia 155, 771–783. https://doi.org/10.1007/s00442-008-0962-9

Coleman, M.D., Bledsoe, C.S., Lopushinsky, W., 2011. Pure culture response of ectomycorrhizal fungi to imposed water stress. Canadian Journal of Botany. https://doi.org/10.1139/b89-005

Corrêa, A., Strasser, R.J., Martins-Loução, M.A., 2008. Response of plants to ectomycorrhizae in N-limited conditions: which factors determine its variation? Mycorrhiza 18, 413–427. https://doi.org/10.1007/s00572-008-0195-0

Courty, P.-E., Franc, A., Pierrat, J.-C., Garbaye, J., 2008. Temporal changes in the ectomycorrhizal community in two soil horizons of a temperate oak forest. Appl. Environ. Microbiol. 74, 5792–5801. https://doi.org/10.1128/AEM.01592-08

Courty, P.-E., Pritsch, K., Schloter, M., Hartmann, A., Garbaye, J., 2005. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. New Phytologist 167, 309–319. https://doi.org/10.1111/j.1469-8137.2005.01401.x

Courty, P.E., Smith, P., Koegel, S., Redecker, D., Wipf, D., 2015. Inorganic nitrogen uptake and transport in beneficial plant root-microbe interactions. Critical Reviews in Plant Sciences 34, 4–16. https://doi.org/10.1080/07352689.2014.897897

Courty, P.-E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.-P., Uroz, S., Garbaye, J., 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biology and Biochemistry 42, 679–698. https://doi.org/10.1016/j.soilbio.2009.12.006

Dannenmann, M., Simon, J., Gasche, R., Holst, J., Naumann, P.S., Kögel-Knabner, I., Knicker, H., Mayer, H., Schloter, M., Pena, R., Polle, A., Rennenberg, H., Papen, H., 2009. Tree girdling provides insight on the role of labile carbon in nitrogen partitioning between soil microorganisms and adult European beech. Soil Biology and Biochemistry 41, 1622–1631. https://doi.org/10.1016/j.soilbio.2009.04.024

de Witte, L.C., Rosenstock, N.P., van der Linde, S., Braun, S., 2017. Nitrogen deposition changes ectomycorrhizal communities in Swiss beech forests. Science of The Total Environment 605–606, 1083–1096. https://doi.org/10.1016/j.scitotenv.2017.06.142

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nature Communications 7, 10541. https://doi.org/10.1038/ncomms10541

Dickie, I.A., Martínez-García, L.B., Koele, N., Grelet, G.-A., Tylianakis, J.M., Peltzer, D.A., Richardson, S.J., 2013. Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. Plant Soil 367, 11–39. https://doi.org/10.1007/s11104-013-1609-0

Dickie, I.A., Reich, P.B., 2005. Ectomycorrhizal fungal communities at forest edges. Journal of Ecology 93, 244–255.

Diedhiou, A.G., Dupouey, J.-L., Buée, M., Dambrine, E., Laüt, L., Garbaye, J., 2010. The functional structure of ectomycorrhizal communities in an oak forest in central France witnesses ancient Gallo-Roman farming practices. Soil Biology and Biochemistry 42, 860–862. https://doi.org/10.1016/j.soilbio.2010.01.011

Finlay, R.D., Ek, H., Odham, G., Süderström, B., 1989. Uptake, translocation and assimilation of nitrogen from ^{15}N-labelled ammonium and nitrate sources by intact ectomycorrhizal systems of Fagus sylvatica infected with Paxillus involutus. New Phytologist 113, 47–55. https://doi.org/10.1111/j.1469-8137.1989.tb02394.x

Finlay, R.D., Frostegård, Å., Sonnerfeldt, A.-M., 1992. Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with Pinus contorta Dougl. ex Loud. New Phytologist 120, 105–115. https://doi.org/10.1111/j.1469-8137.1992.tb01063.x

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.-D., Weisser, W.W., 2010. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic and Applied Ecology 11, 473–485. https://doi.org/10.1016/j.baae.2010.07.009

Fox, J., Wisberg, S., 2021. An R companion to applied regression. SAGE Publications Inc.

Friedman, J.H., Hastie, T., Tibshirani, R., 2010. Regularization paths for generalized linear models via coordinate descent. Journal of Statistical Software 33, 1–22. https://doi.org/10.18637/jss.v033.i01

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Geßler, A., Jung, K., Gasche, R., Papen, H., Heidenfelder, A., Börner, E., Metzler, B., Augustin, S., Hildebrand, E., Rennenberg, H., 2005. Climate and forest management influence nitrogen balance of European beech forests: microbial N transformations and inorganic N net uptake capacity of mycorrhizal roots. Eur J Forest Res 124, 95–111. https://doi.org/10.1007/s10342-005-0055-9

Gessler, A., Schneider, S., Sengbusch, D.V., Weber, P., Hanemann, U., Huber, C., Rothe, A., Kreutzer, K., Rennenberg, H., 1998. Field and laboratory experiments on net uptake of nitrate and ammonium by the roots of spruce (Picea abies) and beech (Fagus sylvatica) trees. New Phytologist 138, 275–285. https://doi.org/10.1046/j.1469-8137.1998.00107.x

Gobert, A., Plassard, C., 2008. The Beneficial Effect of Mycorrhizae on N Utilization by the Host-Plant: Myth or Reality?, Mycorrhiza. Springer, Berlin, Heidelberg, pp. 209–240. https://doi.org/10.1007/978-3-540-78826-3_11

Gruffman, L., Jämtgård, S., Näsholm, T., 2014. Plant nitrogen status and co-occurrence of organic and inorganic nitrogen sources influence root uptake by Scots pine seedlings. Tree Physiology 34, 205–213. https://doi.org/10.1093/treephys/tpt121

Guo, C., Simon, J., Gasche, R., Naumann, P.S., Bimüller, C., Pena, R., Polle, A., Kögel-Knabner, I., Zeller, B., Rennenberg, H., Dannenmann, M., 2013. Minor contribution of leaf litter to N nutrition of beech (Fagus sylvatica) seedlings in a mountainous beech forest of Southern Germany. Plant Soil 369, 657–668. https://doi.org/10.1007/s11104-013-1603-6

Hawkins, B.J., Jones, M.D., Kranabetter, J.M., 2015. Ectomycorrhizae and tree seedling nitrogen nutrition in forest restoration. New Forests 46, 747–771. https://doi.org/10.1007/s11056-015-9488-2

Heijden, M.G.A. van der, Martin, F.M., Selosse, M.-A., Sanders, I.R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist 205, 1406–1423. https://doi.org/10.1111/nph.13288

Hobbie, E.A., Diepen, L.T.A. van, Lilleskov, E.A., Ouimette, A.P., Finzi, A.C., Hofmockel, K.S., 2014. Fungal functioning in a pine forest: evidence from a 15N-labeled global change experiment. New Phytologist 201, 1431–1439. https://doi.org/10.1111/nph.12578

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., O'Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486, 105–108. https://doi.org/10.1038/nature11118

Horton, B.M., Glen, M., Davidson, N.J., Ratkowsky, D., Close, D.C., Wardlaw, T.J., Mohammed, C., 2013. Temperate eucalypt forest decline is linked to altered ectomycorrhizal communities mediated by soil chemistry. Forest Ecology and Management 302, 329–337. https://doi.org/10.1016/j.foreco.2013.04.006

Ishida, T.A., Nara, K., Hogetsu, T., 2007. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer–broadleaf forests. New Phytologist 174, 430–440. https://doi.org/10.1111/j.1469-8137.2007.02016.x

Jacob, A., Leuschner, C., 2015. Complementarity in the use of nitrogen forms in a temperate broad-leaved mixed forest. Plant Ecology & Diversity 8, 243–258. https://doi.org/10.1080/17550874.2014.898166

Jing, X., Sanders, N.J., Shi, Yu, Chu, H., Classen, A.T., Zhao, K., Chen, L., Shi, Yue, Jiang, Y., He, J.-S., 2015. The links between ecosystem multifunctionality and above- and belowground biodiversity are mediated by climate. Nature Communications 6, 8159. https://doi.org/10.1038/ncomms9159

Johnson, D., Martin, F., Cairney, J.W.G., Anderson, I.C., 2012. The importance of individuals: intraspecific diversity of mycorrhizal plants and fungi in ecosystems. New Phytologist 194, 614–628. https://doi.org/10.1111/j.1469-8137.2012.04087.x

Jones, M.D., Phillips, L.A., Treu, R., Ward, V., Berch, S.M., 2012. Functional responses of ectomycorrhizal fungal communities to long-term fertilization of lodgepole pine (Pinus contorta Dougl. ex Loud. var. latifolia Engelm.) stands in central British

Columbia. Applied Soil Ecology, Selected Papers from the 2011 Soil Ecology Society Conference 60, 29–40. https://doi.org/10.1016/j.apsoil.2012.01.010

Jones, M.D., Twieg, B.D., Ward, V., Barker, J., Durall, D.M., Simard, S.W., 2010. Functional complementarity of Douglas-fir ectomycorrhizas for extracellular enzyme activity after wildfire or clearcut logging. Functional Ecology 24, 1139–1151. https://doi.org/10.1111/j.1365-2435.2010.01699.x

Jonsson, L.M., Nilsson, M.-C., Wardle, D.A., Zackrisson, O., 2001. Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. Oikos 93, 353–364. https://doi.org/10.1034/j.1600-0706.2001.930301.x

Jumpponen, A., Brown, S.P., Trappe, J.M., Cázares, E., Strömmer, R., 2012. Twenty years of research on fungal–plant interactions on Lyman Glacier forefront – lessons learned and questions yet unanswered. Fungal Ecology, Fungi in Extreme Environments 5, 430–442. https://doi.org/10.1016/j.funeco.2012.01.002

Kipfer, T., Wohlgemuth, T., Heijden, M.G.A. van der, Ghazoul, J., Egli, S., 2012. Growth response of drought-stressed pinus sylvestris seedlings to single- and multi-species inoculation with ectomycorrhizal fungi. PLOS ONE 7, e35275. https://doi.org/10.1371/journal.pone.0035275

Köhler, J., Yang, N., Pena, R., Raghavan, V., Polle, A., Meier, I.C., 2018. Ectomycorrhizal fungal diversity increases phosphorus uptake efficiency of European beech. New Phytologist 220, 1200–1210. https://doi.org/10.1111/nph.15208

Koide, R.T., Shumway, D.L., Xu, B., Sharda, J.N., 2007. On temporal partitioning of a community of ectomycorrhizal fungi. New Phytologist 174, 420–429. https://doi.org/10.1111/j.1469-8137.2007.02000.x

Kranabetter, J.M., 2014. Ectomycorrhizal fungi and the nitrogen economy of conifers — implications for genecology and climate change mitigation. Botany. https://doi.org/10.1139/cjb-2013-0198

Lang, C., Polle, A., 2011. Ectomycorrhizal fungal diversity, tree diversity and root nutrient relations in a mixed Central European forest. Tree Physiology 31, 531–538. https://doi.org/10.1093/treephys/tpr042

Lang, C., Seven, J., Polle, A., 2011. Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. Mycorrhiza 21, 297–308. https://doi.org/10.1007/s00572-010-0338-y

Leberecht, M., Dannenmann, M., Gschwendtner, S., Bilela, S., Meier, R., Simon, J., Rennenberg, H., Schloter, M., Polle, A., 2015. Ectomycorrhizal communities on the roots of two beech (Fagus sylvatica) populations from contrasting climates differ in nitrogen acquisition in a common environment. Appl Environ Microbiol 81, 5957–5967. https://doi.org/10.1128/AEM.01481-15

Leberecht, M., Tu, J., Polle, A., 2016. Acid and calcareous soils affect nitrogen nutrition and organic nitrogen uptake by beech seedlings (Fagus sylvatica L.) under drought, and

their ectomycorrhizal community structure. Plant Soil 409, 143–157. https://doi.org/10.1007/s11104-016-2956-4

Li, X., Rennenberg, H., Simon, J., 2016. Seasonal variation in N uptake strategies in the understorey of a beech-dominated N-limited forest ecosystem depends on N source and species. Tree Physiology 36, 589–600. https://doi.org/10.1093/treephys/tpv132

Lilleskov, E.A., Fahey, T.J., Horton, T.R., Lovett, G.M., 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in alaska. Ecology 83, 104–115. https://doi.org/10.1890/0012-9658(2002)083[0104:BEFCCO]2.0.CO;2

Lilleskov, E.A., Hobbie, E.A., Horton, T.R., 2011. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecology, Conservation Underground: Fungi in a Changing World 4, 174–183. https://doi.org/10.1016/j.funeco.2010.09.008

Lilleskov, E.A., Kuyper, T.W., Bidartondo, M.I., Hobbie, E.A., 2019. Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: A review. Environmental Pollution 246, 148–162. https://doi.org/10.1016/j.envpol.2018.11.074

Loreau, M., 2004. Does functional redundancy exist? Oikos 104, 606–611. https://doi.org/10.1111/j.0030-1299.2004.12685.x

Marquard, E., Weigelt, A., Roscher, C., Gubsch, M., Lipowsky, A., Schmid, B., 2009. Positive biodiversity–productivity relationship due to increased plant density. Journal of Ecology 97, 696–704. https://doi.org/10.1111/j.1365-2745.2009.01521.x

Martin, F.M., Uroz, S., Barker, D.G., 2017. Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria. Science 356. https://doi.org/10.1126/science.aad4501

Meinshausen, N., Bühlmann, P., 2006. High-dimensional graphs and variable selection with the Lasso. The Annals of Statistics 34, 1436–1462. https://doi.org/10.1214/009053606000000281

Melin, E., Nilsson, H., 1953. Transfer of labelled nitrogen from glutamic acid to pine seedlings through the mycelium of boletus variegatus (Sw.) Fr. Nature 171, 134–134. https://doi.org/10.1038/171134a0

Nacry, P., Bouguyon, E., Gojon, A., 2013. Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant Soil 370, 1–29. https://doi.org/10.1007/s11104-013-1645-9

Näsholm, T., Högberg, P., Franklin, O., Metcalfe, D., Keel, S.G., Campbell, C., Hurry, V., Linder, S., Högberg, M.N., 2013. Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? New Phytologist 198, 214–221. https://doi.org/10.1111/nph.12139

Nehls, U., Plassard, C., 2018. Nitrogen and phosphate metabolism in ectomycorrhizas. New Phytologist 220, 1047–1058. https://doi.org/10.1111/nph.15257

Nguyen, D.Q., Pena, R., Polle, A., 2017. Impact of ectomycorrhizal community composition and soil treatment on inorganic nitrogen nutrition and performance of beech (Fagus sylvatica L.) provenances. Trees 31, 1891–1904. https://doi.org/10.1007/s00468-017-1594-7

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2013. vegan: Community ecology package.

Pena, R., Lang, C., Lohaus, G., Boch, S., Schall, P., Schöning, I., Ammer, C., Fischer, M., Polle, A., 2017. Phylogenetic and functional traits of ectomycorrhizal assemblages in top soil from different biogeographic regions and forest types. Mycorrhiza 27, 233–245. https://doi.org/10.1007/s00572-016-0742-z

Pena, R., Offermann, C., Simon, J., Naumann, P.S., Geßler, A., Holst, J., Dannenmann, M., Mayer, H., Kögel-Knabner, I., Rennenberg, H., Polle, A., 2010. Girdling Affects Ectomycorrhizal fungal (EMF) Diversity and reveals functional differences in EMF community composition in a beech forest. Appl. Environ. Microbiol. 76, 1831. https://doi.org/10.1128/AEM.01703-09

Pena, R., Polle, A., 2014. Attributing functions to ectomycorrhizal fungal identities in assemblages for nitrogen acquisition under stress. The ISME Journal 8, 321–330. https://doi.org/10.1038/ismej.2013.158

Pena, R., Simon, J., Rennenberg, H., Polle, A., 2013a. Ectomycorrhiza affect architecture and nitrogen partitioning of beech (Fagus sylvatica L.) seedlings under shade and drought. Environmental and Experimental Botany 87, 207–217. https://doi.org/10.1016/j.envexpbot.2012.11.005

Pena, R., Tejedor, J., Zeller, B., Dannenmann, M., Polle, A., 2013b. Interspecific temporal and spatial differences in the acquisition of litter-derived nitrogen by ectomycorrhizal fungal assemblages. New Phytologist 199, 520–528. https://doi.org/10.1111/nph.12272

Perry, D.A., Amaranthus, M.P., Borchers, J.G., Borchers, S.L., Brainerd, R.E., 1989. Bootstrapping in Ecosystems: Internal interactions largely determine productivity and stability in biological systems with strong positive feedback. BioScience 39, 230–237. https://doi.org/10.2307/1311159

R development Core Team., 2020. R: The R project for statistical computing: R foundation for statistical computing: Vienna, Austria, 2020. https://www.r-project.org/ (accessed 4.13.21).

Rennenberg, H., Dannenmann, M., 2015. Nitrogen nutrition of trees in temperate forests—the significance of nitrogen availability in the pedosphere and atmosphere. Forests 6, 2820–2835. https://doi.org/10.3390/f6082820

Rennenberg, H., Dannenmann, M., Gessler, A., Kreuzwieser, J., Simon, J., Papen, H., 2009. Nitrogen balance in forest soils: nutritional limitation of plants under climate change stresses. Plant Biology 11, 4–23. https://doi.org/10.1111/j.1438-8677.2009.00241.x

Schleppi, P., Bucher-Wallin, I., Hagedorn, F., Körner, C., 2012. Increased nitrate availability in the soil of a mixed mature temperate forest subjected to elevated CO2 concentration (canopy FACE). Global Change Biology 18, 757–768. https://doi.org/10.1111/j.1365-2486.2011.02559.x

Schröter, K., Wemheuer, B., Pena, R., Schöning, I., Ehbrecht, M., Schall, P., Ammer, C., Daniel, R., Polle, A., 2019. Assembly processes of trophic guilds in the root mycobiome of temperate forests. Molecular Ecology 28, 348–364. https://doi.org/10.1111/mec.14887

Stoelken, G., Simon, J., Ehlting, B., Rennenberg, H., 2010. The presence of amino acids affects inorganic N uptake in non-mycorrhizal seedlings of European beech (Fagus sylvatica). Tree Physiology 30, 1118–1128. https://doi.org/10.1093/treephys/tpq050

Stuart, E.K., Plett, K.L., 2020. Digging deeper: In search of the mechanisms of carbon and nitrogen exchange in ectomycorrhizal symbioses. Front. Plant Sci. 10. https://doi.org/10.3389/fpls.2019.01658

Taylor, A.F.S., Högbom, L., Högberg, M., Lyon, A.J.E., Näsholm, T., Högberg, P., 1997. Natural ^{15}N abundance in fruit bodies of ectomycorrhizal fungi from boreal forests. New Phytologist 136, 713–720. https://doi.org/10.1046/j.1469-8137.1997.00788.x

Tedersoo, L., Bahram, M., 2019. Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes. Biological Reviews 94, 1857–1880. https://doi.org/10.1111/brv.12538

Tedersoo, L., Kõljalg, U., Hallenberg, N., Larsson, K.-H., 2003. Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytologist 159, 153–165. https://doi.org/10.1046/j.1469-8137.2003.00792.x

Tilman, D., Isbell, F., Cowles, J.M., 2014. Biodiversity and ecosystem functioning. Annual review of ecology, evolution, and systematics 45, 471–493. https://doi.org/10.1146/annurev-ecolsys-120213-091917

Tilman, D., Lehman, C.L., Thomson, K.T., 1997. Plant diversity and ecosystem productivity: Theoretical considerations. PNAS 94, 1857–1861. https://doi.org/10.1073/pnas.94.5.1857

Trappe, J.M., 1964. Mycorrhizal host and distribution of Cenococcum graniforme. Lloydia 27, 100–106.

Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11, 1111–1120. https://doi.org/10.1111/j.1461-0248.2008.01230.x

Trudell, S.A., Rygiewicz, P.T., Edmonds, R.L., 2004. Patterns of nitrogen and carbon stable isotope ratios in macrofungi, plants and soils in two old-growth conifer forests. New Phytologist 164, 317–335. https://doi.org/10.1111/j.1469-8137.2004.01162.x

Urbanová, M., Šnajdr, J., 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. https://doi.org/10.1016/j.soilbio.2015.02.011

van der Linde, S., Suz, L.M., Orme, C.D.L., Cox, F., Andreae, H., Asi, E., Atkinson, B., Benham, S., Carroll, C., Cools, N., De Vos, B., Dietrich, H.-P., Eichhorn, J., Gehrmann, J., Grebenc, T., Gweon, H.S., Hansen, K., Jacob, F., Kristöfel, F., Lech, P., Manninger, M., Martin, J., Meesenburg, H., Merilä, P., Nicolas, M., Pavlenda, P., Rautio, P., Schaub, M., Schröck, H.-W., Seidling, W., Šrámek, V., Thimonier, A., Thomsen, I.M., Titeux, H., Vanguelova, E., Verstraeten, A., Vesterdal, L., Waldner, P., Wijk, S., Zhang, Y., Žlindra, D., Bidartondo, M.I., 2018. Environment and host as large-scale controls of ectomycorrhizal fungi. Nature 558, 243–248. https://doi.org/10.1038/s41586-018-0189-9

Velmala, S.M., Rajala, T., Haapanen, M., Taylor, A.F.S., Pennanen, T., 2013. Genetic host-tree effects on the ectomycorrhizal community and root characteristics of Norway spruce. Mycorrhiza 23, 21–33. https://doi.org/10.1007/s00572-012-0446-y

Velmala, S.M., Rajala, T., Heinonsalo, J., Taylor, A.F.S., Pennanen, T., 2014. Profiling functions of ectomycorrhizal diversity and root structuring in seedlings of Norway spruce (Picea abies) with fast- and slow-growing phenotypes. New Phytologist 201, 610–622. https://doi.org/10.1111/nph.12542

Venables, W.N., Ripley, B.D., 2002. Modern applied statistics with s, 4th ed, Statistics and Computing. Springer-Verlag, New York. https://doi.org/10.1007/978-0-387-21706-2

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E., van der Heijden, M.G.A., 2019. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nature Communications 10, 4841. https://doi.org/10.1038/s41467-019-12798-y

Warton, D.I., Shipley, B., Hastie, T., 2015. CATS regression – a model-based approach to studying trait-based community assembly. Methods in Ecology and Evolution 6, 389–398. https://doi.org/10.1111/2041-210X.12280

White, T. j., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR protocols; Academic Press, Inc.: Cambridge, MA, USA, 1990; pp. 315–322. ISBN 978-0-12-372180-8. [WWW Document].

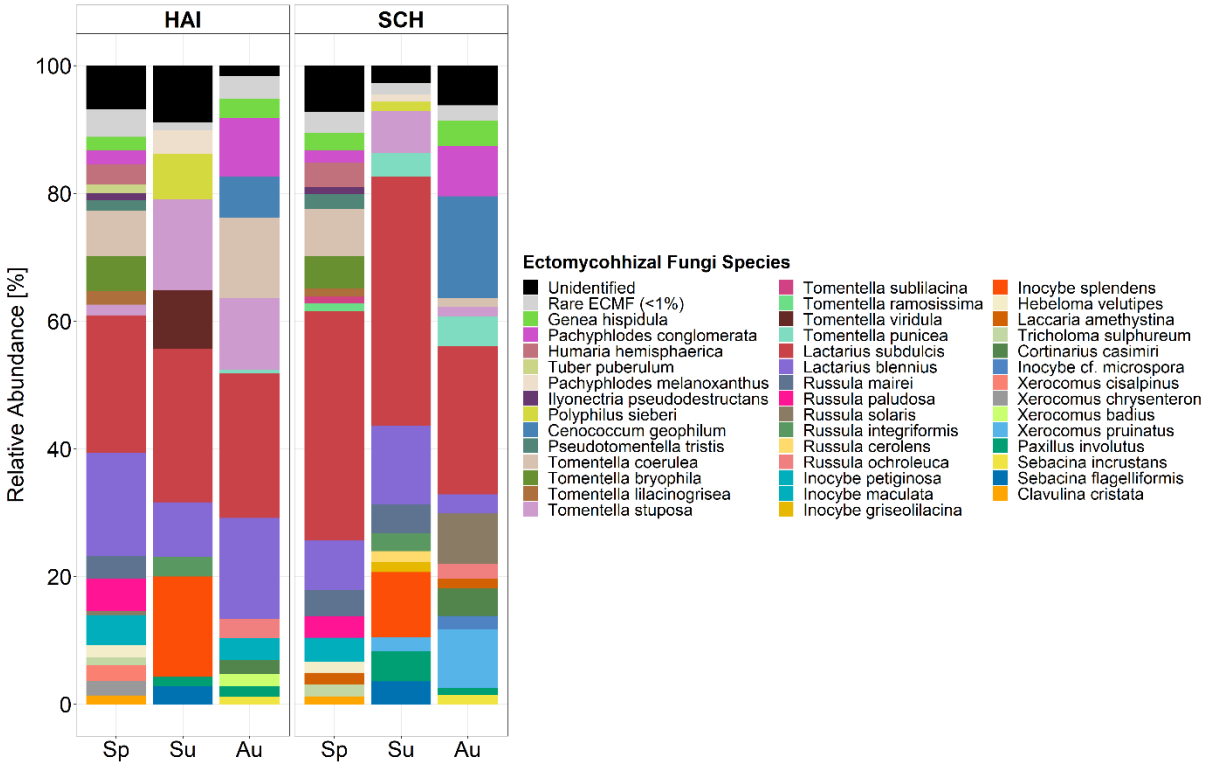
Wickham, H., 2009. ggplot2: Elegant graphics for data analysis, Springer-Verlag, New York. https://doi.org/10.1007/978-0-387-98141-3

Winkler, J.B., Dannenmann, M., Simon, J., Pena, R., Offermann, C., Sternad, W., Clemenz, C., Naumann, P.S., Gasche, R., Kögel-Knabner, I., Gessler, A., Rennenberg, H., Polle, A., 2010. Carbon and nitrogen balance in beech roots under competitive pressure of soil-borne microorganisms induced by girdling, drought and glucose application. Functional Plant Biol. 37, 879–889. https://doi.org/10.1071/FP09309

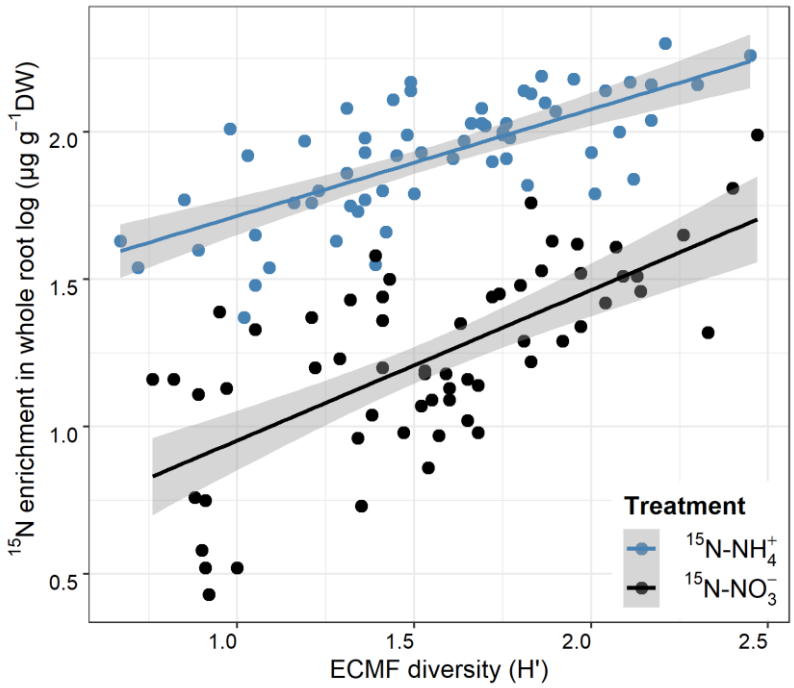
Zeller, B., Colin-Belgrand, M., Dambrine, E., Martin, F., 2001. Fate of nitrogen released from 15N-labeled litter in European beech forests. Tree Physiology 21, 153–162. https://doi.org/10.1093/treephys/21.2-3.153

4.9 Supplementary materials – Chapter 4

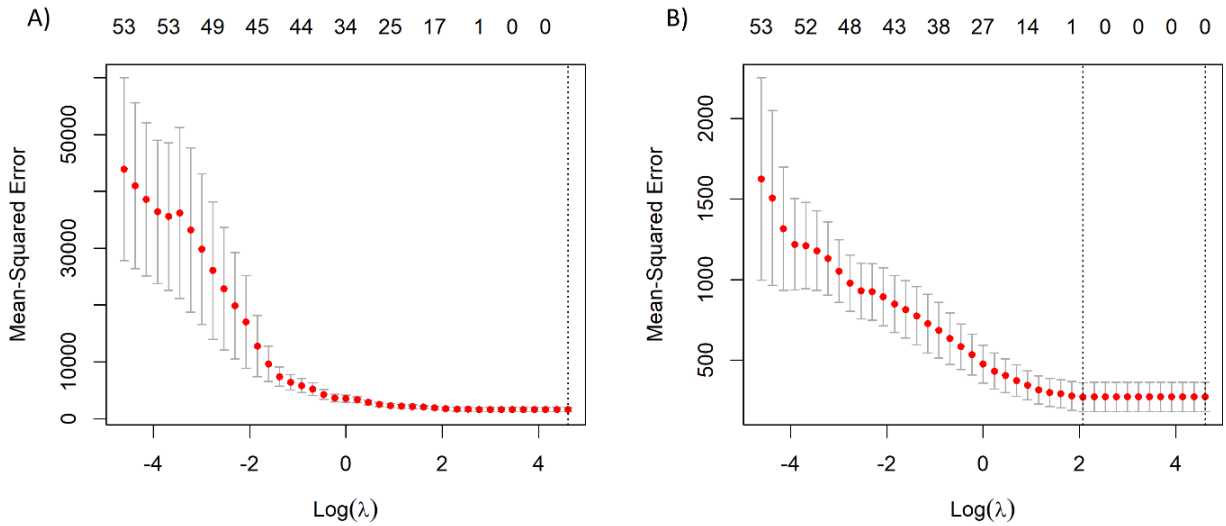
Supplement Figure S4.1: Relative abundance of the ectomycorrhizal fungi (EMF) species in different seasons and regions. HAI = Hainich-Dün, SCH = Schorfheide-Chorin, Sp = Spring, Su = Summer, Au = Autumn. "Rare taxa", EMF species with less than 1% of the total abundance of each season were summarized. Unidentified, EMF species which were not possible to identify them until species level were summarized. A total number of 30 (Sp), 14 (Su) and 19 (Au) EMF species were found in HAI. A total number of 28 (Sp), 17 (Su) and 22 (Au) EMF species were found in SCH. EMF community dissimilarities among season based on ANOSIM (HAI, $R = 0.31$, $p < 0.001$ and SCH, $R = 0.43$, $p < 0.001$) and among the regions, $R = 0.04$, $p = 0.006$).



Supplement Figure S4.2: Dependence of beech N uptake on ectomycorrhizal fungi (EMF) diversity. Pearson correlation analysis was used to calculate the relationship between EMF Shannon diversity (H′) and ^{15}N uptake in the whole root segment. Blue coloured dots refer to $NH4^+$ and black to NO_3^- uptake. Data are n = 62 for $^{15}N\text{-}NH_4^+$ and n = 60 for $^{15}N\text{-}NO_3^-$. Statistical information: ($^{15}N\text{-}NH_4^+$: y = 0.39x + 1.49, R^2= 0.50, p = < 0.001, and $^{15}N\text{-}NO_3$: y = 0.51x + 0.49, R^2 = 0.46, p < 0.001; slope: t = 1.37, p = 0.17).



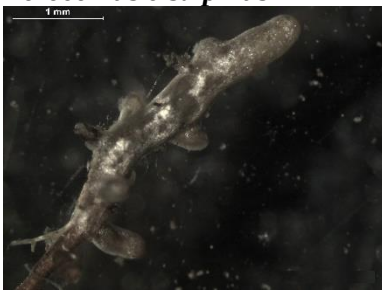
Supplement Figure S4.3: Ectomycorrhizal fungal (EMF) taxon-specific influence on N uptake in the whole root segment from NH_4^+ (a) and for NO_3^- (b). Least absolute shrinkage and selection operator (LASSO) logistic regression models were used to uncover the impact of the individual EMF species on root N uptake. A total of 53 different EMF species were included in the LASSO model to test EMF taxon-specific influence on root N-uptake. The vertical line indicates the total number of EMF species that potentially contribute to N uptake in the whole root segment.



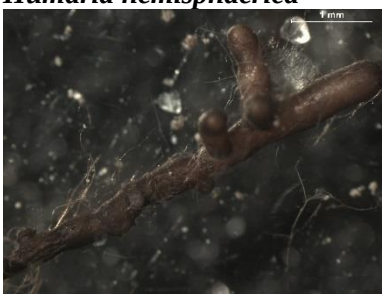
Supplement Table S4.1: Key characteristics of the study plots and sites. Hainich-Dün = HAI and Schorfheide-Chorin = SCH, dry weight = DW, carbon = C, nitrogen = N, ammonium = NH_4^+, nitrate = NO_3^-. Location and climate data were taken from (http://www.biodiversity-exploratories.de). Soil chemistry data are available in the BeXis database (Dataset 26226 and 26227).

Parameters	**Hainich-Dün (HAI)**			**Schorfheide-Chorin (SCH)**		
Plot number	HAI 7	HAI 20	HAI 22	SCH 6	SCH 43	SCH 44
Location						
Longitudes-Latitude	51.1311-10.3854	51.3339-10.3603	51.3372-10.3593	52.9074-13.8417	52.9010-13.9283	52.9185-13.8592
Average temperature (°C) during labelling time (8 a.m. to 2 p.m.)						
Spring	13.36	16.38	10.76	3.48	5.35	4.86
Summer	27.52	28.60	25.77	20.84	23.96	28.97
Autumn	9.11	10.62	9.76	0.58	0.64	1.79
Average photosynthetically active radiation (µmol m^{-2} s^{-1}) during labelling time (8 a.m. to 2 p.m.)						
Spring	303.84	143.39	39.88	108.29	249.02	161.71
Summer	56.91	57.10	88.62	37.31	22.17	35.30
Autumn	39.43	23.53	43.22	15.03	34.08	16.42
Stand Characteristics						
Tree stands	*Fagus sylvatica*	*Fagus sylvatica*	*Fagus sylvatica*	*Fagus sylvatica*	*Fagus sylvatica*	*Fagus sylvatica*
Soil moisture (%)	39.08	45.25	36.68	13.05	12.88	9.85
Soil chemical properties						
pH	4.16	6.30	4.69	3.67	3.72	3.64
Organic soil layers						
C (mg g^{-1} DW)	376.10	401.90	399.10	237.50	170.50	266.70
N (mg g^{-1} DW)	14.60	19.20	16.10	11.70	9.30	11.90
C:N	25.76	20.93	24.79	20.30	18.33	22.41
Mineral soil layers						
NH_4^+ (µmol kg-1 DW)	137.01	90.69	141.28	54.48	45.17	35.20
NO_3^- (µmol kg-1 DW)	52.95	166.89	75.52	47.75	36.43	29.74

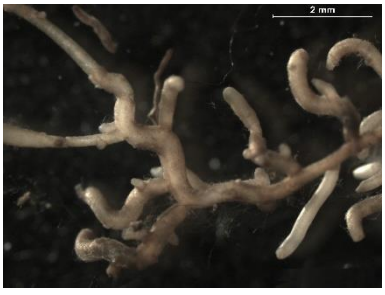
Supplement Table S4.2: Molecular identification and morphological characterization of ectomycorrhizal fungi species associated with European beech (*Fagus sylvatica*). Best match indicates the accession number in the NCBI database obtained by BLAST search of the nucleotide sequence. No of bases and % similarity indicates independent of query sequence length and base-pair size and percent sequence identity. NCBI No = NCBI accession number under which the sequences can be found in the NCBI Genbank database.

Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Lactarius subdulcis  NCBI No: MN947338	HM189800	756/758 99%	Monopodial-pinate ramification, brown, nest-like in abundance, rhizomorphs absence, hydrophobicity absents, straight and cylindric ends shape, cortical cell not visible, mantel surface visibility present, mantel dots smooth and light brownish, unramified ends mantel surface densely grainy, contact exploration type.	Spring, Summer, Autumn
Pezizales sp.1  NCBI No: MN947339	AJ969617.1	639/639 100%	Monopodial-pinate ramification, dark brown, rhizomorphs absent, unramified tapering end shape, visible and smooth cortical cells, short distance exploration type.	Spring
Tomentella coerulea  NCBI No: MN947340	MK431005.1	636/638 99%	Monopodial-pinate ramification, black, infrequent rhizomorphs, cylindric end shape, cortical cells not visible, hyphae most at the tip, short distance exploration type.	Spring, Autumn
Xerocomus cisalpinus  NCBI No: MN947341	HM190054.1	776/781 99%	Monopodial-pyramidal ramification, whitish, cylindric end shape, cortical cells not visible, silvery mantle surface, short distance exploration type.	Spring

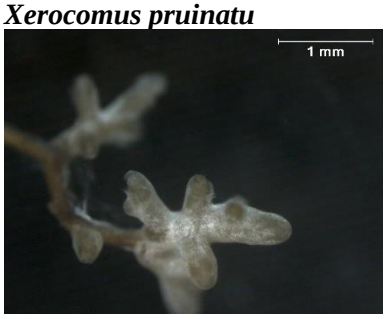
Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Laccaria amethystina  NCBI No: MN947342	HM189773.1	723/723 100%	Ramification absent, Whitish, rhizomorphs absent, tapering end shape, visible cortical cells, smooth ends mantle surface, short distance exploration type.	Spring, Autumn
Genea hispidula  NCBI No: MN947343	HM189754.1	676/677 99%	Dichotomous ramification, dark brown, rhizomorph absent, straight and cylindric end shape, invisible cortical cells, smooth mantle surface, hyphae not specifically distribute, short distance exploration type.	Spring, Autumn
Inocybe maculata  NCBI No: MN947344	MN947344.1	615/615 99%	Monopodial-pyramidal ramification, brownish, rhizomorph absent, cylindric end shape, cortical cells not visible, mantel surface cover with soil particle, short distance exploration type.	Spring
Tomentella bryophila  NCBI No: MN947345	KM576657.1	627/630 99%	Irregularly-pinnate ramification, brownish, rhizomorph absent, cylindrical end shape, smooth and shiny mantle surface, contact exploration type.	Spring
Hebeloma velutipes  NCBI No: MN947346	MH931275.1	729/730 99%	Irregularly-pinnate ramification, whitish, abundant rhizomorph, sinuous end shape, concentrated proximally hyphae distribution, densely woolly mantle surface, short distance exploration type.	Spring

Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Pachyphlodes conglomerata  NCBI No: MN947347	JN102487.1	1230/1230 100%	Monopodial-pyramidal ramification, whitish and nest-like in abundance, fan-like rhizomorph present, not specifically hyphae distribution, densely woolly and shiny mantle surface, visible cortical cells, straight and sinuous end shape, short distance exploration type.	Spring, Autumn
Humaria hemisphaerica  NCBI No: MN947348	KT692924.1	685/690 99%	Monopodial-pinnate ramification, dark brown, nest-like in abundance, rhizomorph present, not specifically distributed hyphae. Smooth mantle surface, cylindric end shape, cortical cells not visible, short distance exploration type.	Spring
Clavulina cristata  NCBI No: MN947349	EU862223.1	720/724 99%	Irregularly-pinnate ramification, whitish, rhizomorph absent, tortuous end shape, visible cortical cells, silvery mantle surface, short distance exploration type.	Spring
Byssocorticium sp.  NCBI No: MN947350	KM576308.1	669/672 99%	Ramification absent, black, solitary or in small numbers in abundance, rhizomorph absent, cylindric end shape, cortical cells not visible, densely cottony and silvery mantle surface, short distance exploration type.	Spring
Russula solaris  NCBI No: MN947351	AF418627.1	697/698 99%	Dichotomous ramification, yellowish, nest-like in abundance, rhizomorph absent, sinuous end shape, visible cortical cells, not specifically distributed hyphae, shiny mantle ends dot, short distance exploration type.	Spring. Autumn

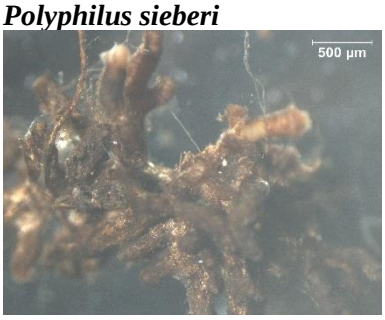
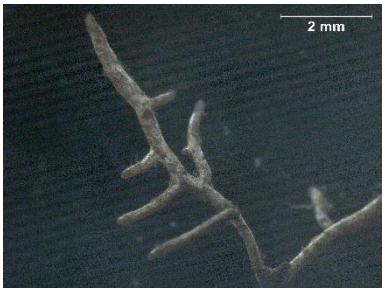
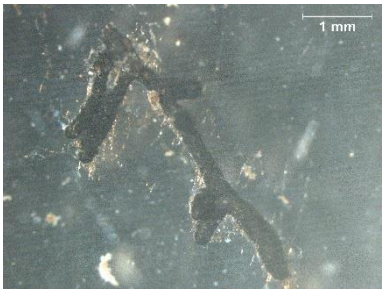
Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Russula mairei  NCBI No: MN947352	AF418620.1	711/713 99%	Monopodial-pyramidal ramification, whitish, nest-like in abundance, infrequent rhizomorph, inflated end shape, cortical cells not visible, densely grainy or warty and rough mantel surface, contact exploration type.	Spring. Summer
Lactarius blennius  NCBI No: MN947353	AY606944.1	730/734 99%	Monopodial-pyramidal ramification, whitish, nest-like in abundance, infrequent rhizomorph, not-inflated ends shape, cortical cells visible, smooth and shiny mantle surface, contact exploration type.	Spring, Summer, Autumn
Pseudotomentella tristis  NCBI No: MN947354	AF274771.1	658/660 99%	Irregularly-pinnate ramification, grey, rhizomorph present, sinuous ends shape, cortical cells not visible, not specifically and densely woolly distributed hyphae and covered with soil particles, short distance exploration type.	Spring
Tricholoma sulphureum  NCBI No: MN947355	LT000056.1	709/710 99%	Monopodial-pinnate ramification type, brownish, abundant in rhizomorph, straight end shape, cortical cells not visible, densely woolly and silvery mantle surface, medium distance exploration type.	Spring
Pezizaceae sp.  NCBI No: MN947356	KM576471.1	643/644 99%	Monopodial-pinnate, brownish, rhizomorph presence and fan-like, ends shape-constricted between older and younger parts, cortical cells not visible, not specifically distributed hyphae, orange ends dots, short distance exploration type.	Spring

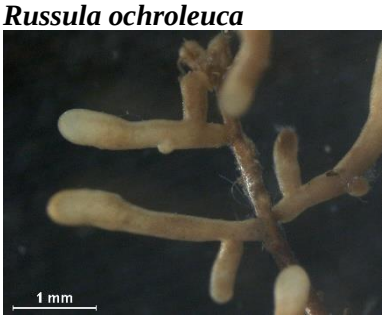
Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Ilyonectria pseudodestructans  NCBI No: MN947357	JF735291.1	561/561 100%	Irregularly-pinnate ramification, dark brown, dense in abundance, rhizomorph absence, cylindric ends shape, cortical cells not visible, not specifically distributed hyphae, smooth mantle surface, short distance exploration type.	Spring
Tomentella sublilacina  NCBI No: MN947358	KC952702.1	670/680 99%	Irregularly-pinnate ramification, rhizomorph absence, cylindric ends shape, cortical cells not visible, whitish dots on the mantle ends, short distance exploration type.	Spring
Russula paludosa  NCBI No: MN947359	KT934000.1	714/715 99%	Monopodial-pinnate ramification, rhizomorph absence, cylindric ends shape, cortical cells not visible, densely grainy and rough mantle surface, contact exploration type.	Spring
Tuber puberulum  NCBI No: MN947360	AJ969625.1	561/562 99%	Monopodial-pyramidal ramification, black, abundant, rhizomorph absence, straight and cylindric ends shape, cortical cells not visible, densely grainy and glistening mantle surface, short distance exploration type.	Spring, Autumn
Tomentella ramosissima  NCBI No: MN947361	U83480.1	669/674 99%	Irregularly-pinnate ramification, whitish, nest-like abundance, rhizomorph absence, tortuous ends shape, visible cortical cells, smooth and shiny mantle surface, contact exploration type.	Spring

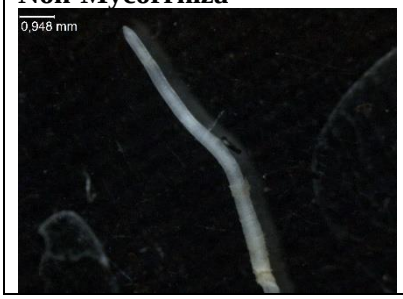
Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Inocybe petiginosa  NCBI No: MN947362	HQ586862.1	611/613 99%	Monopodial-pyramidal ramification, whitish, solitary in abundance, rhizomorph absence, cylindric ends shape, visible cortical cells, smooth mantle surface, contact exploration type.	Spring, Autumn
Xerocomus chrysenteron  NCBI No: MN947363	HQ207691.1	761/769 99%	Irregularly-pinnate ramification, rhizomorph absence, cylindric ends shape, cortical cells not visible, silvery mantle surface with brown ends dots, short distance exploration type.	Spring
Pachyphlodes nemoralis  NCBI-No: MN947364	JN102486.1	708/710 99%	Monopodial-pinnate ramification, whitish, rhizomorph absence, straight ends shape, cortical cells not visible, mantle surface- densely grainy and covered with soil particles, short distance exploration type.	Spring
Tomentella stuposa  NCBI No: MN947365	KY693712.1	680/682 99%	Monopodial-pyramidal ramification, brown, nest-like in abundance, rhizomorph absence, straight and cylindrical ends shape, cortical cells not visible, rough mantle surface, short distance exploration type.	Spring, Summer, Autumn
Tomentella lilacinogrisea  NCBI No: MN947366	KM576631.1	659/660 99%	Ramification absence, single, black, solitary abundance, rhizomorph absence, cylindric ends shape, not specifically distributed hyphae. Rough mantle surface, short distance exploration type.	Spring

Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Xerocomus pruinatu  NCBI No: MN947367	HM190089.1	790/794 99%	Monopodial-pyramidal ramification, whitish, solitary in abundance, rhizomorph absence, cylindric ends shape, cortical cells not visible, silvery mantel surface, contact exploration type.	Spring, Summer, Autumn
Helotiales sp.1  NCBI No: MN947368	MK431012.1	596/602 99%	Monopodial-pinnate ramification, brown, solitary in abundance, rhizomorph absence, sinuous ends shape, cortical cells not visible, densely woolly mantel surface, short distance exploration type.	Spring
Uncultured Thelephoraces sp.  NCBI No: MN947370	U83466.1	656/659 99%	Single, brown, solitary or small numbers in abundance, rhizomorphs absence, cortical cells not visible, mantle surface not visible, not specifically distributed hyphae, short distance exploration type.	Spring
Russula integriformis  NCBI No: MN947372	KP783458.1	706/730 97%	Ramification absence, black, solitary or small in numbers in abundance, rhizomorph absence, cylindric ends shape, cortical cells not visible, mantel surface are not smooth, contact exploration type.	Summer
Pachyphlodes melanoxanthus  NCBI No: MN947373	JX414223.1	685/687 99%	Coralloid ramification, brownish, infrequent rhizomorph, cylindric ends shape, cortical cells not visible, densely grainy and covered with soil particles, short distance exploration type.	Summer

Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Tomentella viridula  NCBI No: MN947374	HF675488.1	670/670 100%	Dichotomous ramification, brown, infrequent rhizomorph presence, cylindric ends shape, cortical cells not visible, shiny browns dots in mantel ends, short distance exploration type.	Summer
Inocybe griseolilacina  NCBI No: MN947375	AM882728.2	729/738 99%	Ramification absence, whitish, solitary or small numbers in abundance, rhizomorph absence, cylindric ends shape, cortical cells not visible, silvery mantle surface, contact exploration type.	Summer
Sebacina flagelliformis  NCBI No: MN947376	KF000463.1	635/636 99%	Single or ramification absence, black, solitary or small numbers in abundance, rhizomorph presence, cylindric ends shape, cortical cells not visible, densely woolly mantel surface, short distance exploration type.	Summer
Inocybe splendens  NCBI No: MN947377	JF908119.1	728/730 99%	Monopodial-pyramidal ramification, brownish, nest-like in abundance, rhizomorph absence, cylindric and bent ends shape, visible cortical cells, smooth and transparent mantle surface, contact exploration type.	Summer
MT 13 =Pezizales sp.2  NCBI No: MN947378	KM576477.1	602/623 97%	Dichotomous ramification, whitish, rhizomorph absence, tapering ends shape, cortical cells not visible, silvery mantel surface, contact exploration type.	Summer

Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Polyphilus sieberi  NCBI No: MN947379	MG719688.1	573/580 97%	coralloid ramification, brownish, rhizomorphs presence, cylindric ends shape, cortical cells not visible, mantel surface are not smooth, short distance exploration type.	Summer
Paxillus involutus  NCBI No: MN947380	GQ389624.1	754/755 99%	Single or ramification absence, silvery, solitary or small numbers in abundance, rhizomorph absence, tapering ends shape, cortical cells are not visible, mantle surface are not smooth and silvery, short distance exploration type.	Summer, Autumn
Russula cerolens  NCBI No: MN947381	KX449208.1	693/708 98%	Single or ramification absence, black, solitary or small numbers in abundance, rhizomorph presence, cylindric ends shape, cortical cells not visible, densely woolly mantel surface, short exploration type.	Summer
Tomentella punicea  NCBI No: MN947382	MK431003.1	677/679 99%	Monopodial pinnate ramification, brown, infrequent rhizomorph, cylindric ends shape, cortical cells not visible, not specifically distributed hyphae, slight silvery mantel surface, short distance exploration type.	Summer, Autumn
Cenococcum geophilum  NCBI No: MN947383	LC095192.1	1059/1059 100%	Single or ramification absence, black, solitary or small numbers in abundance, tapering ends shape, cortical cells not visible, mantel surface are not smooth, short distance exploration type.	Autumn

Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Russula ochroleuca  NCBI No: MN947384	KX449448.1	714/714 100%	Monopodial-pinnate ramification, brown, rhizomorph absence, inflated ends shape, cortical cells not visible, light brown ends dots of the mantle, contact exploration type.	Autumn
Tomentella sp.  NCBI No: MN947385	KU924841.1	670/670 100%	Single or ramification absence, brownish, rhizomorphs absence, sinuous ends shape, cortical cells not visible, not specifically distributed hyphae, light whitish mantle ends dots, short distance exploration type.	Autumn
Cortinarius casimiri  NCBI No: MN947386	HQ604710.1	616/618 99%	Single or ramification absence, whitish, rhizomorph absence, bent ends shape, cortical cells not visible, silvery and densely woolly mantle surface, medium distance exploration type.	Autumn
Helotiales sp.2  NCBI No: MN947387	MG820050.1	515/515 100%	Single and monopodial pinnate ramification, brownish, rhizomorphs absence, cylindric ends shape, cortical cells not visible, densely grainy mantle surface, contact exploration type.	Autumn
Xerocomus badius  NCBI No: MN947388	HM190022.1	628/628 100%	Monopodial-pyramidal ramification, whitish, rhizomorph absence, cylindric ends shape, cortical cells not visible, silvery mantle surface, short distance exploration type.	Autumn

Ectomycorrhizal fungi species (NCBI accession No.)	Best match in NCBI	No. of bases % similarity	Species description	Presence (Months)
Inocybe cf. Microspora  NCBI No: MN947389	AM882808.2	716/722 99%	Monopodial-pyramidal ramification, brown, rhizomorph absence, cylindric ends shape, cortical cells not visible, smooth mantle surface, contact exploration type.	Autumn
Sebacina incrustans  NCBI No: MN947392	KF000440.1	618/619 99%	Single or ramification absence, brownish, rhizomorph absence, ends shape-cylindric and constricted between older and younger parts, cortical cells not visible, contact exploration type.	Autumn
Non-Mycorrhiza 				Spring, Summer, Autumn

Supplement Table S4.3: Chemical properties in ectomycorrhizal root tips (EMRTs), fine roots (fine), coarse roots (coarse), transport and whole root segments. HAI = Hainich-Dun, SCH = Schorfheide-Chorin, NA = not available. Root chemistry: C = Carbon, N = Nitrogen, $^{15}N\text{-}NH_4^+$ = labelled with NH_4^+ and $^{15}N\text{-}NO_3^-$ = labelled with NO_3^-. Data are means of n = 30 ± SE. Linear mixed-effect models were used to compare the means of each variable with either season (spring, summer and autumn) or treatment groups ($^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$) as a random factor. Kruskal-Wallis test was used to compare the means of the C : N ratio. Significant differences at $p \leq 0.05$ are indicated with bold letters.

Variables	Units	HAI			SCH			p values		
		Spring	Summer	Autumn	Spring	Summer	Autumn	HAI - SCH	HAI (season)	SCH (season)
EMRTs										
C	mg g^{-1} DW	434.8 ± 6.81	NA	432.54 ± 8.22	456.36 ± 4.53	NA	415.03 ± 15.59	0.763	0.787	**0.006**
N	mg g^{-1} DW	27.81 ± 0.87	22.72 ± 0.84	26.03 ± 0.57	30.64 ± 0.77	24.18 ± 1.19	28.21 ± 0.88	0.109	**<0.001**	**0.001**
C:N		16.14 ± 0.38	NA	16.73 ± 0.33	15.45 ± 0.25	NA	15.38 ± 0.88	**0.018**	0.191	0.231
$^{13}C/^{12}C$	(‰)	-29.83 ± 0.21	NA	-31.26 ± 0.15	-29.23 ± 0.39	NA	-29.69 ± 0.23	**<0.001**	**<0.001**	0.325
$^{15}N\text{-}NH_4^+$	µg g^{-1} DW	561.89 ± 31.44	257.64 ± 21.92	472.35 ± 18.38	699.62 ± 40.52	317.61 ± 32.06	432.51 ± 56.65	0.438	**<0.001**	**<0.001**
$^{15}N\text{-}NO_3^-$	µg g^{-1} DW	97.65 ± 13.15	14.86 ± 2.3	78.96 ± 11.12	102.02 ± 18.12	39.19 ± 7.21	49.63 ± 12.01	0.895	**<0.001**	**0.024**
Fine										
C	mg g^{-1} DW	473.35 ± 2.53	NA	467.21 ± 4.19	483.9 ± 4.36	NA	429.14 ± 17.73	0.075	0.186	**0.004**
N	mg g^{-1} DW	16.87 ± 0.42	12.89 ± 0.35	14.97 ± 0.35	18.35 ± 0.35	14.19 ± 0.46	16.11 ± 0.54	**<0.001**	**<0.001**	**<0.001**
C:N		28.81 ± 0.67	NA	31.65 ± 0.74	26.95 ± 0.5	NA	27.38 ± 1.46	**<0.001**	**0.007**	0.695
$^{13}C/^{12}C$	(‰)	-32.46 ± 0.25	NA	-30.2 ± 0.19	-30.71 ± 0.3	NA	-29.96 ± 0.43	**0.002**	**<0.001**	**<0.001**
$^{15}N\text{-}NH_4^+$	µg g^{-1} DW	221.66 ± 13.62	129.49 ± 6.91	184.79 ± 13.22	242.35 ± 20.18	158.79 ± 13.34	173.29 ± 15.34	0.466	**<0.001**	**0.006**
$^{15}N\text{-}NO_3^-$	µg g^{-1} DW	50.06 ± 6.76	9.91 ± 0.97	37.69 ± 6.32	48.92 ± 8.76	21.6 ± 4.09	26.39 ± 6.04	0.616	**<0.001**	**0.048**
Coarse										
C	mg g^{-1} DW	477.54 ± 2.01	NA	469.84 ± 1.39	476.04 ± 12.74	NA	432.98 ± 15.82	**0.041**	**0.001**	0.128
N	mg g^{-1} DW	13.22 ± 0.35	8.29 ± 0.31	10.65 ± 0.34	13.25 ± 0.4	8.59 ± 0.41	10.87 ± 0.45	0.716	**<0.001**	**<0.001**
C:N		36.78 ± 0.98	NA	45.17 ± 1.17	37.22 ± 1.44	NA	41.84 ± 2.42	0.159	**<0.001**	0.205
$^{13}C/^{12}C$	(‰)	-32.4 ± 0.28	NA	-28.3 ± 0.11	-30.71 ± 0.35	NA	-30.86 ± 0.32	0.178	**<0.001**	**0.011**
$^{15}N\text{-}NH_4^+$	µg g^{-1} DW	113.62 ± 12.41	47.9 ± 4.54	90.77 ± 10.47	104.73 ± 11.53	49.87 ± 6.16	67.87 ± 9.34	0.143	**<0.001**	**<0.001**
$^{15}N\text{-}NO_3^-$	µg g^{-1} DW	36.47 ± 4.86	7.64 ± 0.6	22.14 ± 3.1	29.15 ± 6.65	12.76 ± 1.43	11.15 ± 1.98	0.076	**<0.001**	0.373

Supplement Table S4.3 (continued)

Variables	Units	HAI	SCH	p values						
		Spring	Summer	Autumn	Spring	Summer	Autumn	HAI - SCH	HAI (season)	SCH (season)
Transport										
C	mg g^{-1} DW	431.56 ± 7.92	NA	440.29 ± 8.14	457.46 ± 5.8	NA	418.58 ± 23	**0.032**	0.501	**0.033**
N	mg g^{-1} DW	11.13 ± 0.37	7.19 ± 0.39	8.08 ± 0.35	11.75 ± 0.46	9.2 ± 0.49	9.5 ± 0.57	**<0.001**	**<0.001**	**<0.001**
C:N		39.98 ± 1.5	NA	57.19 ± 2.49	41.08 ± 1.97	NA	47.4 ±3.42	0.031	**<0.001**	0.177
$^{13}C/^{12}C$	(‰)	-32.29 ± 0.25	NA	-29.44 ± 0.4	-30.79 ± 0.33	NA	-29.73 ±0.4	0.063	**<0.001**	**<0.001**
$^{15}N\text{-}NH_4^+$	µg g^{-1} DW	31.78 ± 4.08	13.07 ± 3.08	11.47 ± 1.78	20.74 ± 4.66	23.72 ± 3.27	15.1 ± 2.21	0.494	**0.001**	0.327
$^{15}N\text{-}NO_3^-$	µg g^{-1} DW	12.38 ± 1.32	6.82 ± 1.14	3.84 ± 1.16	13.98 ± 3.1	15.01 ± 1.5	5.22 ± 1.43	**0.015**	**<0.001**	**<0.001**
Whole root										
$^{15}N\text{-}NH_4^+$	µg g^{-1} DW	105.13 ± 8.9	50.71 ± 5.11	101.05 ± 8.69	116.46 ± 10.56	65.08 ± 7.42	92.74 ± 7.8	0.428	**<0.001**	**<0.001**
$^{15}N\text{-}NO_3^-$	µg g^{-1} DW	30.25 ± 3.03	7.83 ± 0.72	20.89 ± 2.81	30.18 ± 6.41	15.9 ± 1.74	14.58 ± 3.16	0.763	**<0.001**	**0.033**

Supplement Table S4.4: Mean ^{15}N enrichment (µg g^{-1} DW) of different ectomycorrhizal fungi (EMF) species in different seasons after exposure either with NH_4^+ or NO_3^-. EMF species ordered according to their mean relative abundance (RA) in different season separately. Data are shown as means ± SE (n = 3-5). Kruskal-Wallis test was used to compare the RA. Linear mixed-effect model was used with regions as a random factor to compare the means ^{15}N enrichment among EMF species.

			Mean ^{15}N enrichment		
EMF species	**Season**	**RA (%)**	**^{15}N-NH_4^+**	**^{15}N-NO_3^-**	**$^{15}NH_4^+$: $^{15}NO_3^-$**
Lactarius subdulcis	Spring	28.3	704.6 ± 14.0	30.8 ± 0.3	22.9
Lactarius blennius	Spring	12.2	748.9 ± 4.4	76.9 ± 2.2	9.7
Tomentella coerulea	Spring	7.3	433.9 ± 0.8	53.9 ± 0.5	8.0
Tomentella bryophila	Spring	5.2	420.2 ± 2.6	195.8 ± 0.8	2.1
Russula paludosa	Spring	4.3	451.7 ± 2.7	246.9 ± 1.4	1.8
Russula mairei	Spring	3.8	489.3 ± 0.9	59.6 ± 0.5	8.2
Humaria hemisphaerica	Spring	3.5	566.4 ± 5.0	287.8 ± 2.1	2.0
Inocybe petiginosa	Spring	2.9	447.0 ± 2.2	320.1 ± 1.0	1.4
Helotiales sp. 1	Spring	2.6	756.3 ± 27.6	1086.0 ± 3.0	0.7
Genea hispidula	Spring	2.4	446.8 ± 0.6	171.3 ± 0.1	2.6
Pachyphlodes conglomerata	Spring	2.1	424.2 ± 21.7	32.3 ± 0.1	13.1
Pseudotomentella tristis	Spring	1.9	452.7 ± 3.5	127.9 ± 2.2	3.5
Hebeloma velutipes	Spring	1.9	541.4 ± 4.9	48.6 ± 0.4	11.1
Pezizales sp. 1	Spring	1.8	466.7 ± 60.2	66.4 ± 17.7	7.0
Tomentella lilacinogrisea	Spring	1.8	730.4 ± 3.6	151.1 ± 1.6	4.8
Pezizaceae sp.	Spring	1.7	712.5 ± 9.2	86.4 ± 2.5	8.2
Xerocomus cisalpinus	Spring	1.6	917.5 ± 0.1	65.0 ± 1.0	14.1
Tricholoma sulphureum	Spring	1.5	433.0 ± 1.1	187.4 ± 2.4	2.3
Byssocorticium sp.	Spring	1.4	180.4 ± 0.3	146.5 ± 1.6	1.2
Inocybe maculata	Spring	1.4	1070.4 ± 8.2	37.9 ± 0.4	28.3
Clavulina cristata	Spring	1.3	1366.2 ± 2.2	42.2 ± 0.3	32.4
Xerocomus chrysenteron	Spring	1.2	1030.3 ± 5.8	117.0 ± 1.3	8.8
Tuber puberulum	Spring	1.2	771.2 ± 6.7	149.6 ± 2.5	5.2
Ilyonectria pseudodestructans	Spring	1.1	943.3 ± 10.5	237.4 ± 1.7	4.0
Tomentella stuposa	Spring	1.1	788.5 ± 2.2	131.8 ± 1.2	6.0
Tomentella ramosissima	Spring	1.0	1150.8 ± 22.2	57.5 ± 0.7	20.0
Laccaria amethystina	Spring	0.9	713.2 ± 0.6	125.3 ± 1.0	5.7
Russula solaris	Spring	0.6	475.3 ± 2.3	120.6 ± 0.3	3.9
Pachyphlodes nemoralis	Spring	0.6	596.7 ± 3.1	127.0 ± 0.8	4.7
Xerocomus pruinatus	Spring	0.5	668.5 ± 7.1	258.6 ± 1.6	2.6
Tomentella sublilacina	Spring	0.5	790.2 ±3.3	67.0 ± 0.3	11.8
Uncultured Thelephoraceae	Spring	0.5	547.5 ± 3.6	76.5 ± 0.1	7.2
F Value		***3.1531***	***56.489***	***43.645***	***21.759***
P Value		***<0.001***	***0.01754***	***<0.001***	***<0.001***
Lactarius subdulcis	Summer	32.0	527.1 ± 41.7	44.5 ± 6.6	11.9
Inocybe splendens	Summer	12.8	537.4 ± 0.8	18.9 ± 0.1	28.5

Supplement Table S4.4 (continued)

		Mean ^{15}N enrichment			
EMF species	**Season**	**RA (%)**	**^{15}N-NH_4^+**	**^{15}N-NO_3^-**	**$^{15}NH_4^+$: $^{15}NO_3^-$**
Lactarius blennius	Summer	10.6	524.0 ± 2.8	52.4 ± 0.4	10.0
Tomentella stuposa	Summer	10.2	385.6 ± 6.7	21.4 ± 0.2	18.0
Pezizales sp. 2	Summer	5.3	187.4 ± 0.8	13.4 ± 0.1	14.0
Tomentella viridula	Summer	4.7	476.3 ± 4.0	8.4 ± 0.1	56.7
Polyphilus sieberi	Summer	4.1	142.0 ± 0.6	2.6 ± 0.1	54.7
Sebacina flagelliformis	Summer	3.2	576.4 ± 3.1	114.6 ± 0.6	5.0
Paxillus involutus	Summer	3.2	524.2 ± 1.6	51.0 ± 0.2	10.3
Russula integriformis	Summer	2.9	212.9 ± 20.6	10.1 ± 0.3	21.1
Russula mairei	Summer	2.6	534.5 ± 6.9	23.2 ± 0.3	23.0
Pachyphlodes melanoxanthus	Summer	2.4	569.4 ± 1.2	22.8 ± 0.2	24.9
Tomentella punicea	Summer	2.0	385.0 ± 6.4	225.2 ± 1.9	1.7
Inocybe griseolilacina	Summer	1.2	678.8 ± 2.1	78.7 ± 1.8	8.6
Xerocomellus pruinatus	Summer	1.1	441.7 ± 12.0	50.0 ± 15.8	8.8
Russula cerolens	Summer	0.9	522.5 ± 2.6	37.3 ± 0.6	14.0
Pezizales sp. 1	Summer	0.4	443.0 ± 2.2	10.7 ± 0.2	41.5
MTSU6	Summer	0.3	437.7 ± 0.9	93.5 ± 0.9	4.7
F Value		***3.8064***	***240.21***	***7.9429***	***229.69***
P Value		***<0.001***	***<0.001***	***<0.001***	***<0.001***
Lactarius subdulcis	Autumn	22.9	680.0 ± 23.0	42.7 ± 5.3	15.9
Cenococcum geophilum	Autumn	10.8	302.2 ± 11.0	76.2 ± 23.5	4.0
Lactarius blennius	Autumn	10.0	812.9 ± 29.9	132.0 ± 10.3	6.2
Pachyphlodes conglomerata	Autumn	8.6	512.7 ± 49.7	153.1 ± 8.1	3.3
Tomentella coerulea	Autumn	7.4	155.9 ± 23.6	51.7 ± 3.3	3.0
Tomentella stuposa	Autumn	6.8	504.8 ± 83.1	442.3 ± 80.3	1.1
Xerocomus pruinatus	Autumn	4.2	452.8 ± 41.2	197.5 ± 23.4	2.3
Russula solaris	Autumn	3.6	463.1 ± 22.0	82.5 ± 12.0	5.6
Genea hispidula	Autumn	3.5	901.2 ± 86.8	116.5 ± 20.0	7.7
Cortinarius casimiri	Autumn	3.2	534.2 ± 8.9	22.1 ± 5.9	24.2
Russula ochroleuca	Autumn	2.7	754.7 ± 67.5	115.8 ± 6.4	6.5
Tomentella punicea	Autumn	2.5	813.5 ± 138.0	124.2 ± 5.1	6.6
Inocybe petiginosa	Autumn	2.2	842.4 ± 23.7	76.5 ± 14.3	11.0
Tomentella sp.	Autumn	1.8	530.0 ± 88.4	125.8 ± 36.3	4.2
Paxillus involutus	Autumn	1.4	536.9 ± 64.0	23.6 ± 4.6	22.7
Xerocomus badius	Autumn	1.4	368.3 ± 16.1	91.5 ± 23.6	4.0
Sebacina incrustans	Autumn	1.3	502.6 ± 37.9	55.5 ± 7.9	9.1
Uncultured Thelephoraceae	Autumn	1.3	991.3 ± 100.9	313.9 ± 63.1	3.2
Laccaria amethystina	Autumn	1.2	599.0 ± 47.8	205.3 ± 22.9	2.9
Inocybe cf. microspora	Autumn	0.9	958.7 ± 28.4	224.4 ± 16.1	4.3
Tuber puberulum	Autumn	0.9	727.2 ± 10.7	66.8 ± 8.3	10.9
MTAU37	Autumn	0.9	654.2 ± 40.7	58.0 ± 1.7	11.3
Helotiales sp. 2	Autumn	0.7	462.8 ± 47.2	20.5 ± 18.3	22.6
F Value		***2.6514***	***17.091***	***7.9429***	***6.759***
P Value		***<0.001***	***<0.001***	***<0.001***	***<0.001***

Supplement Table S4.5: Nutrient concentrations in different ectomycorrhizal fungi (EMF) species. Carbon = C, Nitrogen = N. EMF species ordered according to their mean relative abundance (RA) in different season separately. Data are shown as means ± SE (n = 3-5). Kruskal-Wallis test was used to compare the means of RA, C : N and $^{13}C/^{12}C$ among EMF species. Linear mixed-effect models were used to compare the means of C and N among the EMF species with regions as a random factor.

ECMF species	RA (%)	$^{13}C/^{12}C$ (‰)	C (mg g^{-1} DW)	N (mg g^{-1} DW)	C:N
Spring					
Lactarius subdulcis	28.3	-29.92 ± 0.54	467.25 ± 2.83	33.8 ± 0.57	13.85 ± 0.31
Lactarius blennius	12.2	-27.29 ± 0.18	454.41 ± 5.45	34.18 ± 0.49	13.32 ± 0.34
Tomentella coerulea	7.3	-29.07 ± 0.26	429.9 ± 6.29	24.21 ± 2.05	18.31 ± 1.32
Tomentella bryophila	5.2	-28.96 ± 0.22	428.17 ± 2.16	31.05 ± 2.43	14.2 ± 1.05
Russula paludosa	4.3	-29.67 ± 0.07	428.51 ± 13.36	36.27 ± 2.07	11.9 ± 0.32
Russula mairei	3.8	-28.71 ± 0.41	447.69 ± 3.66	32.19 ± 0.42	13.91 ± 0.08
Humaria hemisphaerica	3.5	-28.68 ± 0.19	450.21 ± 1.56	30.91 ± 1.11	14.65 ± 0.49
Inocybe petiginosa	2.9	-27.96 ± 0.34	447.17 ± 2.26	33.74 ± 0.21	13.26 ± 0.15
Helotiales sp. 1	2.6	-30.05 ± 0.03	445.07 ± 9.35	29.6 ± 1.96	15.27 ± 0.69
Genea hispidula	2.4	-29.76 ± 0.22	424.27 ± 7.96	30 ± 0.22	14.14 ± 0.16
Pachyphlodes conglomerata	2.1	-27.5 ± 0.47	393.81 ± 16.44	35.62 ± 1.88	11.09 ± 0.16
Pseudotomentella tristis	1.9	-28.21 ± 0.39	416.68 ± 2.03	27.59 ± 0.46	15.12 ± 0.29
Hebeloma velutipes	1.9	-29.45 ± 0.58	424.71 ± 1.09	24.24 ± 1.47	17.84 ± 1.05
Pezizales sp. 1	1.8	-29.52 ± 0.61	456.95 ± 3.8	26.87 ± 1	17.31 ± 0.76
Tomentella lilacinogrisea	1.8	-29.14 ± 0.31	446.57 ± 4.16	29.4 ± 1.98	15.59 ± 1.19
Pezizaceae sp.	1.7	-29.23 ± 0.47	446.03 ± 1.41	27.32 ± 2.75	17.17 ± 1.68
Xerocomus cisalpinus	1.6	-26.93 ± 0.5	453.13 ± 2.93	34.52 ± 2.89	13.64 ± 1.23
Tricholoma sulphureum	1.5	-27.23 ± 0.02	466.25 ± 9.87	27.89 ± 3.6	18.47 ± 2.72
Byssocorticium sp.	1.4	-28.05 ± 0.11	451.83 ± 11.06	25.74 ± 2.35	18.52 ± 2.12
Inocybe maculata	1.4	-28.87 ± 0.97	423.43 ± 11.53	29.98 ± 3.83	15.11 ± 1.55
Clavulina cristata	1.3	-29.44 ± 1.06	448.23 ± 0.9	29.58 ± 2.83	15.87 ± 1.49
Xerocomus chrysenteron	1.2	-28.78 ± 0.2	422.26 ± 1.09	34.07 ± 2.44	12.67 ± 0.95
Tuber puberulum	1.2	-27.23 ± 0.37	461.71 ± 1.22	33.8 ± 0.35	13.67 ± 0.12
Ilyonectria pseudodestructans	1.1	-29.72 ± 0.37	460.55 ± 2.39	36.86 ± 0.43	12.51 ± 0.2
Tomentella stuposa	1.1	-28.66 ± 0.16	435.67 ± 8.43	31.59 ± 0.12	13.79 ± 0.26
Tomentella ramosissima	1.0	-28.87 ± 0.39	456.09 ± 2.59	33.71 ± 0.3	13.53 ± 0.11
Laccaria amethystina	0.9	-27.82 ± 0.67	429.01 ± 8.28	28.63 ± 0.63	14.99 ± 0.05
Russula solaris	0.6	-30.01 ± 0.32	443.9 ± 4.59	36.79 ± 0.64	12.07 ± 0.09
Pachyphlodes nemoralis	0.6	-28.45 ± 0.43	435.06 ± 2.91	35.92 ± 0.75	12.13 ± 0.18
Xerocomus pruinatus	0.5	-29.76 ± 0.21	427.91 ± 3.95	34.57 ± 0.26	12.38 ± 0.13
Tomentella sublilacina	0.5	-29.07 ± 0.07	410.51 ± 2.8	29.65 ± 0.75	13.9 ± 0.45
Uncultured Thelephoraceae	0.5	-29.16 ± 1.22	460.24 ± 5.2	34.86 ± 1.97	13.37 ± 0.61
F value		***3.1531***	***7.1757***	***4.3369***	***4.7619***
P value		***<0.001***	***<0.001***	***<0.001***	***<0.001***
Summer					
Lactarius subdulcis	32.0	-29.78 ± 0.44	416.09 ± 9.05	27.47 ± 1.29	15.56 ± 0.9
Inocybe splendens	12.8	-29.96 ± 0.23	370.76 ± 4.3	23.27 ± 0.66	15.97 ± 0.27

Supplement Table S4.5 (continued)

ECMF species	RA (%)	$^{13}C/^{12}C$ (‰)	C (mg g^{-1} DW)	N (mg g^{-1} DW)	C:N
Lactarius blennius	10.6	-29.74 ± 0.29	441.62 ± 9.15	27.86 ± 0.83	15.88 ± 0.17
Tomentella stuposa	10.2	-30.00 ± 0.33	375.06 ± 1.57	23.42 ± 0.87	16.13 ± 0.61
Pezizales sp. 2	5.3	-31.31 ± 0.17	413.53 ± 7.75	20.31 ± 1.03	20.53 ± 0.68
Tomentella viridula	4.7	-31.63 ± 0.26	465.59 ± 2.71	22.43 ± 0.34	20.78 ± 0.43
Polyphilus sieberi	4.1	-29.44 ± 0.63	346.5 ± 7.29	16.85 ± 0.25	20.55 ± 0.14
Sebacina flagelliformis	3.2	-30.11 ± 0.01	452.21 ± 1.2	22.97 ± 0.4	19.72 ± 0.39
Paxillus involutus	3.2	-28.77 ± 0.06	434.81 ± 7.15	28 ± 0.5	15.58 ± 0.53
Russula integriformis	2.9	-32.03 ± 0.4	460.7 ± 8.8	22.77 ± 1.49	20.7 ± 1.43
Russula mairei	2.6	-28.16 ± 0.55	456.87 ± 1.03	29.59 ± 1.13	15.55 ± 0.59
Pachyphlodes melanoxanthus	2.4	-30.54 ± 0.32	360.37 ± 45.32	24.71 ± 3.44	14.74 ± 0.22
Tomentella punicea	2.0	-29.47 ± 0.69	449.02 ± 3.51	26.52 ± 1.31	17.17 ± 0.99
Inocybe griseolilacina	1.2	-29.58 ± 0.17	443.06 ± 3.27	29.95 ± 0.98	14.87 ± 0.61
Xerocomellus pruinatus	1.1	-28.92 ± 1.33	531.01 ± 81.05	47.42 ± 10.67	11.84 ± 0.94
Russula cerolens	0.9	-29.92 ± 0.75	425.28 ± 13.8	26.41 ± 2.53	16.62 ± 1.07
Pezizales sp. 1	0.4	-29.20 ± 0.37	396.55 ± 0.93	27.28 ± 0.56	14.56 ± 0.27
MTSU6	0.3	-29.18 ± 0.55	476.57 ± 3.48	26.87 ± 1.93	18.16 ± 1.18
F value		***3.8064***	***6.5852***	***7.858***	***10.188***
P value		***<0.001***	***<0.001***	***<0.001***	***<0.001***
Autumn					
Lactarius subdulcis	22.9	-27.8 ± 0.73	452.82 ± 5.98	29.22 ± 1.93	16.05 ± 1.07
Cenococcum geophilum	10.8	-30.82 ± 0.65	452.7 ± 9.48	23.77 ± 0.66	19.12 ± 0.61
Lactarius blennius	10.0	-30.56 ± 0.38	466.51 ± 8.33	30.76 ± 1.16	15.28 ± 0.5
Pachyphlodes conglomerata	8.6	-30.17 ± 0.22	425.57 ± 14.32	31.84 ± 1.51	13.55 ± 0.75
Tomentella coerulea	7.4	-31.47 ± 0.32	447.19 ± 10.46	21.44 ± 0.49	20.89 ± 0.43
Tomentella stuposa	6.8	-29.63 ± 0.31	478.2 ± 25.66	39.88 ± 2.3	12.03 ± 0.26
Xerocomus pruinatus	4.2	-28.09 ± 0.61	453.36 ± 9.57	29.14 ± 1.14	15.67 ± 0.41
Russula solaris	3.6	-30.62 ± 0.37	482.67 ± 8.07	28.6 ± 0.58	16.92 ± 0.48
Genea hispidula	3.5	-30.45 ± 0.64	449.98 ± 13.08	27.11 ± 1.51	16.8 ± 0.71
Cortinarius casimiri	3.2	-29.66 ± 0.59	414.1 ± 15.53	27.02 ± 1.77	16.15 ± 20
Russula ochroleuca	2.7	-29.98 ± 0.92	477.18 ± 5.84	34.37 ± 2.41	14.46 ± 1.18
Tomentella punicea	2.5	-29.83 ± 0.59	467.94 ± 34.81	32.47 ± 2.84	14.6 ± 0.69
Inocybe petiginosa	2.2	-30.4 ± 0.55	465.95 ± 10.66	30.4 ± 1.91	15.81 ± 1.16
Tomentella sp.	1.8	-29.39 ± 0.64	444.88 ± 13.54	28.05 ± 1.28	16.2 ± 0.87
Paxillus involutus	1.4	-29.53 ± 0.6	431.05 ± 12.63	27.48 ± 1.08	15.75 ± 0.28
Xerocomus badius	1.4	-29.7 ± 1.01	440.04 ± 11.96	26.51 ± 1.11	16.7 ± 0.52
Sebacina incrustans	1.3	-31.31 ± 0.36	442.82 ± 16.59	25.57 ± 1.6	17.48 ± 0.63
Uncultured Thelephoraceae	1.3	-28.84 ± 0.61	410.39 ± 22.57	29.07 ± 1.34	14.18 ± 0.83
Laccaria amethystina	1.2	-29.22 ± 0.36	450.01 ± 8.7	30.63 ± 1.76	15.07 ± 0.89
Inocybe cf. microspora	0.9	-30.41 ± 0.09	482.98 ± 2.82	34.21 ± 1.15	14.22 ± 0.51
Tuber puberulum	0.9	-29.76 ± 0.89	416.93 ± 42.38	30.8 ± 3.66	13.71 ± 0.52
MTAU37	0.9	-30.67 ± 0.29	475.14 ± 9.11	32.79 ± 0.95	14.6 ± 0.67
Helotiales sp. 2	0.7	-29.23 ± 0.33	457.88 ± 4.52	29.62 ± 1.96	15.93 ± 1.02
F value		***2.6514***	***1.8637***	***5.4979***	***4.9117***
P value		***<0.001***	***<0.001***	***<0.001***	***<0.001***

Supplement Table S4.6: Average length (cm) of the transport root segment. Data are shown as means ± SE (n = 5). Different letters denote significant differences of the means among the treatment groups ($^{15}N\text{-}NH_4^+$, $^{15}N\text{-}NO_3^-$, $NH_4^+NO_3^-$) of the respective season and plot.

Parameters	**Hainich-Dün (HAI)**			**Schorfheide-Chorin (SCH)**		
Plot numbers	HAI 7	HAI 20	HAI 22	SCH 6	SCH 43	SCH 44
Spring						
$^{15}N\text{-}NH_4^+$	6.44 ± 0.69 (a)	7.82 ± 2.12 (a)	9.20 ± 3.05 (a)	6.30 ± 2.02 (a)	7.28 ± 0.82 (a)	6.66 ± 1.72 (a)
$^{15}N\text{-}NO_3^-$	6.02 ± 1.60 (a)	7.40 ± 1.39 (a)	7.60 ± 1.21 (a)	7.78 ± 0.68 (a)	9.00 ± 3.03 (a)	8.60 ± 2.23 (a)
$NH_4^+NO_3^-$	6.68 ± 1.36 (a)	7.08 ± 1.45 (a)	7.62 ± 1.71 (a)	6.78 ± 2.18 (a)	9.44 ± 2.28 (a)	8.13 ± 1.29 (a)
Summer						
$^{15}N\text{-}NH_4^+$	7.10 ± 2.07 (a)	6.31 ± 2.99 (a)	7.72 ± 3.36 (a)	7.48 ± 2.30 (a)	8.64 ± 2.33 (a)	6.60 ± 1.11 (a)
$^{15}N\text{-}NO_3^-$	5.82 ± 1.27 (a)	5.44 ± 1.40 (a)	7.34 ± 0.30 (a)	6.88 ± 1.34 (a)	7.14 ± 1.58 (a)	8.08 ± 1.99 (a)
$NH_4^+NO_3^-$	8.28 ± 1.70 (a)	7.58 ± 2.85 (a)	7.88 ± 1.79 (a)	7.96 ± 1.36 (a)	7.82 ± 2.47 (a)	9.12 ± 2.88 (a)
Autumn						
$^{15}N\text{-}NH_4^+$	5.78 ± 1.97 (a)	8.22 ± 2.59 (a)	6.30 ± 1.29 (a)	6.04 ± 1.56 (a)	5.36 ± 1.79 (a)	9.54 ± 2.29 (a)
$^{15}N\text{-}NO_3^-$	8.40 ± 2.22 (a)	6.34 ± 1.29 (a)	8.48 ± 1.81 (a)	8.24 ± 1.86 (a)	7.26 ± 2.23 (a)	8.10 ± 1.10 (a)
$NH_4^+NO_3^-$	7.30 ± 1.77 (a)	6.90 ± 1.27 (a)	7.00 ± 1.62 (a)	5.54 ± 0.99 (a)	6.74 ± 0.90 (a)	7.32 ± 1.38 (a)

Scientific activities during the Ph.D. studies

External research Poster

Relationship between belowground tree species and root-associated fungal diversity, 17th Assembly of the Biodiversity Exploratories, 2020, Wernigerode, Germany.

Beech and ectomycorrhizal ammonium and nitrate uptake across biogeographic areas, 16th Assembly of the Biodiversity Exploratories, 2019, Wernigerode, Germany.

Assessment of the below-ground tree species on the Exploratories, 15th Assembly of the Biodiversity Exploratories, 2018, Wernigerode, Germany.

External research talk

Below ground nutrient resources drive root-associated fungal community composition in different soil layers in temperate forest, 18th Assembly of the Biodiversity Exploratories, 2020, Germany (Online).

Diversity of root-associated fungi in relation to root resource, morphological traits and plant species composition, 16th Assembly of the Biodiversity Exploratories, 2019, Wernigerode, Germany.

Functional diversity of mycorrhiza in relation to land-use changes and ecosystems functions, 15th Assembly of the Biodiversity Exploratories, 2018, Wernigerode, Germany.

Nitrogen uptake by roots is driven by ectomycorrhizal fungal diversity, DFH-Summer school 2019 Functions of microbial communities in soils, Helmholtz Zentrum München, Germany.

Internal research talk

Beech and Ectomycorrhizal ammonium and nitrate uptake across biogeographic areas, Forest Botany Seminar, Department of Forest Botany and Tree Physiology, University of Göttingen, summer semester, 2019.

Temporal variation of plant and mycorrhizal ammonium and nitrate uptake across two different regions, Forest Botany Seminar, Department of Forest Botany and Tree Physiology, University of Göttingen, winter semester 2019-20.

Root associated fungal community composition and their function in relation to nutrient and tree species composition, Forest Botany Seminar, Department of Forest Botany and Tree Physiology, University of Göttingen, winter semester, 2020-21.

Acknowledgements

PhD, it was probably the difficult but surely the most significant learning period in my life. I will remember all those PhD moments. I am grateful to all my colleagues who help me to make this a reality.

First of all, I would like to thank my main supervisor Prof. Dr. Andrea Polle for giving me the opportunity to work on this exciting functional biodiversity research project and for her excellent supervision throughout my PhD. I have learned a lot from her about life and science, and my deepest thanks for that. I am thankful to Prof. Dr. Christian Ammer for being second referee of my doctoral examination board and Prof. Dr. Rolf Daniel for being part of my thesis committee member and granting access to the Göttingen Genomics Laboratory for molecular analysis.

I am thankful to all my colleagues from the Department of Forest Botany and Tree Physiology for their kind supports and helps both in the field and lab. I would like to thank Dr. Rodica Pena for teaching me a lot about ectomycorrhizal fungi and for her great scientific advice during her stay in our group and even today. I want to thank Dr. Dennis Janz for his support with the data analysis and for teaching me various advanced statistical methods and programming in R. I would like to thank Dr. Johannes Ballauff and Likulund Emanauel Likulunga for the great discussion and suggestions about data analysis. I want to thank Dr. Simon Clausing for his help in root carbohydrate measurements. I would like to thank Steven Dreischhoff for his help to translate my English thesis summary into German. I want to thank Dr. Quang Dung Nguyen, Dr. Bin Song, Aisjah Ryadin and Michael Reichel for their help during the soil sampling campaign, 2017. I am thankful to Ronny Thoms, Dr. Huanying Feng, Jens Peter, Sven Hansen, Magnus Niemeyer, Ashkan Amirkhosravi and Huli Shi for their help in harvest during seasonal stable isotope labelling experiments in the field. I am grateful to Thomas Klein for extracting DNA from the root samples, to Merle Fastenrath for preparing the artificial soil solution, Monika Franke-Klein for conducting ICP measurements, and Bernd Kopka for solving computer-related problems, and to Gabriele Lehmann for her support in measuring soil ammonium and nitrate.

I would like to thank Dr. Dominik Schneider for his support in DNA sequence processing and bioinformatics analysis. I want to thank Reinhard Langel and Larz Szwec for their technical supports in measuring stable isotopes from the root samples and ectomycorrhizal fungi.

I would like to thank to Graduate School Forest and Agricultural Sciences (GFA) for allowing me to pursue my PhD degree within the Forest Science and Forest Ecology program. Moreover,

I want to thank German Research Foundation for providing the fund of this project as part of the DFG Priority Program 1374 "Infrastructure-Biodiversity-Exploratories" (DFG- PO362/18-4, ECTOMYC). I want to thank the managers of the Biodiversity Exploratories: Kirsten Reichel-Jung, Swen Renner, Katrin Hartwich, Sonja Gockel, Kerstin Wiesner, and Martin Gorke for maintaining the experimental plots; project infrastructure: Christiane Fischer and Victoria Grießmeier for their support through central coordination office, Cornelia Fürstenau for her help with data upload in BExIS and Markus Fischer, Karl Eduard Linsenmair, Dominik Hessenmöller, Jens Nieschulze, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser and the late Elisabeth Kalko for setting up the Biodiversity Exploratories project. Likewise, I am thankful to Markus Fischer, Marion Schrumpf, Christiane Fischer, Jörg Hailer, Juliane Vogt, and Miriam Teuscher for allowing me to conduct the stable isotope experiments in Hainich and Schorfheide-Chorin. Further, I want to thank Ulf Pommer, Victoria Groß, Frederic Van Broeck and Markus Rubenbauer for their help in setting up the subplots and maintaining the plots for stable isotope labelling experiments.

Finally, I would like to express my deepest gratitude to all my lovely family members, especially my father (Jahangir Alam), my mother (Sufia Begum) and my wife (Nisrat Zerin) for their supports, encouragement and love and to my beloved daughter, Shaonita Khokon.

Declaration of originality and certificate of authorship

I, Anis Mahmud Khokon, hereby confirm that I am the original author of this doctoral dissertation entitled “Assembly of root-associated fungi in different soil layers and nitrogen uptake by ectomycorrhizae in temperate forests”. This doctoral dissertation has not been submitted elsewhere for another degree. All the author's contributors in this doctoral dissertation have been properly acknowledged.

…….

Anis Mahmud Khokon
Göttingen, 26-07-2021