Functional diversity of mycorrhiza in relation to land-use changes and ecosystem functions

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“The study of plants without their mycorrhizas
is the study of artefacts.

The majority of plants, strictly speaking,
do not have roots; they have mycorrhizas.”

BEG Committee, 25th May, 1993

(http://www.i-beg.eu/)

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List of abbreviations

ACC-nr	=	Accession number
ACE	=	Abundance-based coverage estimators
Al	=	Aluminium
AG	=	Joint-stock company
AM	=	Arbuscular Mycorrhiza
Anova	=	Analysis of Variance
ANOSIM	=	Analysis of Similarities
a.s.l.	=	above sea level
Aug	=	August
BExIS	=	Biodiversity Exploratories Information System

Biodiversity Exploratories:

ALB	=	Swabian Alb
HAI	=	Hainich Dün
SCH	=	Schorfheide Chorin

bp	=	Base pairs
C	=	Carbon
c(prefix)	=	centi (10^{-2})
°C	=	degree Celsius
Ca	=	calcium
DBH	=	Diameter at breast height (1.30 m)
DF	=	Degrees of freedom
DGGE	=	Denaturing gradient gel electrophoresis
d (prefix)	=	deci (10^{-1})
DNA	=	Deoxyribonucleic acid
dNTP	=	Deoxynucleotide
e.g.	=	For example (exempli gratia)
EM	=	Ectomycorrhiza

et al.	=	And others (et alii)			
ET	=	Exploration types:	C	=	Contact
			SD	=	Short Distance
			MD	=	Medium Distance
			LD	=	Long Distance
Fe	=	Iron			
ForMI	=	Forest Management Index			
g	=	Gramm			
gam	=	Generalized adaptive model			
GCV	=	Generalized cross validation			
glm	=	Generalized linear model			
GmbH	=	Companionship with limited liability			
H’	=	Shannon diversity index			
ICP	=	Inductively Coupled Plasma			
Iharv	=	Amount of harvested tree biomass			
ITS	=	Internal Transcript Spacer			
K	=	Kalium			
k(prefix)	=	Kilo (10^3)			
KCl	=	Kaliumchloride			
KM	=	Michaelis constant			
l	=	Litre			
M	=	Molar			
m	=	Metre			
m (prefix)	=	Milli (10^{-3})			
MA	=	Massachusetts			
Mg	=	Magnesium			
MID	=	Multiplex Identifier			
min	=	Minute			

MMF	=	Michaelis Menten Fit
Mn	=	Manganese
MTH	=	Morphotype number
N	=	Nitrogen
n	=	Number of replicates used
Na	=	Natrium
NA	=	Not available (data point)
NADP	=	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	=	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
norm.	=	Normalization/normalized
Nov	=	November
NY	=	New York
OTU	=	Operational Taxonomic Unit
p	=	Probability of error
P	=	Phosphorus
PCA	=	Principal component analysis
PCR	=	Polymerase Chain Reaction
pH	=	Negative log of the activity of the hydrogen ion
RFLP	=	Restrict fragment length polymorphism
rpm	=	Rotations/revolutions/rounds per minute
S	=	Sulphur
SMI	=	Silvicultural Management Index
sp.	=	Species

Stand structures :

DI	=	Thicket, taller than 2 m, but DBH smaller than 7 cm
JB	=	Pole stage forest, average DBH between 7 and 15 cm
sBH	=	Average DBH over 30 cm
wBH	=	Average DBH between 15 and 30 cm
UGL	=	great span of age and DBH

Treatment within the Trenching experiment:		C	=	Control
		In	=	Ingrowth
		No In	=	No Ingrowth
UFZ	=	Helmholtz centre for environmental research		
USA	=	United States of America		
W	=	Wilkox test		
wrp	=	was removed from the model at … position		
ws	=	without singletons		
Zn	=	Tin		

Summary

Managing forest ecosystems has a long tradition in Germany. However, the influence of this intervention on the most important symbiosis partners of the trees, mycorrhizal fungi, is poorly understood. The fungal partner profits from the supply of plant carbohydrates. The plant partner receives nutrients, for example nitrogen (N). In temperate forests, ectomycorrhizas (EM) are the dominant type of this symbiosis on trees. The "*plant-economic theory*" predicts that trees invest fewer carbohydrates in their EM partners when nutrients are readily available.

In addition to EM, fungi with other lifestyles including endophytes, saprophytes or pathogens grew in and on the tree roots, forming a community of root-associated fungi. As information has been very rare until now, it is an important issue to understand how forest management impacts the root-associated fungal community, with special regard on its different fungal lifestyles.

The overarching goal of this thesis was to explore the community structures of root-associated fungi with different lifestyles, and specifically to investigate the effects of environmental variables and forest management on these communities. Root disturbance caused by tree harvesting was simulated by cutting roots; the resulting degradation of root litter and recolonization by living roots and associated EM community were observed. Taxonomic and functional diversity, represented by EM hyphal exploration types, were addressed. The Biodiversity Exploratories (Swabian Alb, Hainich Dün and Schorfheide Chorin) with differences in management intensity, tree species composition, climate conditions and soil properties provide an excellent opportunity to address the following hypothesis and research aims:

(I) To characterise the root-associated fungal community in temperate forests in Germany. The root-associated fungal community was observed by 454 pyrosequencing on tree roots from forest plots of the Biodiversity Exploratories. The lifestyles of specific fungi were assigned by an intensive literature search. Dominant tree species of the plot was found to have a greater effect on the overall root-associated fungal community than the study region. These differences of the whole fungal community were primarily driven by EMs, as they accounted for more than 60% of the fungi to which a lifestyle could be annotated. In contrast, the saprophytic community, with 20% abundance of all fungi to which a lifestyles could be annotated, was less influenced by dominant tree species than by regional origin. The sequence numbers of other lifestyles such as endophytes or pathogens were underrepresented within the pyrosequencing dataset and could therefore not be used to identify drivers for their community structure.

(II) To investigate the *"plant-economic theory"* on a large scale. Therefore, it was hypothesized that there is a relationship between forest management, root nitrogen and carbohydrate concentrations in roots as well as EM richness, diversity and community structure. (III) Furthermore, it was hypothesized that different abiotic and biotic environmental variables influence the richness, diversity and community of EM and of saprophytes to differing degrees, based on their different lifestyles.

Root carbon (C), N, glucose and fructose concentrations were measured. Information on soil properties, like pH or soil C and N concentration, were provided by other working groups from the Biodiversity Exploratories for the same soil samples. Additionally, indices describing the intensity of forest management were available for the same plots. Generalized linear and adaptive models suggest a dynamic interaction between the EM diversity and community to forest management, root N and carbohydrate concentrations. The present study indicates that under low root N concentrations and higher root glucose concentrations EM fungal diversity was enhanced. Root glucose, rather than fructose concentration, was found to be important for EM richness and community structure. The richness of EM was also positively associated with forest management intensity. In intensively utilized forests a lower N concentration in the roots was detected, probably caused by nutrient export via tree harvesting. Furthermore, root glucose concentration increased with forest management intensity perhaps the result of higher light availability for remaining trees. This large scale study had borne out indications from laboratories studies, that glucose appears to be specifically important for EM fungi.

In contrast to EM, the diversity of the saprophytic fungi was negatively correlated with the intensity of forest management. Their diversity and richness mainly relied on forest management, on root C and both carobhydrate concentration without specification, as well as on some rare elements. Community differences of saprophtic fungi were fewer dependent on dominant tree species than that of the EM community.

(IV) To test whether root litter, resulting for example from tree harvesting, affects EM communities locally. Small scale root disturbance was simulated by severing roots in soil of beech plots in the Hainich Exploratory by a cutting devise. This treatment resulted in patches free from living roots. The degradation of this root litter and the recolonization of those patches were monitored for one year and a half year. For comparison, undisturbed EM communities were also monitored. After a year and a half, the EM community of roots within disturbed patches reached a climax state that was not significantly different from that of undisturbed EM communities anymore. Tis demonstrated on a small scale the high resilience of the EM fungal community against disturbance. Fungi communly present in the undisturbed control soil cores were also those fungi, which were most able to

recolonized disturbed patches. Furthermore differences were identified in functionality, represented by exploration types of the EMs during recolonization. Short distance exploration type was identified to preferably recolonize cut patches during the first year, possibly caused by the release of soluble N from the decomposing roots.

In conclusion, this study demonstrated that forest management influences root-associated fungal community on a large scale, whereas different lifestyles react in different ways. Forest management had a negative influence on the diversity of saprophytic fungi and a positive on the diversity and richness of EM fungi. This negative influence on the saprophytes could be caused by fewer deadwood in intensively managed forests. This positive influence of forest management on EM fungal community may be due to two main drivers: disturbances by tree harvesting may cause short term changes. In the long term, environmental factors such as removal of nutrients due to biomass removal or higher carbohydrate production, due higher light availability, may have stronger impact on EM community structures than root litter or niche occupation. Our small scale root cutting experiment revealed a high resilience of the EM community to disturbance.

Zusammenfassung

Die Nutzung von Waldökosystemen hat eine lange Tradition in Deutschland. Wie allerdings dieser Eingriff in das Ökosystem Wald die wichtigsten Symbiosepartner der Bäume– die Mykorrhizapilze – beeinflusst, ist noch nicht ausreichend verstanden. In temperaten Wäldern bilden die Ektomykorrhiza-Pilze (EM) die vorherrschende Form dieser Symbiose an Bäumen. Bei dieser Symbiose profitiert der Pilz-Partner von der Versorgung mit Kohlehydraten. Der Pflanzen-Partner erhält Nährstoffe wie beispielsweise Stickstoff (N). Die „Pflanzen-Ökonomie-Theorie" impliziert, dass Bäume weniger Kohlehydrate in ihre EM-Partner investieren, wenn Nährstoffe leicht verfügbar sind. In diesem Fall sind sie nicht so sehr auf die Unterstützung ihrer EM-Partner angewiesen. Zusätzlich zu EM-Pilzen wachsen Pilze mit anderen Lebensweisen in oder an Baumwurzeln, wie zum Beispiel Endophyten, Saprophyten oder auch Pathogenen. Zusammen bilden sie die Gemeinschaft der wurzelassoziierten Pilze. Bisher gibt es nur wenig Information darüber, wie diese Gemeinschaft der wurzelassoziierten Pilze auf waldbauliche Maßnahmen reagiert. Es ist deshalb von großer Bedeutung, diesen Einfluss, mit Bezug auf die differenzierten Lebensweisen, besser zu verstehen.

Das übergeordnete Ziel dieser Doktorarbeit war es, Veränderungen in der Struktur der Gemeinschaft wurzelassoziierter Pilze, in Bezug auf waldbauliche Maßnahmen und verschiedene Umweltparameter, zu untersuchen. Relevant war dabei der Bezug zu unterschiedlichen, pilzlichen Lebensweisen.

Untersucht wurden hierzu Einflüsse verschiedener Umweltparameter und Waldbauintensitäten auf die pilzliche Gemeinschaft mit Hilfe von Pyrosequenzierung. Ebenso wurde die Ernte eines Baumes simuliert, indem Bodenbereiche frei von lebenden Wurzeln erzeugt wurden. Die taxonomische und funktionelle Diversität, letztere repräsentiert durch Explorations-Typen der EM-Hyphen, wurde über eineinhalb Jahre beobachtet. Die Untersuchungsflächen der Biodiversitäts-Exploratorien in der Schwäbischen Alb, dem Hainich-Dün und der Schorfheide Chorin unterscheiden sich in der Intensität des Waldbaus, der Baumartenzusammensetzung, dem Klima sowie Bodenparametern und bieten daher eine exzellente Möglichkeit, um folgende Forschungsziele und Hypothesen zu untersuchen:

(I) Die Charakterisierung der wurzelassoziierten Pilze in temperaten Wäldern in Deutschland war eines der Hauptziele dieser Arbeit. Die Gemeinschaft der wurzelassoziierten Pilze wurde mit 454-Pyrosequenzierung auf Waldflächen der Biodiversitäts-Exploratorien untersucht. Diese wurde mittels intensiver Literaturrecherche in verschiedene Lebensgemeinschaften gegliedert. Die gesamte pilzliche Gemeinschaft unterschied sich hauptsächlich je nach dominierender Hauptbaumart der

Fläche. Diese Unterschiede wurden maßgeblich durch EM-Pilze verursacht, da diese über 60% derjenigen Pilze ausmachten, denen eine Lebensweise zugeordnet werden konnte. Die Gemeinschaft der saprophytischen Pilze wurde hingegen mehr durch regionale Herkunft beeinflusst. Die saporphytischen Pilze machten etwa 20% derjenigen Pilze aus, denen eine Lebensweise zugeordnet werden konnte. Andere Lebensweisen, wie beispielsweise Endophyten oder Pathogene, waren im Pyrosequenzierungs-Datensatz unterrepräsentiert, weshalb für sie keine verlässlichen Berechnungen durchgeführt werden konnten.

(II) Ein anderes wichtiges Ziel dieser Arbeit war es, die „Pflanzen-Ökonomie-Theorie" großräumig zu untersuchen. Hierfür wurde die Hypothese aufgestellt, dass es einen Zusammenhang zwischen Intensität der Waldbewirtschaftung, Wurzelstickstoff- und Wurzelkohlehydrat-Konzentration sowie dem Artenreichtum, der Diversität und der Gemeinschaftstruktur der EM-Pilze gibt. (III) Darüber hinaus wurde angenommen, dass verschiedene biotische und abiotische Umweltparameter die Gemeinschaft der EM und der saprophytischen Pilze unterschiedlich beeinflussen, basierend auf ihrer differenten Lebensweise.

Kohlenstoff (C)-, Stickstoff (N)-, Glukose- und Fruktosekonzentrationen der Wurzel wurde hierfür gemessen. Informationen zu Bodenparametern wie beispielsweise pH, C- und N-Gehalt des Bodens der gleichen Flächen wurden dankenswerter Weise von anderen Arbeitsgruppen der Biodiversitäts-Exploratorien bereitgestellt. Zusätzlich waren Indices, welche die Intensität der waldbaulichen Maßnahmen auf diesen Flächen beschreiben, vorhanden und wurden zum Vergleich herangezogen. Die Glukosekonzentration der Wurzeln schien mehr Einfluss auf Artenanzahl und Gemeinschaft der EM-Pilze zu haben, als die Fruktosekonzentration. Generelle lineare und adaptive Modelle deuten eine dynamische Interaktion zwischen der Gemeinschaft der EM, forstlicher Maßnahmen sowie N- und Glukosekonzentration der Wurzeln an. Dies könnte beispielsweise auf den Nährstoff-Austrag bei der Ernte von Bäumen zurückzuführen sein. Darüber hinaus steigt die Glukosekonzentration mit der Intensität des Waldbaus an, was auf eine bessere Lichtversorgung einzelner Bäume hindeuten könnte. Der Artenreichtum von EM-Pilzen war positiv mit der Intensität des Waldbaus korreliert. Die vorliegende Studie zeigte auch, dass unter niedriger N- und hoher Glukosekonzentration in den Wurzeln die Diversität der EM-Pilze erhöht war. Für die saprophytischen Pilze konnte dieser Zusammenhang nicht eindeutig gezeigt werden. Die Diversität saprophytischer Pilze war negativ mit der Intensität des Waldbaus korreliert. Zudem waren sie von dem allgemeinen C-Gehalt der Wurzeln sowie einigen Spurenelementen abhängig und profitierten vermutlich über Wurzelexudate vor allem von Fruktose. Bodenparameter und regionale Herkunft erklärten die Gemeinschaftsstruktur der saprophytischen Pilze besser als die der EM.

Insgesamt wurde gezeigt, dass die Hauptbaumart der Fläche, Bodenparameter wie pH, die Versorgung der Wurzel mit Glukose und der Einfluss durch waldbauliche Maßnahmen die Haupteinflussfaktoren für Artenreichtum, Diversität und Gemeinschaftsstruktur der EM-Pilze sind.

(IV) Mit einem kleinräumigen Störungs-Experiment wurde untersucht, ob Wurzelstreu, welche zum Beispiel bei der Ernte eines Baumes anfällt, lokal die EM-Gemeinschaft beeinflusst. Hierfür wurden auf Buchen dominierten Untersuchungsflächen im Hainich Wurzeln im Boden von ihrem Baum abgetrennt. Aus dieser Behandlung ergaben sich Bereiche, die frei von lebenden Wurzeln waren. Der Abbau der Wurzelstreu sowie die Wiederbesiedlung dieser Bereiche wurden über einen Zeitraum von eineinhalb Jahren beobachtet. Die EM-Gemeinschaft in ungestörten Bereichen diente hierbei als Kontrolle. Nach eineinhalb Jahren erreichte die EM Gemeinschaft wieder den Klimax-Status der ungestörten Kontrollen. Für die Wiederbesiedlung der gestörten Bereiche waren hauptsächlich diejenigen Pilze von Bedeutung, die auch in den ungestörten Kontrollen häufig zu finden waren. Auch Unterschiede in der Funktionalität der wieder besiedelnden EM-Pilze wurden untersucht. Während des ersten Jahres des Wiederbesiedlungsprozesses waren EM mit einem „Kurzen-Distanz“ Hyphen-Explorations-Typ von Bedeutung. Dies wurde möglicherweise durch die Stickstoffabgabe degradierender Wurzeln beeinflusst.

Zusammenfassend zeigte diese Studie in großem Maßstab, dass Waldbau die EM-Pilz-Gemeinschaft positiv und die saprophytische Gemeinschaft negativ beeinflusst. Der positive Einfluss könnte auf zwei Hauptursachen basieren: Kurzfristig verursachen Störungen im Wurzelbereich Veränderungen in der EM-Gemeinschaft. Langfristig sind Umweltparameter, wie der Austrag von Nährstoffen durch die Baumernte, sowie die Erhöhung der Kohlehydratkonzentration durch erhöhte Lichtverfügbarkeit wahrscheinlich ausschlaggebender. Der negative Einfluss auf die saprophytischen Pilze wird vermutlich durch einen geringeren Totholzanteil in stark genutzten Wäldern mit verursacht. Es wurde in großem Maßstab gezeigt, dass Glukose wichtiger für die EM-Gemeinschaft zu sein scheint als Fruktose. Dies war vorher vor allem in Laborstudien untersucht worden. Das kleinräumige Störungs-Experiment zeigte eine hohe Resilienz der EM-Gemeinschaft.

1. Introduction

1 Introduction

1.1 Influence of humankind on biodiversity

Humankind has drastically changed the planet's land surface (Foley et al., 2005). Habitat conversion and degradation, habitat fragmentation, climate change, harvesting and pollution have degraded global biodiversity and species richness more than 8% during the last 500 years (Newbold et al., 2015). This loss of biodiversity undermines ecosystem functions, like ecosystem stability or productivity (Cardinale et al., 2012; Chapin et al., 2000; Hooper et al., 2012). The overall negative impacts of land use vary between different habitats (Baan et al., 2012). Humankind influences biodiversity mainly by nitrogen (N) fertilization, fresh water use and land modification (McGill, 2015; Millennium Ecosystem Assessment, 2005).

In Germany forest utilization has a long tradition, with the result that since the 19th century forest management has been moderate (Röhrig et al., 2006). Many groups of forest organisms including deadwood inhabiting fungi, saprophytic arthropods, herbivores, canopy predators and breeding birds decrease in diversity when forest management and land use increase (Blaser et al., 2013; Gossner et al., 2014). Other groups such as mosses and ground-dwelling predators benefit from increased land use intensity, whereas the overall plant diversity was unaffected by forest management in the study of Gossner et al. (2014). However, not all groups of organisms are influenced by forest management, because for example the community structure of soil inhabiting fungi did not differ between managed and unmanaged beech forests (Wubet et al., 2012).

1.2 Ectomycorrhiza – an important plant symbiont in temperate forests

In temperate forests, the most important tree symbionts are ectomycorrhizal fungi (EM). In general, 90% of all plants form mycorrhizas (Cairney, 2000), which additionally to EM, are mainly arbuscular mycorrhiza (AM), orchid mycorrhiza and ericoid mycorrhiza. Frank (1885) was the first to describe this symbiosis between plants and fungi for EM. EM contribute to ecosystem processes such as the carbon cycling, nutrient mobilization and enhanced plant growth (Finlay, 2008). Morphologically, this symbiosis is formed by a fungal mantle ensheathing the root tip and a Hartig net, which consists of fungal hyphae between cortical and epidermal cells of the host plant root. The Hartig net increases the surface for nutrient exchange (Peterson et al., 2004). Furthermore they form an extramatrical

mycelia for soil exploration. With their extramatrical mycelium EM fungi grow rapidly into soil areas beyond the nutrient depletion zone of the plant roots (Bending and Read, 1995; Carleton and Read, 1991). Furthermore, the hyphal networks of EM has a larger surface area than plant roots (Allen, 1992; Read, 1992) and due to their small diameter, hyphae can explore smaller soil pores than plant root hairs (Finlay, 2008). Additionally, enzymatic activities vary between EM species, resulting in different nutrient availability for host plants (Courty et al., 2010, 2005). Courty et al. (2005) reported, for 14 different EM species to have different hydrolytic and oxidative enzymes, which are involved in the decomposition of lignocellulose, chitin and phosphorus-containing organic compounds.

In the same way that aboveground fruiting bodies of EM fungi can be distinguished by morphological criteria, so can the belowground structures. Agerer (1987) has described EM fungal species by their morphology and anatomy on different host plants. Ectomycorrhizas can be distinguished by color, branching and surface texture of the mantle and emanating mycelia (Figure 1.1). According to their emanating hyphae, EM fungi have been classified in different exploration types: contact type with few emanating hyphae, short-distance type with a lot of emanating hyphae, medium-distance type forming rhizomorphs and long-distance type with few highly differentiated rhizomorphs (Agerer, 2001). Morphological differences also indicate that the species diversity of EM fungi is linked to their functional diversity (Rineau and Courty, 2011). Petchey and Gaston (2006) suggest, that “measuring functional diversity is about measuring functional trait diversity, where functional traits are components of an organism’s phenotype that influence ecosystem level processes”. Different parts of the mantle and emanating hyphae could differ in their hydrophobic/hydrophilic properties and thus influence nutrient and water uptake (Taylor and Alexander, 2005; Unestam and Sun, 1995). Those structures are important for the function of the EM, which supply their host plants with water, nutrients and protection against some pathogens and heavy metal toxicity (Schützendübel and Polle, 2002; Smith and Read, 2008). For example a *Paxillus-Pinus* ectomycorrhiza stimulates the phenolic defense system and can therefore enhance tolerance to cadmium (Schützendübel and Polle, 2002). Different EM species were found to accumulate different element concentrations in their own, and in their associated root cells indicating different functional roles (Seven and Polle, 2014). Species richness or relative abundance of EM fungal species was demonstrated to have a positive correlation to soil peroxidase activity reveling a strong link to functional diversity (Phillips et al., 2014; Talbot et al., 2013).

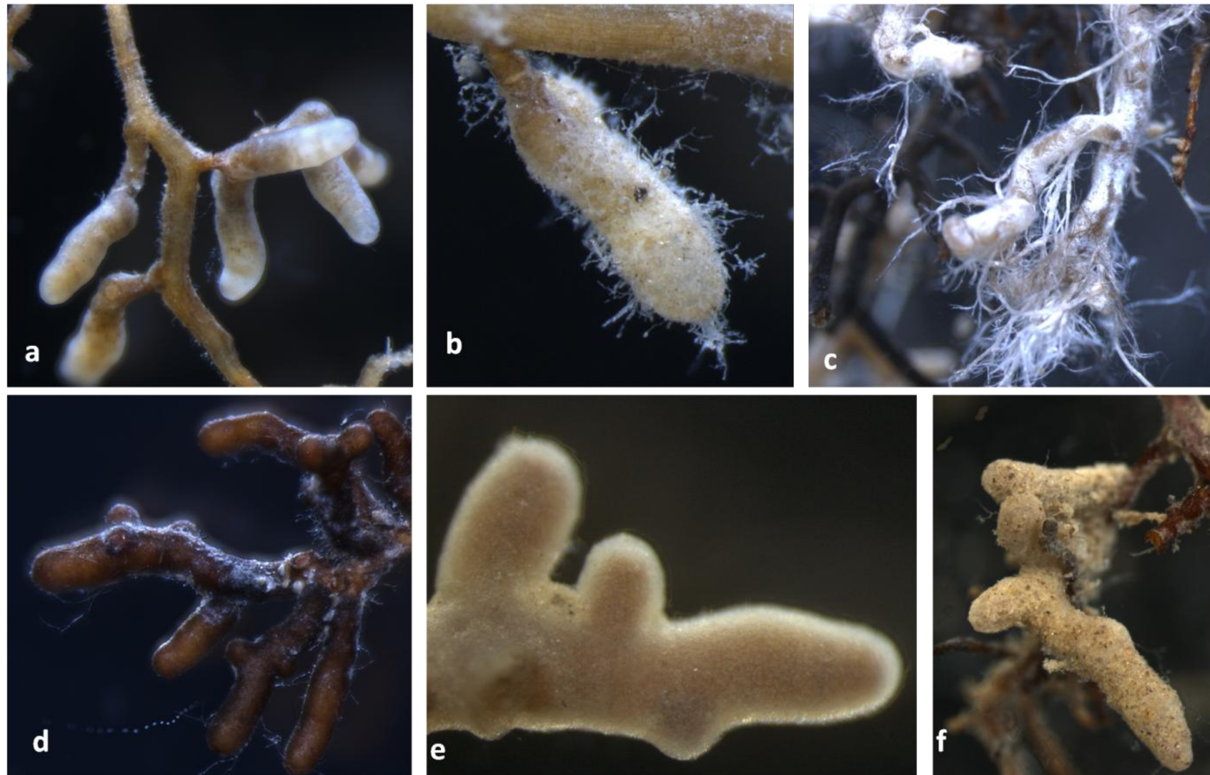


Figure 1.1: Ectomycorrhizal root tips of a) *Inocybe* sp. (exploration type (ET) = short distance (SD)), b) *Amanita* sp. (ET = medium distance (MD)), c) *Tricholoma sciodes* (ET = MD), d) *Genea hispidula* (ET = SD), e) *Lactarius pallidus* (ET= contact (C)) and f) *Hymenogaster griseus* MD. Pictures are not true to scale.

1.3 Carbon supply from the host plant to the mycorrhizal partner

EMs receive carbohydrates from their host plant (Treseder et al., 2006). Carbon flows to the soil via the EM mycelia and therefore EM fungi play a major role in the belowground carbon cycling (Godbold et al., 2006; Simard et al., 2003). Factors changing carbohydrate allocation influence EM richness, diversity and community composition as shown in a girdling experiment (Pena et al., 2010). After girdling, which reduced the carbohydrate flow, mainly cryptic EM species disappeared. The study reported a causal relationship between a reduction of root carbohydrates and the loss of EM diversity. Until now it has only been shown for AM, that plants can select for their best supplying mycorrhizal partner (Kiers et al., 2011). For example *Medicago*, an herbal plant, can detect, discriminate, and reward the best arbuscular mycorrhizal partners and supplied those with more carbohydrates (Kiers et al., 2011). EM are known to be host plant species specific (Bruns et al., 2002; Lang et al., 2011; Tedersoo et al., 2008). As different EM species have different ecological traits and functions, a high EM richness is likely to be important for a stable ecosystem with a high resilience with many ecosystem functions (Courty et al., 2010; Pena and Polle, 2014; Rineau and Courty, 2011). For example, (Rineau and Courty, 2011) showed that functional diversity, represented by secreted

enzymes is strongly correlated with taxonomic diversity. Since taxonomic diversity is strongly correlated with functional diversity of EM plants try to optimize their functional abilities in soil nutrient and water uptake by supporting a high diversity of EM on their roots (Rineau and Courty, 2011). Druebert et al., (2009) showed that plant carbohydrate productivity was the reason for and not the result of high EM diversity. Plants are estimated to deliver 20 to 30% or even 50% of their net primary production to supply their fungal partner (Hobbie and Hobbie, 2006; Simard et al., 2003; Söderström, 2002). Mycorrhizas stimulate the carbon transfer to roots by increasing the below-ground sink strength (Bidartondo et al., 2001; Dosskey et al., 1990; Kaschuk et al., 2009). Bidartondo et al. (2001) demonstrated that a *Paxillus involutus* mycorrhiza on *Pinus muricata* produced low biomass, but consumed proportionally more carbon and transported as twice as much ammonium to the host than other EMs tested. When carbohydrates are transported from the plant to the fungal partners at the plant–fungus interface, fungal hyphae are expected to preferentially utilise glucose from the glucose/fructose mixture (Nehls et al., 2010).

1.4 Nitrogen as a limiting growth factor in temperate forests

In temperate and boreal forests, where N is the limiting factor for tree growth (LeBauer and Treseder, 2008; Vitousek and Howarth, 1991), EM fungi are the most important symbiotic partners as they produce nitrogen-degrading enzymes, allowing them greater access to organic nitrogen sources than AM fungi (Averill et al., 2014; Read and Perez-Moreno, 2003).

About 80% of the plant phosphorous (P) and N contents are acquired via mycorrhizas (van der Heijden et al., 2008). EM fungi have the capacity to use organic N from complex organic substrates (Lilleskov et al., 2002b), explore a bigger soil volume than plant roots, are more efficient in their uptake of N as they can also uptake organic forms as amino acids (reviewed in Deckmyn et al. (2014). Therefore the carbohydrate investment in EM by the plant is more essential on N limited sites. The "*plant-economic theory"* predicts that trees invest fewer carbohydrates in their EM partners when nutrients like N are easily available (Read, 1991). But for carbon (C) and N fluxes direct evidence for this theory was not established, suggesting that many different services provided by the EM community are important for C flux under long term conditions (Valtanen et al., 2014). However, this study was performed under N saturation conditions. Pena and Polle (2014) demonstrated that EM roots provide advantages for uptake of inorganic N in comparison to non-mycorrhized roots under environmental stresses such as drought, but not under unstressed conditions. As this stress activation was different between EM taxa, certain degree of functional diversity was indicated.

Necessarily, the plants are able to control the carbon loss towards the fungal partner to avoid fungal parasitism (Kiers et al., 2011; Nehls et al., 2007).

1.5 Environmental factors and forest management influence fungal root and soil community

Besides N, there are other variables like soil pH and soil texture influencing soil fungal community composition (Wubet et al., 2012). Furthermore climatic conditions, especially drought, can change the community structure of EM (Abbaspour et al., 2012; Shi et al., 2002; Swaty et al., 2004). Additionally soil moisture is known to influence EM community structure as well as seasonality (Buée et al., 2005).

Anthropogenic impacts also play a role; like for example forest management, which aims to optimize several ecosystem services like soil and water protection, climate regulation and wood production. Those impacts via forest management may also influence soil microbial communities (Felsmann et al., 2015; Hartmann et al., 2012; Nacke et al., 2011). Thinning is a widespread forestry practice that enhances the growth of remaining trees by reducing competition for light. Due to the increased light availability a higher production of carbohydrates per single trees is possible. However, to our knowledge only a few studies have addressed the effect of thinning and forest management in general on EM richness and diversity (Buée et al., 2005; Kropp and Albee, 1996; Lin et al., 2011; Teste et al., 2012). Those studies indicate that a moderate forest management increases the overall richness of EM and has either positive or negative effects on different fungal taxa. In these studies forest management was only categorized in “thinned” and “unthinned”. Because the intensity of forest management is more variable than these two categories of thinning, different attempts have been made to categorize it. For moderate land use, such as usual forest management in central Europe, it was difficult to define the intensity, but since 2013 two indices are available. Schall and Ammer (2013) and Kahl and Bauhus (2014) calculated the SMI and ForMI index respectively to describe the intensity of forest management. SMI takes into account the tree species, stand age and aboveground, living and dead wood biomass, whereas ForMI is based on the proportion of harvested tree volume, the proportion of non-native tree species in comparison to the natural forest community and the amount of dead wood showing signs of saw cuts. For a comparison both were used within this study. Both indices are available for 150 forest plots in the Biodiversity Exploratories (Fischer et al., 2010). The Biodiversity Exploratories are a large scale and long term project to investigate how taxonomical and functional diversity reacts on land use for many taxonomical groups. With their standardized field plots the Biodiversity Exploratories provide ideal conditions to

study whether a relationship between the intensity of forest management and root-associated fungal diversity exists.

1.6 Root-associated fungal community

Mycorrhizas are not the only fungal lifestyles associated with roots. There are endophytic fungi, living within the root (Ahlich and Sieber, 1996) known to increase resistance to drought (Richardson et al., 1992) and insects (Cheplick and Clay, 1988). Furthermore parasitic fungi and saprophytes are expected to grow near and on the roots (Kernaghan et al., 2003a; Tedersoo et al., 2009). Litter and humus layer in forest soils are rich in complex carbohydrates (e.g., cellulose and lignin). Using them as an energy source requires highly specialized microorganisms that possess effective degradation enzymes. However, simple carbohydrates like glucose or fructose are necessary for most microbes in forest soils (Nehls et al., 2007). The exudates of living plants, which are rich in simple carbohydrates, may not only be an important resource for EM, but also for saprophytes. EM fungi are known to have originated from a saprophytic lifestyle and still retain some saprophytic enzymatic capabilities (Lindahl and Tunlid, 2014).

1.7 Hypothesis and aims of this thesis

The overarching goal of this thesis was to investigate the community structures of root-associated fungi with different lifestyles, with special regard to EM and their functional diversity in relation to ecosystem functions and land-use intensity.

The aims and hypotheses of this thesis were:

- Characterisation of the root-associated fungal communities - separated by different lifestyles (richness, diversity and community structure).
    - Region and dominant tree species influence fungal community of different lifestyles differently.
    - Biotic and abiotic environmental variables differentially influence different lifestyles.

  - Forest management influences EM via root nitrogen and carbohydrate concentration in roots – investigation of the “*plant-economic theory”* on the large scale.
- Local disturbance affects EM communities:
  - Duration until disturbed EM communities returned to an undisturbed state.
  - Differences in functionality of the EMs during recolonization.

To investigate these hypotheses and aims, soil and roots were sampled on all 150 experimental plots within the Biodiversity Exploratories. The three Exploratories (http://www.biodiversity-exploratories.de/; Fischer et al. (2010)) provide an excellent opportunity to address these hypothesis as they differ in management intensity, tree species composition, climate conditions and soil properties (Fischer et al., 2010). High through put sequencing was used to detect the root-associated fungi present. They were separated to different lifestyles by intensive literature search, as a separation was necessary if effects on different lifestyles should be addressed. Root glucose and fructose as well as carbon, nitrogen and other root element concentrations were measured. Soil parameters were measured by other working groups and could kindly be used for comparison. The impact of intensive land use is mainly negative on biodiversity, as mentioned above. For moderate land use since 2013 two indices (SMI and ForMI) have become available and can therefore be used for comparisons. Additionally an experiment was installed within beech forests in the Hainich Exploratory by cutting roots within the forest soil. When a tree is harvested, the belowground parts remain within the forest soil and degrade, thereby serving as supplementary nutrient source. The increased production of root litter as a consequence of forest utilization may create new niches. Additionally, Peay et al. (2011) hypothesized soil disturbances to maintain community diversity at a high level by creating additional habitats for EM species. Within our experiment a disturbance was simulated which resulted in patches free from living roots and the disturbed patches were monitored to study recolonization. Those patches are assumed to be recolonized by roots of surrounding trees, seeking to utilise the newly available space and nutrients. The EM fungal community within ingrowth and within undisturbed control cores were observed by morphotyping and Sanger sequencing over 18 months. This experiment was expected to obtain information about functional differences of the EM community using exploration types as markers.

2. Material and Methods

2 Material and Methods

2.1 Study sites

Samples were taken in the frame of the German Biodiversity Exploratories. The three areas are located in the south west of Germany - Swabian Alb =ALB, the middle of Germany - Hainich Dün = HAI and the north east - Schorfheide Chorin = SCH (Table 2.1, Figure 2.1 a; Fischer et al. (2010)). The plots vary in management intensity, soil and tree species composition (Fischer et al., 2010; Schall and Ammer, 2013).

Table 2.1: Main geographical and environmental characters of the three Biodiversity Exploratories, modified from Fischer et al. /2010) and Solly et al. (2014)

	Schwäbische Alb	**Hainich-Dün**	**Schorfheide-Chorin**
Abbreviation	ALB	HAI	SCH
Location	South West Germany	Central Germany	North Eeast Germany
Coordinates precise	lat= 48.3639617-48.5000527; lon= 9.22239205-9.50193186	lat= 51.0460522-51.369932 ; lon= 10.207728-10.5340491	lat= 52.8619726-53.1922476; lon= 13.6329537-14.0017904
Size	~422 km2	~1300 km2	~1300 km2
Geology	Calcareous bedrock with karst phenomena	Calcareous bedrock	Young glacial landscape
Soil type in forests	Cambisol (eutric)-Leptosol	Luvisol	Cambisol (dystric)
Annual mean temperature	6–7 °C	6.5–8 °C	8–8.5 °C
Annual mean precipitation	700–1000mm	500–800mm	500–600mm
Altitude a.s.l.	460–860m	285–550m	3–140m
dominant tree species on plots	beech (*Fagus sylvatica*) and spruce (*Picea abies*)	beech and spruce	beech, pine (*Pinus sylvstris*) and oak (*Quercus robur*)

2.1.1 Study sites for root sampling on 150 plots

Soil/root samples for the pyrosequencing study were taken on all 150 Experimental forest plots (EP; 100 x 100 m)in all three study regions of the Biodiversity Exploratories. The plots ranged from unmanaged to highly used plots and harboured different dominant tree species: beech (*Fagus sylvatica*) in all three Exploratories, spruce (*Picea abies*) in the Swabian Alb and the Hainich and oak (mainly *Quercus robur*) and pine (*Pinus sylyvestris;* Table 2.1). For additional information and supporting data from the same soil sampling campaign and/or the same study plots see Supplementary tablel S1.

2.1.2 Study sites for Root-Trenching-Experiment

Six forest EPs (Hew 19, HEW 21, HEW 35, HEW 36, HEW 41 and HEW 47) within the Hainich Exploratory were chosen based on the following criteria: beech dominated, Luvisol soil, similar age class structure and silvicultural system (uniform shelterwood). Within each plot, five beech trees were selected according to the following criteria: similar height (24 to 30 m) and diameter at breast height (DBH, 0.35 to 0.55 m), absence of direct (minimum distance of 4 m) bigger neighbouring trees. Presence of other tree species and stocks of dead wood were kept as low as possible around the sampling trees.

2.2 <u>Sampling and experimental setup</u>

2.2.1 Sampling and experimental setup for root sampling on 150 plots

In each study region (Exploratory), 50 forest experimental plots were sampled in the beginning of May 2011 (see also Solly et al., (2013); sampling date: 02.05-12.05.2011). Soil cores with a diameter of 5 cm and a depth of 10 cm were collected using a split tube along two transects of 40 m length from north to south and from west to east at 1, 7, 13, 19, 31 and 37 m each, resulting in 14 samples. Organic layers were removed prior to soil sampling. The soil of a fixed sampling depth of the upper 10 cm was selected within each soil core when opening the split tube. The samples of each plot were mixed. Directly next to every second sampling point an additional soil sample was taken using a

zylindric plastic tube (3 x 10 cm, Figure 2.1 b). Those tubes were stored at 4°C until further processing.

With a distance of 6 m between the samples an optimal distance was chosen, to avoid multiple sampling of the same individual. An individual ectomycorrhizal mycelium could reach several decimetres or even meters (Agerer, 2001; Douhan et al., 2011; Smith et al., 1992). For example Lilleskov et al. (2004) found that most of the dominant EM taxa showed a patchiness of less than 3 m, with a range from 0 to 17 m. Therefore with this distance the same EM individual should not be detected many times. Another advantage was that we sampled the upper 10 cm of the mineral soil. As nutrient cycling is most intense in the upper 10 cm, trees also use those resources with their fine roots (Bruns, 1995).

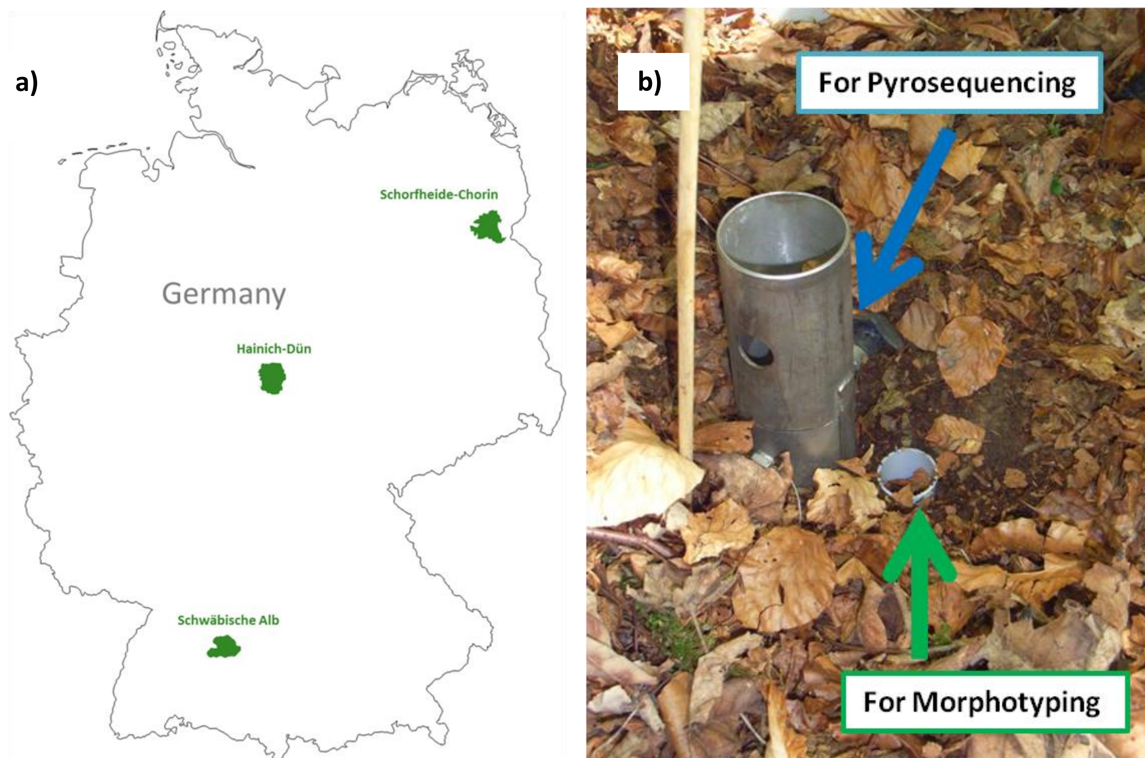


Figure 2.1: Soil sampling in May 2011. a) Location of the three Biodiversity Exploratories(Fischer et al., (2010) modified by Kezia Goldmann UFZ Halle) b) Next to every second split tube sampling, containing the sample for all groups, for the present study roots for pyrosequencing, a second small sample were taken for morphotyping.

2.2.2 Sampling and experimental setup for Root-Trenching-Experiment

Two types of soil cores were installed on 28-29.04.2011 and 02.05.2011. For the first core type ("Ingrowth") cylindrical soil cores were cut with an electrical jigsaw, 0.2 m deep and 0.08 m in diameter (1 L). Thereby the roots were severed from the plant, but other soil parameters were not affected. Ingrowth of new roots was not prevented. These cores were marked at the top with 0.02 m deep x 0.08 m wide plastic rings to allow future discovery and accurate harvesting. For the second type ("No Ingrowth") a soil core was cut as described above, but a plastic tube was inserted around the core and covered at the bottom with a nylon mesh (mesh size of 30 µm, A. Hartenstein GmbH, Würzburg, Germany) to close the core and to prevent ingrowth of new roots. Five of each core type (Ingrowth and No Ingrowth) were installed around the base of each tree in a distance of 1.5-2.0 m, as the highest root density is expected within this distance (Ammer and Wagner, 2002). At each harvest an untreated 1 L control soil core (d = 0.08 m, depth 0.2 m) was taken between two harvested corresponding cores of type Ingrowth and No ingrowth (Figure 2.2).

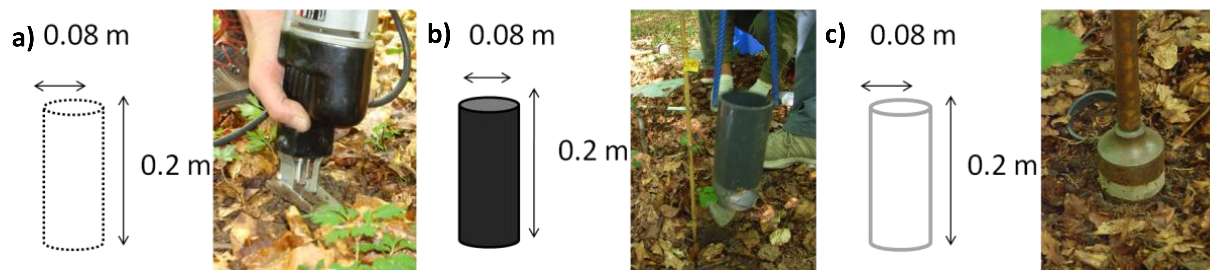


Figure 2.2: Experimental setup of the different core types: a) "Ingrowth", b) "No Ingrowth" and c) undisturbed soil cores as "Controls". Five Ingrowth and No Ingrowth soil cores respectively were installed on six beech dominated forest plots of the Biodiversity Exploratories in May 2011.

Five times the recolonization process were investigated. After three, six, twelve, 15 and 18 months (sampling dates: 2.-3.8.2011, 1.-2.11.2011, 2.5.2012, 30.7-02.08.2012 and 12-13.11.2012) one sample of each treatment from each sampling tree was harvested. This resulted in 30 samples per treatment Ingrowth, No ingrowth and undisturbed Controls respectively. Only in May 2012 samples were taken of only two of the five beech trees per plot. At the experiment installation in Mai 2011, five undisturbed soil samples were collected in each plot (Table 2.2). The collected soil samples were cooled and kept in polyethylene bags at 4°C until further analysis. Freezing of soil and roots were done within the two weeks after sampling, whereas morphotyping took up to two months. Only the samples from the last harvest were first frozen at -20°C and were thawed again. Soil moisture reflects the temperature in proportion to the precipitation and soil capability to hold water and is therefore

important for the plant. Soil moistures and soil temperature at 10 cm depth of the six plots over the whole sampling period are available in Supplementary Figure S2.

Table 2.2: Number of samples used/redetected per harvest and treatment.

	May 11	Aug 11	Nov 11	May 12	Aug 12	Nov 12
Control	30	30	30	12	30	28
Ingrowth	-	30	30	12	30	23
No Ingrowth	-	30	30	12	30	16

2.3 Sample processing

2.3.1 Sample processing for root sampling on 150 plots

Fine roots (<2 mm in diameter) were randomly chosen, stored at 4°C and transported to the laboratory. Directly next to every second sampling point an additional soil sample was taken using a cylindrical plastic tube (3 x 10 cm; Figure 2.1). Those tubes were also stored at 4°C, before being soaked in water for a minimum of half an hour. The roots were washed out carefully and used for Morphotyping.

About 2 g of roots from the split tubes were washed in deionised, sterile 4°C cold water (USF Seral - Seralpur, Seral, Ransbach-Baumbach, Germany with filter Delta Supor® DCF CHS92DE, Pall Cooperations, Washington, NY, USA) and frozen in liquid nitrogen. The roots were stored at -80°C. For further analysis, the roots were freeze-dried (P4K-S, Dieter Piatkowski Forschungsgeräte, Munich, Germany and PK4D vacuum pump Type 302051, ILMVAC GmbH, Ilmenau, Germany, starting with -60°C rising up to -20°C for four days) and then ground in a ball mill (Type MM2, Retsch, Haan, Germany).

2.3.2 Sample processing for Root-Trenching-Experiment

The fresh soil cores were weighed and homogenized. An aliquot of the soil sample was frozen at -20°C. About 1 g randomly chosen roots were washed and stored at -80°C. To collect the roots the remaining soil was soaked, and the roots were carefully washed. Adherent soil was removed and they were kept moist in wet tissue paper at 4°C until morphotyping.

2.4 Chemical analysis of roots and soil

2.4.1 Root element concentrations for root sampling on 150 plots

For carbon and nitrogen measurements, aliquots (0.700-0.900 mg) of those freeze dried and milled root material was weighed (Supermicro, Satorius, Göttingen, Germany) and filled into Zn capsules (HEKAtech GmbH, Wegberg, Germany) which were subsequently transferred into a CHNS-O EA1108 Element analyser (Carlo Erba Instruments, Lancashire, UK). Mineral element concentration of Al, Ca, Fe, K, Mg, Mn, Na, P and S were determined using iCAP 6300 Duo VIEW ICP Spectrometer (Thermo Fischer Scientific GmbH, Waltham, MA, USA) after pressure digestion of samples in 65% HNO_3 for 12 h (Heinrichs et al., 1986).

2.4.2 Carbohydrate analysis for root sampling on 150 plots

For carbohydrate measurements, 25 mg freeze dried root material was used for analysis. Glucose and fructose concentrations within the roots were determined spectrophotometrically after enzymatic conversion of NADP to NADPH by the consumption of carbohydrates (Schopfer, 1989) as described in (Danielsen, 2013; Luo et al., 2006). A sugar solution with a known concentration of 100mg/l of each carbohydrate tested was used as reference. The following enzymes were used: for Glucose: Hexkoinase/Glucose -6-Photphate Dehydrogenase from Roche, Ref: 10737275001; 30 mg/10 ml and for fructose: phosphoglucose isomerase (PGI from Roche Ref: 10128139001; 10mg/ml). For detailed description see Supplementary: Detailed carbohydrate analysis.

2.4.3 Soil moisture for Root-Trenching-Experiment

For estimation of soil moisture, soil aliquots were weighed, dried at 60°C for 96 hours and left to cool in a desiccator for at least 2 hours before being re-weighed.

2.4.4 pH measuerment for Root-Trenching-Experiment

Bulk soil pH was measured using 5 g of dried soil from each sample by adding 12.5 ml distilled water. The solution was shaken at 200 rpm for 2 h before being measured with a calibrated pH meter (Multical® pH 538, WTW, Weilheim, Germany). For limiting seasonal effects, 12.5 ml 0.1 M KCl was added to the soil/water solution, the samples were shaken at 200 rpm for 30 min and measured again.

2.5 Processing of roots and Morphotyping

The carefully washed roots were morphologically classified to tree species level (Hölscher et al., 2002; Korn, 2004) and separated into size categories , where roots smaller than 2 mm in diameter were defined as fine roots. Coarse roots were weighed, dried and re-weighed as described for the soil samples.

2.5.1 Processing of roots for root sampling on 150 plots

Roots from the additional small samples of the sampling on 150 plots were soaked in water for a minimum of half an hour and tree roots were washed out carefully. The roots were inspected using a binocular (M205 FA, Leica Microsystems, Wetzlar, Germany). Tree roots were separated to roots with ectomycorrhizal and arbuscular mycorrhiza. Roots with EM were frozen in a 10% glycerin solution to prevent cell burst. This method was tested before to prevent a change in surface structure and colour. The roots were used for Morphotyping after carefully thawed at 4°C. Within each sample, the numbers of dead and vital root tips were counted until 500 vital root tips were reached. Vital tips were divided into mycorrhized and non-mycorrhized tips.

2.5.2 Processing of roots for Root-Trenching-Experiment

Roots belonging to other plant species than beech, mainly *Acer* sp., *Fraxinus excelsior* or herbal plants were not considered for further analysis. The amount of these roots was less than 2% of the total fine root biomass.

The root tips of beech fine roots were inspected using a binocular (M205 FA, Leica Microsystems, Wetzlar, Germany). Within each sample up to 1000 root tips were counted and classified as dead and vital tips (according to the method of Allen et al. (2000)) for calculating the ratio between dead and vital tips (Percent vital tips= number of vital tips/total number of root tips*100). All vital tips were further divided in mycorrhized (with hyphal mantle) or non-mycorrhized (without hyphal mantel, white and thin) ones. Mycorrhization rate was calculated after (Lang et al., 2011).

2.5.3 Morphotyping

Vital mycorrhized root tips were classified into morphotypes based upon a simplified description scheme of the morphotyping system developed by (Agerer, 1987). This is based on morphological observations of colour, surface texture, branching and the appearance and properties of emanating hyphae and rhizomorphs. Of each morphotype, pictures were taken (Leica DFC 420C, Wetzlar, Germany) to allow future recognition (Supplementary Figure S4). Of each morphotype 3-20 tips were collected and frozen at -20°C for further ITS sequencing. Roots were weighed and dried for 48 hours at 60°C. The samples were left to cool in a desiccator for at least 2 hours before being re-weighed for assessing the root dry mass.

2.5.4 DNA extraction, PCR amplification and ITS sequencing of morphotypes

The frozen morphotype tips were used for ITS sequencing. The whole process was performed similarly as described in Druebert et al., (2009) or Lang et al. (2011)) with the exception that the DNA extraction kit, innuPREP Plant DNA Kit (Analytik Jena AG, AJ Innuscreen GmbH, Jena, Germany), was used according to the manufacturer’s instructions. As forward primer either ITS1F (5’-TCC GTA GGT GAA CCT GCG G-3’) (Gardes and Bruns, 1993) or ITS1 (5’TCCGTAGGTGAACCTGCGG3’) and as reverse primer ITS4 (5’-TCC TCC GCT TAT TGA TAT GC-3’)(White et al., 1990) was used for all PCR steps. This change in the primers was due to a change of the general laboratory process during experiment duration.

Purified DNA was either sequenced as described in (Druebert et al., 2009; Lang et al., 2011) or by “Seqlab” – Sequence Laboratories Göttingen GmbH (Göttingen, Germany) after the isopropanol purification step, depending on the date of sequencing as the general laboratory process changed during experiment duration.

2.5.5 Species and exploration type assignment

Alignment of forward and reverse DNA strands and subsequent editing were carried out using seqtrace 0.9.0 (Stucky (2012); https://code.google.com/p/seqtrace/). For fungal identification BLAST searches were carried out against the UNITE (Kõljalg et al., 2013); http://unite.ut.ee) and the NCBI public sequence databases (Sayers et al. (2009); http://www.ncbi.nlm.nih.gov). The results were compared and the best consensus for species name or a higher taxonomic range was chosen. For further analysis all morphotypes resulting in the same species were re-checked against the pictures and compared to http://deemy.de/ and http://www.uni-goettingen.de/de/goe-fungi/92389.html and if this was in accordance they were grouped together. The Accession numbers of sequence deposition at NCBI database (KT020767-KT020824), the taxonomical classification and the groupings are listed in Supplementary Table S7.

To the EM species/genus the exploration types were annotated according to the method of Agerer (2001), resulting in contact (C), short-distance (SD), medium-distance (MD), and long-distance (LD) types. Additionally Courty et al. (2008) and http://deemy.de/ were used. Morphotypes which exploration types were not found in the literature were assigned based on the length of hyphae and rhizomorphs (own descriptions and pictures).

2.6 Pyrosequencing for root sampling on 150 plots

2.6.1 DNA Extraction, sample preparation and Pyrosequencing

The ITS 2 region or the fungal rRNA gene was used for community analysis. DNA was extracted from 50 mg freeze dried and milled root powder using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions.

The Polymerase Chain Reaction (PCR; 50 µl) for amplification of the ITS 2 region contained: 5 µl 10x*Pfu*-PCR-buffer with $MgSO_4$, 1 µl dNTP Mix (10mM) 0.5 µl *Pfu* DNA polymerase (#EP 0572, 2.5u/µl; all reagents from Thermo Fisher Scientific, Waltham, MA, USA), 2 µl of each primer and 4 µl of DNA template (diluted 1:10). The thermal cycling scheme described by (Wubet et al., 2012) was used for amplification.

The ITS 2 region was amplified with the following set of primers containing the Roche 454 pyrosequencing adaptors (underlined) followed by a key for data processing (bold) and a unique Multiplex Identifier (MIDs of 10 bp): ITS4 (White et al., 1990) 5'-CCATCTCATCCCTGCGTGTCTCCGAC-

TCAG-(dN)$_{10}$-TCCTCCGCTTATTGATATGC-3’ and ITS3 (White et al., 1990) 5’-CCTATCCCCTGTGTGCCTTGGCAGTC-**TCAG-**GCATCGATGAAGAACGCAGC-3’ (ordered at Sigma Albrich, St. Louis, MO, USA).

PCR reactions were performed in triplicate and subsequently purified by gel extraction using the Quiagen QIAquick Gel extraction Kit, (Qiagen, Hilden, Germany) following the manufacturer’s instructions. DNA concentrations of the three replicates were measured using a NanoDrop ND 1000 (Peqlab, Thermo Fischer Scientific GmbH, Waltham, MA, USA) and pooled in equimolar amounts. Sequences of the ITS 2 region were determined by the Goettingen Genomics Laboratory using a Roche GS-FLX 454 pyrosequencer (Roche, Mannheim, Germany) with Titanium chemistry (Roche, Mannheim, Germany).

2.6.2 Bioinformatical analysis

Sequence data were processed with QIIME (Caporaso et al., 2010) and other tools as descripted as follows: After raw data extraction, reads shorter than 200 bp, possessing long homopolymer stretches (> 8 bp), or primer mismatches (> 5) were removed. Subsequently, sequences were denoised employing the free software Acacia (http://sourceforge.net/projects/acaciaerrorcorr/?source= navbar Bragg et al. (2012)). Remaining primer sequences were truncated employing *cutadapt* (Martin, 2011). Chimeric sequences were removed using UCHIME in the reference mode with the most recent UNITE ITS database ((http://unite.ut.ee/; v 6.0) as reference dataset (Edgar et al., 2011; Kõljalg et al., 2013). Processed sequences of all samples were combined, sorted by decreasing length and clustered in operational taxonomic units (OTUs) at 3% genetic distance employing the UCLUST algorithm (Edgar, 2010).

Taxonomy of each OTU was determined by BLAST alignment using the QIIME assign_taxonmy.py script (Caporaso et al., 2010). A database consisting of the most recent UNITE database and all plant ITS sequences available at the NCBI database (http://www.ncbi.nlm.nih.gov/; 04.11.2014) was used as reference database to simultaneously determine the phylogenetic composition of the sample and to separate fungal and plant ITS sequences. This combination of databases was necessary to remove plant sequences.

Sequences were classified with respect to the UNITE (fungi) and NCBI (plants) taxonomy of their best hit, respectively. OTUs and corresponding sequences affiliated to plants were excluded from further

analysis. OTUs with a taxonomic assignment at the species level were classified according to their ecological lifestyle by manual literature search (for lifestyle and literature see Supplementary Table S2). If the ecological life style at the genus was known, it was added to Table S2. OTUs were categorized as arbuscular mycorrhiza (AM), ectomycorrhizal (EM), ericoid mycorrhiza, orchid mycorrhiza, endophtic, saprophytic, plant pathogen, animal pathogen, lichens, mycoparasites or nematophagous. The OTU was categorized as "unknown", if no supporting literature was found for an appropriate classification.

2.6.3 Sequence data deposition

Raw sequence data obtained by pyrosequencing was deposited in the sequence read archive (SRA) of the National Center for Biotechnology Information (NCBI) under accession number SRP049044. Those sequences contain plant und fungal originated sequences.

2.7 Statistical data analysis

Most of the statistical analyses were conducted using R 3.1.2. (2014 The R Foundation for Statistical Computing). Histogram and a Shapiro Wilk test were used to check the variables for normal distribution. If necessary they were transformed accordingly. Correlations were calculated with Pearson correlation test if data were normal distributed, if not Spearman correlation test with Bonferroni correction was used. ANOVA and Student's Tukey tests were performed. ANOVA was accepted if normal distribution of the data, the residuals of the Anova or if no pattern within the residual plot was detected. If data were not normal distributed, the variable was square root or log transformed. Percentages were arcsin root transformed before being used for statistical analysis. If after this transformation the data or residuals were not normal distribution Kruskal Wallis/ Pairwise Wilkox Test were used. For all calculations p-values <0.05 were accepted as significant. Also the boxplots for Michaelis Menten Fit (MMF) and Shannon were drawn using R. For figures, not drawn with R, Origin Pro 8.5 (Origin Lab Corp., Northampton, USA) was used.

2.7.1 Statistical data analysis for root sampling on 150 plots

As we wanted to show general biological trends, we did not split our dataset according to origin or plant species composition on plots. Alpha diversity indices (Michaelis Menten Fit (MMF) and Shannon were finally used) were calculated at 3% genetic distance and 494 sequences per plot according to Wemheuer et al. (2014). An abundance matrix with 494 sequences per plot was used for further calculations. The number of plots used per Exploratory or dominant tree species is listed in Table 2.3.

Table 2.3 Number of plots used for diversity/richness calculations, according to Exploratory and dominant tree species on the plot for the 150 plot sampling

		all fungi	EM	Saprophytes	unknown
ALB	**beech**	19	14	18	16
	spruce	12	12	11	10
HAI	**beech**	31	28	30	30
	spruce	3	3	3	3
SCH	**beech**	20	20	13	21
	pine	21	19	20	17
	oak	5	4	5	3

Some additional data, e.g. about soil characteristics, plant diversity or management indices used for calculations, particularly for all models and correlations, were generated by other working groups within the Biodiversity Exploratories (for details see Supplementary Table S1).

Rarefaction curves were calculated in R 3.1.2. (2014 The R Foundation for Statistical Computing) using the function *accumresults* within the BiodiversityR (Kindt, 2014) package. The heatmaps for the taxonomical distribution were generated with Excel 2007.

The values for venn diagrams were calculated using Venny (http://bioinfogp.cnb.csic.es/tools/venny/), but the diagrams were drawn in R using the package "VennDiagram" (Chen, 2014). To check if rare species (singletons) were important for community structure a Procrustes correlation analysis form the non-metric multidimensional scaling (NMDS) ordinations using the *protest* function (Peres-Neto and Jackson, 2001) within the *vegan* package (Oksanen et al., 2015) was performed. According pairwise ANOSIMs, were calculated in PAST (Hammer et al., 2001). For all similarity or distance calculations Bray Curtis was used. NMDS with ordisurf structures and GCV scores were calculated using the *vegan* package (Oksanen et al., 2015).

The *envfit* function from the same package was used to calculate the vectors for different variables (therefore normalized values, were calculated: $x_i_n = (x_i - mean(x))/standarddeviation(x)$).

Generalized adaptive models (gam) with quasi Poisson distribution were calculated for comparison within one variable, as this distribution fitted best in comparison to Gaussian distribution (smallest GCV score) for this data. Alternatively for a comparison between the variables gams with Gaussian distribution and z-scored variables were calculated as it was not possible to calculate quasi Poisson distribution with z-scores. The smaller the GCV score the better the model fits.

Different models were tested to filter for important variables like soil or root properties for MMF and Shannon of different lifestyles. After testing different model structures it turned out that generalized linear models (glm) with a quasi Poisson distribution were the most appropriate ones for the data (no linear Gaussian distribution (counting data, plotted as histogram → no linear models (lm); and overdispersion in glms with Poisson distribution). A model is overdispersed, if there is a greater variability within the data set than the model predicts. Therefore sigma2 is calculated: sigma2=sum(residuals(model)^2/residuals degrees of freedom). If sigma2 is much larger than 1 than the model is overdispersed. The finally used glms with quasi Poisson distribution were reduced in the number of variables by the highest p-value until all remaining variables were significant. The p-values for the variables were calculated via maximum likelihood ratio tests.

2.7.2 Statistical data analysis for Root-Trenching-Experiment

A taxonomic tree of nucleotide sequences alignments for the ITS regions was computed by MEGA6 software (Tamura et al., 2013), http://www.megasoftware.net/). Phylogenies were inferred by the Neighbour–Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. The analysis involved 57 nucleotide sequences. A *Fagus sylvatica* sequence was used as an outlier and to display not sequenced morphotypes within the phylogenetic tree. The taxonomic tree generated in MEGA6 was displayed using ITOL (Letunic and Bork, 2011), http://itol.embl.de) with additional data on their abundance during experiment duration in different treatments. Past 3.01 (Hammer et al., 2001), http://folk.uio.no/ohammer/past/) was used to perform nonmetric multidimensional scaling (NMDS) based on Bray Curtis (Bray and Curtis, 1957), Analysis of Similarities (ANOSIM) and Principal component analysis (PCA). Species Richness, Shannon diversity index (H'), Morisita Horn similarity index, Chao 1 and Chao 2 were calculated using EstimateS 190 V. 9.1 (Colwell, 2013).

2.7.3 Data deposition within the Biodiversity Exploratory database – BexIS

All data were deposited in the Biodiversity Exploratory database Bexis, with the following dataset Identification numbers (IDs; Table 2.4).

Table 2.4: BExIS IDs and names of the datasets

BExIS ID	Name of the dataset
	Sampling on 150 plots
19230	C and N concentrations of tree fine roots from soil sampling May 2011
18346	Carbohydrates in fine roots May 2011 all Forest EPs
19229	ICP conzentration of elements in fine roots soil sampling May 2011
19186	Root associated fungal community - normalized to 494 seq per plot
19168	Root associated fungal community allseq 454 Pyrosequencing
	Root Trenching Experiment
19226	Root Trenching Experiment in Hainich beech plots - succession of Ectomycorrhiza
19228	Root Trenching Experiment in Hainich beech plots - succession of Ectomycorrhiza - Exploration types
13987	Ectomycorrhiza_Trenches_Experiment (old version of the first harvest)

3. Results

3.1. Results of characterizing the root-associated fungal community in temperate forests

3.2. Results of the disturbance root trenching experiment

3 Results

3.1 Results of characterizing the root-associated fungal community in temperate forests

The aim of the present pyrosequencing study was on the one hand a characterization of the root-associated fungi. On the other hand, to investigate to what extent environmental variables such as soil properties and forest management, as well as individual tree variables such as root carbohydrate concentrations influence the root-associated fungal community. Specifically the effects on fungi with an ectomycorrhizal (EM) or a saprophytic lifestyle were addressed. First the results of the basic processing steps and the assignment of operational taxonomic units (OTUs) to different lifestyles are described. The fungal richness, diversity and community structure of the three Biodiversity Exploratory study locations, Alb, Hainich and Schorfheide, is presented before differences between the dominant tree species on the sampling plots will be regarded. Finally the main drivers for the richness, diversity and community structure of the root-associated fungal community and the different lifestyles are addressed.

3.1.1 Sequence quality control and characterisation

Pyrosequencing yielded over 1.6 million raw ITS2 sequences, of which 60% remained after processing (Figure 3.1a). De-noising and screening out chimeras removed another 1.5% of the sequences leaving around 978 000 for further analysis. A blast search in the NCBI database revealed that over 66% were of plant origin; within individual plots the percentage of sequences from plants rather than fungi varied between 0.12% and 99.90%, (Figure 3.1b) or in quantitative numbers between 4 and 16 911 sequences (mean = 4149,8 seq). As a result plots containing fewer than 494 fungal sequences (explained later) were removed from further analysis. The number of sequences per plot, remaining after quality filtering, trimming, denoising, chimera check and singleton removal is shown in Supplementary Table S3.

After this exclusion 325,797 fungal sequences remained for further analysis, of which fewer than 3% were singletons. A procrustes test showed that the NMDS ordinations from the abundance matrices with either the presence or absence of singletons were significantly correlated, so the singletons had no significant effect on the fungal community ordination (Procrustes correlation coefficient = 0.255; Procrustes sum of squares = 0.934; p=0.004).

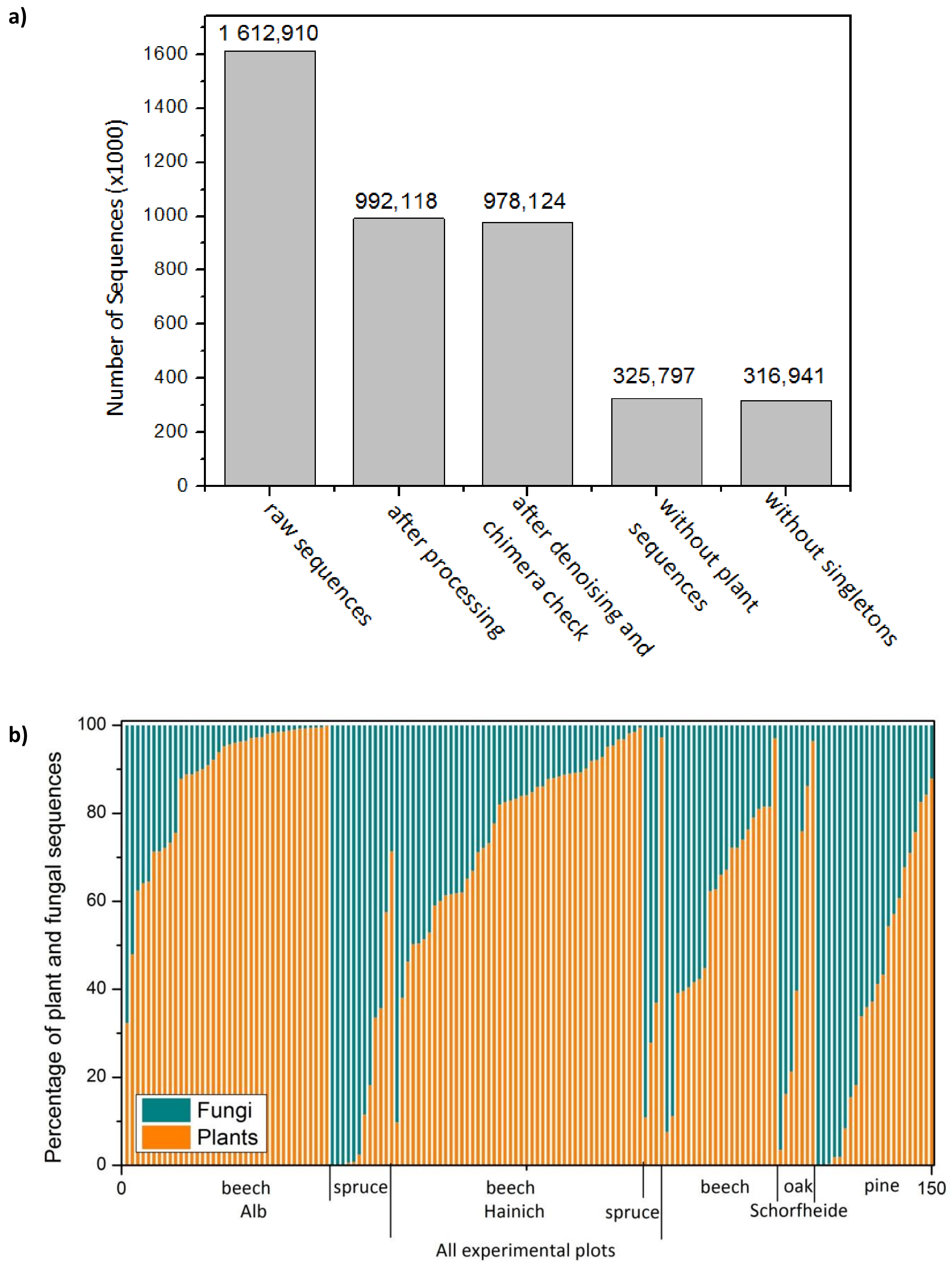


Figure 3.1: a) Numbers of sequences after different processing steps, which have been described in Material and Methods 2.6.2. b) Percentage of plant or fungal sequences per plot respectively (n=150).

3.1.2 OTU clustering and lifestyle annotation

The remaining sequences were clustered into 4,544 OTUs, of which 1,885 were annotated as ectomycorrhizal (EM) lifestyle (Table 3.1). Together with a few other mycorrhizal lifestyles, such as arbuscular mycorrhiza, ericoid mycorrhiza and orchid mycorrhiza, EM accounted for 42.9% of all OTUs and 55.5% of all sequences. Saprophytic fungi were the second most abundant group, accounting for 14.3% of all OTUs. Endophytes accounted for less than 5% (213 OTUs) and plant pathogens for less than 2% (88 OTUs). 35.2% of all OTUs could not be assigned to any lifestyle and remained unknown (Figure 3.2,Table 3.1).

Table 3.1: Total number of sequences per lifestyle (for details see Supplementary S2), resulting number of OTUs, number of sequences which were used for diversity calculations per plot (without singletons) and number of remaining plots, where enough sequences were available to calculate diversity indices.

Lifestyle	Number of Sequences	Number of OTUs	Sequences used for α-Diversity calculations per plot	Number of Plots
all fungi	325,797	4,544	494	111
ectomycorrhizal	179,250	1,885	296	100
all mycorrhiza (EM, AM, ericoid- and orchid mycorrhiza)	180,837	1,950	314	100
saprophytic	39,016	651	63	100
plant pathogen	2,461	88	-	-
endophytic	10,714	213	-	-
unknown	92,398	1,601	104	100
antagonistic to plant pathogens*	61	11		
colonizing other fungi*	17	1		
animal (specific insect) pathogen*	79(25)	12(7)		
lichen*	7	3		
mycoparasites*	22	2		
myconodules*	2	1		
nematophagous*	10	4		
root-associated without known function*	173	7		
* **summarized as “others”**	371	41	-	-

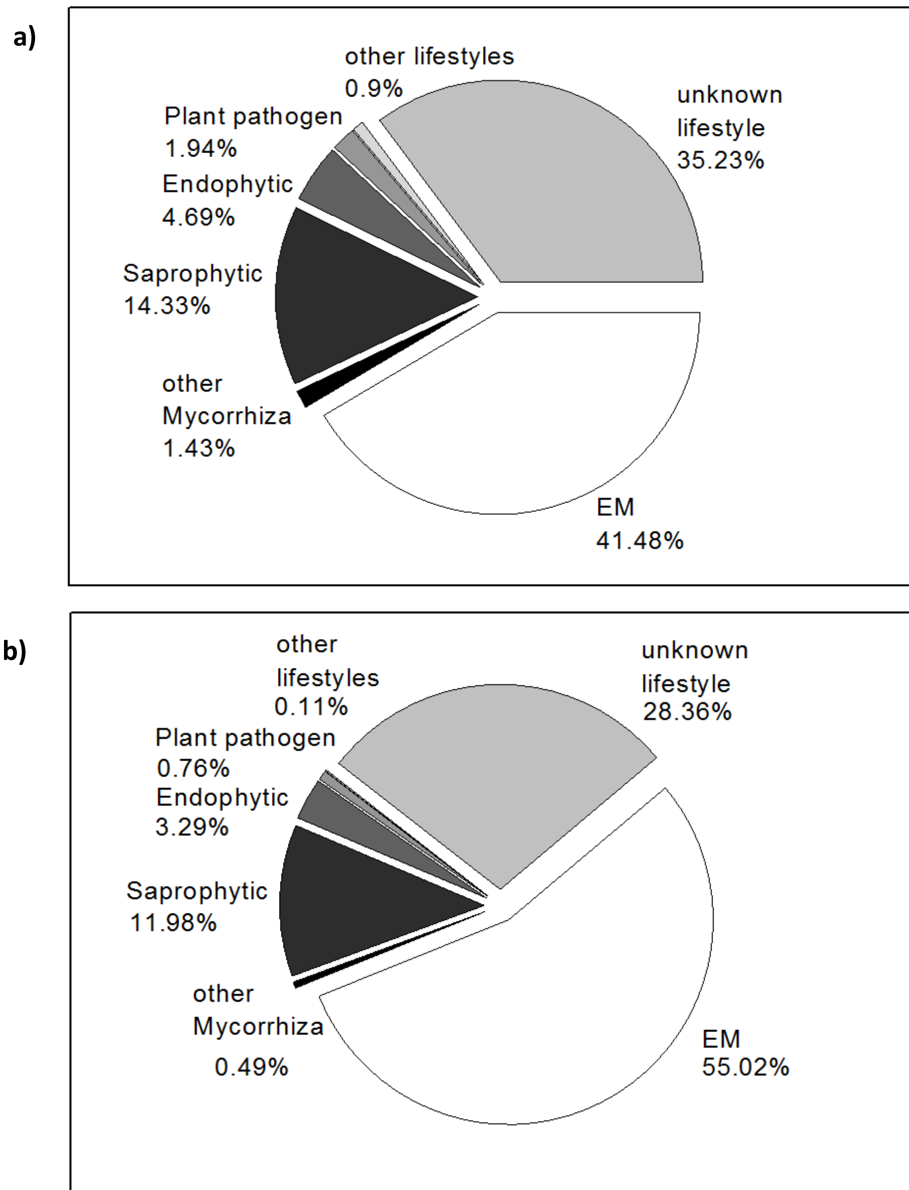


Figure 3.2: Percentage of different fungal lifestyles of the root-associated fungal community; a) percentage according to number of OTUs (100% = 4544 OTUs, b) Percentage of number of sequences (100% =325 795 sequences)

3.1.3 Root-associated fungi within the three Exploratories

For normalization the number of sequences per plot was reduced to 494 sequences, covering 96% of all OTUs. Therefore this appeared to be an appropriate cut-off for normalization and richness/diversity calculation. On this basis richness and diversity estimators as well as an abundance matrix (mean of 10 repetitions) were calculated resulting in the use of 4359 OTUs. For the different lifestyles an appropriate number of sequences were used (Table 3.1). As this abundance matrix was calculated 10 times the mean of each hit was used and could therefore lead to non- integer sequence numbers.

With the reduction on 494 Sequences per plot, for the Alb 31 plots, for Hainich 34 and for Schorfheide 46 plots remained for further analysis. At first glance this might seem unbalanced, but considering the different dominant tree species on the plots within the Exploratories within Alb from the 38 beech plots 19 and from the twelve spruce plots all remained. Within the Hainich from the 46 beech plots 31 and from the four spruce plots three remained. In Schorfheide 21 from 22 pine plots and five from seven oak plots remained. From the 21 beech plots in the Schorfheide 20 remained. Therefore the host plant communities were all sufficiently represented. Overall with the 111 remaining plots for all fungi and the 100 plots for the different lifestyles, we covered a good range of plots in relation to the dominant tree species distribution within the Biodiversity Exploratories.

3.1.3.1 Overlap of OTUs between the three Exploratories

In order to compare the OTUs between Exploratories or tree species the number of plots analysed were reduced to the lowest number of plots in this category for venn diagrams. A subset of 31 plots remained, limited by Alb. Plots from non limiting Exploratories were randomly chosen.

Nearly 400 OTUs were shared between the three Exploratories. The main overlap was found between Alb and Hainich, which shared more than 900 OTUs. Schorfheide shared around 650 OTUs with Alb and around 690 OTUs with the Hainich region. The total number of OTUs was lowest in Hainich with 1748, followed by Schorfheide with 1968 and most OTUs were obtained for Alb with 2246 (Figure 3.3 a). The EM showed a similar pattern with most OTUs shared between Alb and Hainich (441 EM OTUs), followed by the comparison between Hainich and Schorfheide (284 EM OTUs) and finally between Alb and Schorfheide (271 EM OTUs, Figure 3.3 b). Most saprophytic fungi are shared between Alb and Hainich (158 saprophytic OTUs, Figure 3 c) and again nearly the same amount between Schorfheide and Hainich (98 saprophytic OTUs) and between Schorfheide and Alb (107 saprophytic OTUs, Figure 3.3 c). The fungi with unknown lifestyle show a similar pattern like all OTUs, with most of them shared between Alb and Hainich (284 OTUs with unknown lifestyle second between Hainich and Schorfheide (243 OTUs with unknown lifestyle) and least between Alb and Schorfheide (215 OTUs with unknown lifestyle, Figure 3.3 d).

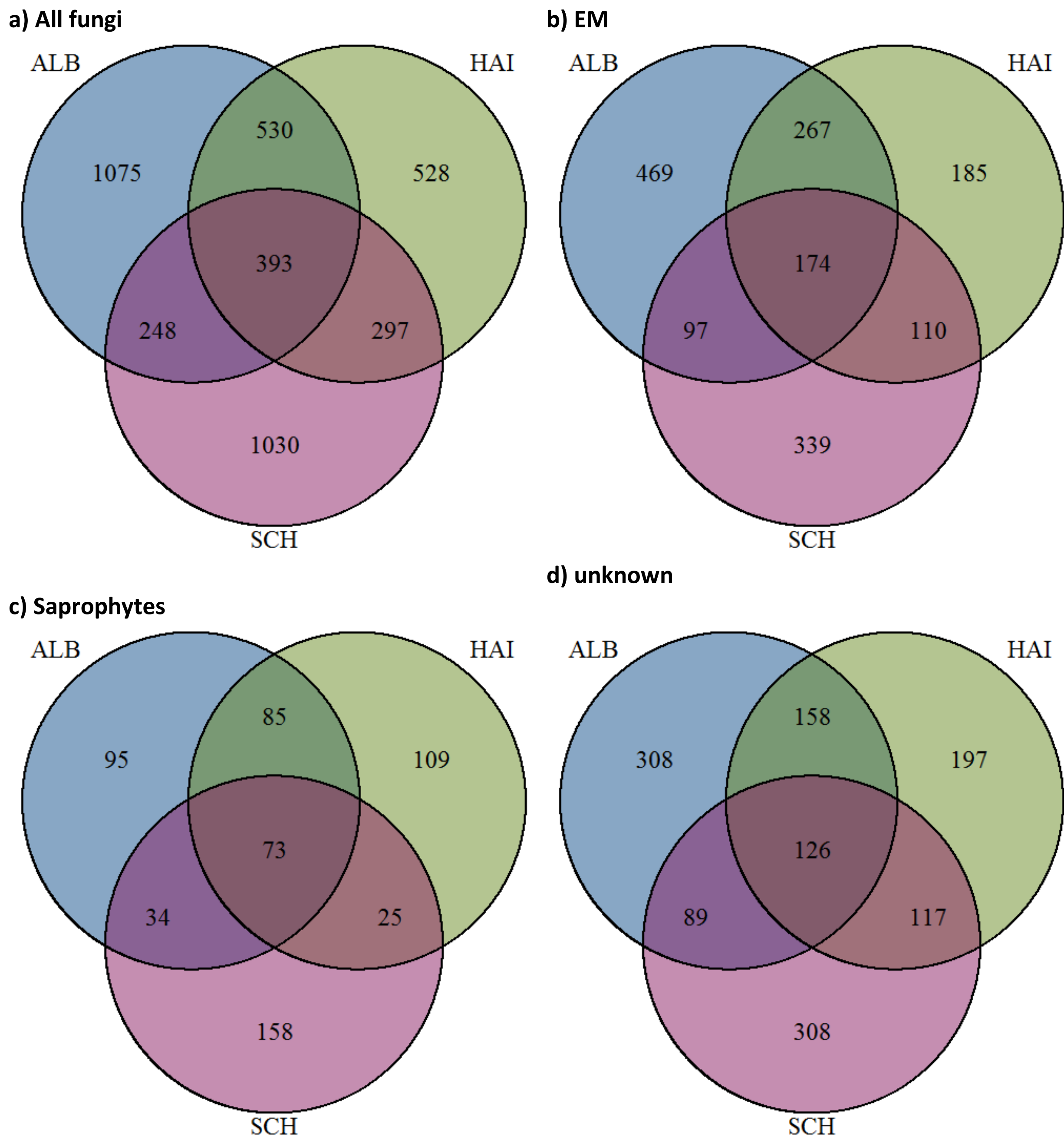


Figure 3.3: Venn diagrams representing the number and overlap of OTUs, categorised by Exploratory (n=31). a) all fungi, b) EM, c) saprophytes and d) unknown lifestyle.

3.1.3.2 Taxonomic distribution between the three Exploratories

The most abundant phylum with more than 68% of sequences and ~48% of all OTUs was the Basidiomycota, whereas Ascomycota accounted for 25.2% of sequences and nearly 39% of all OTUs (Table 3.2 a). Ascomycota were completely absent in some plots, independent from Exploratory or tree species (Figure 3.4). Around 5.4% of all sequences could not be assigned to any fungal phylum. Zygomycota accounted for 0.8% of sequences and 2.7% of all OTUs. The smallest group were the Glomeromycota with 0.04% of sequences and 0.08% of the OTUs. Within Alb there were four times more Basidiomycota sequences than Ascomycota one. Within Hainich and Schorfheide there only were twice as much Basidiomycota than Ascomycota sequences. When regarding the mean number of OTUs there was only 1.7 times more Basidiomycota than Ascomycota in the Alb and nearly the same mean number of OTUs per plot within Hainich and Schorfheide (Table 3.2 a).

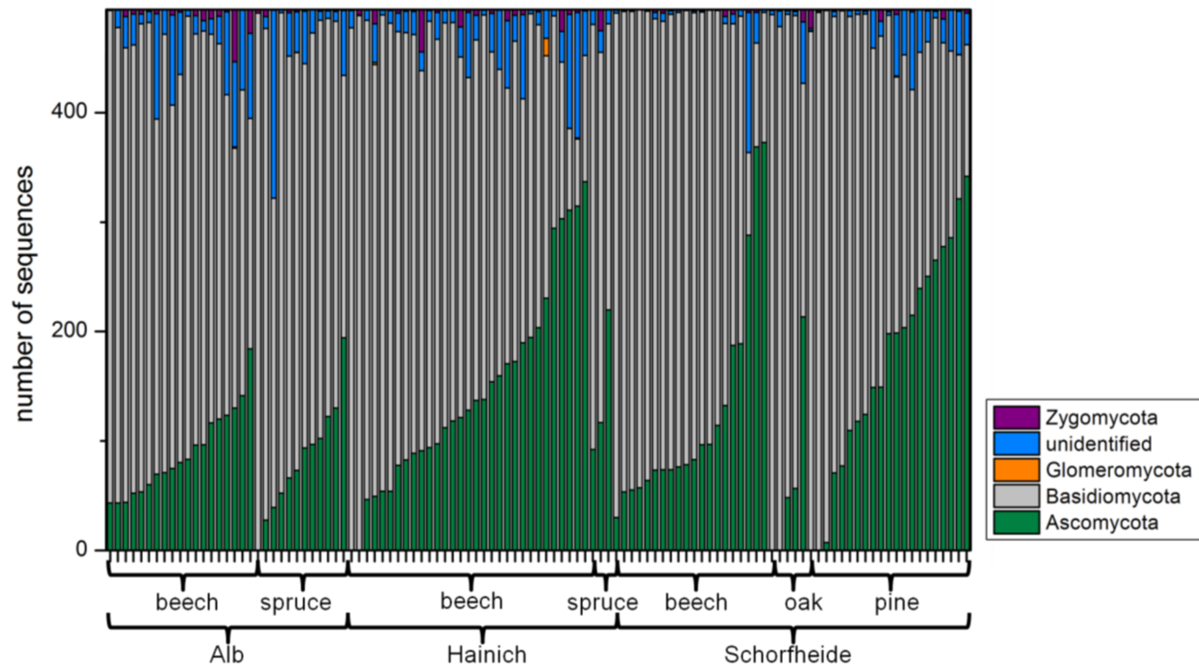
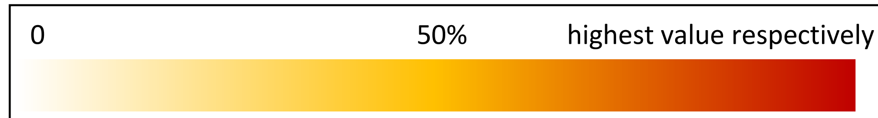


Figure 3.4: Number of Sequences per phylum per plot (total number of sequences = 494; n= 111).

Within the Basidiomycota the Agaricomycetes were the most abundant class, with nearly 68% of the sequences and 47% of all OTUs (Table 3.2 b) of which Russulales and Agaricales were the most abundant orders (Table 3.2 c). The Leotiomycetes, the most abundant order within the Ascomycetes, with 14% of sequences and 20% of all OTUs were as twice as much abundant in Schorfheide than in Alb, mostly owing to high numbers of the order Helotiales (12% of the sequences and 16% of OTUs). The class Pezizomycetes with the order Pezizales (nearly 6% of the sequences and 4.8% of the OTUs) were most abundant in Hainich.

The most abundant family were the Russulaceae (which contained 37% of sequences and 17.7% of OTUs). Most of the OTUs (Table 3.2 d, e and f) were assigned to the genus *Lactarius* (mainly an uncultured one, *Lactarius quietus* and some other species with less than 0.5% of the sequences) with more than 25% of the sequences and ~7% of all OTUs. Additionally the genus *Russula* (mainly an uncultured one, *Russula ochroleuca, Russula formula* and *Russula integra*) with 9.3% of the sequences and 7.3% of the OTUs was highly abundant. In the Hainich there was twice the number of *Lactarius* OTUs than in the other two Exploratories. The genus *Russula* was most abundant in Alb (more than three times more sequences than in Hainich and ~30% more than in Schorfheide). Also *Hygrophorus pustulatus* with 1.5% of the sequences was a highly abundant species. *Xerocomus* species reached 1.5% of the sequences and 0.7% of the OTUs and *Sebacina* species around 1.8% of the sequences and 1% of OTUs. Additionally to the genera already mentioned *Tomentella, Cenococcum* and *Tuber* were under the most abundant OTUs. All of them were affiliated to ectomycorrhizal lifestyle. *Mycena* (more than 3% of all sequences and more than 2.5% of the OTUs) and *Trechispora* (0.6% of sequences and 0.4% of OTUs) were the most abundant saprophytic genera. *Phialocephala* (1.1% of the sequences and 1.4% of OTUs), *Lachnum* species (1% of the sequences and 0.6% of OTUs) and *Tetracladium* (1.6% of the sequences and 1.5% of OTUs) were the most abundant endophytic genera. All other taxa accounted for less than 1% of the sequences and OTUs (for details see Supplementary S4.2.1).

Table 3.2: Distribution of sequences and number of OTUs according to taxonomic levels per Exploratory. This calculation is based on standardized abundance matrix with 494 sequences per plot. a) Phylum, b) Class, c) Order, d) Family, e) Genus, f) Species (mean). All taxa with a total abundance (sum of the percentage from mean number of sequences and mean number of OTUs) with more than 1% are shown (Taxa with less than 1% are additionally shown in Supplementary TableS 4.1).



a) Phylum

	Mean number of sequences per phylum per plot				Mean number of OTUs per phylum per plot			
	ALB	HAI	SCH	%	ALB	HAI	SCH	%
Basidiomycota	365.7	314.7	334.9	68.5	89.4	62.1	69.1	47.9
Ascomycota	86.6	145.7	141	25.2	52.1	62.9	63.7	38.8
unidentified	36.8	28	15.9	5.4	17.9	15.9	14.7	10.5
Zygomycota	4.8	4.9	2.2	0.8	4.3	4.6	3.5	2.7
Glomeromycota	0	0.6	0	04	06	0.24	07	08

b) Class

	Mean number of sequences per class per plot				Mean number of OTUs per class per plot			
	ALB	HAI	SCH	%	ALB	HAI	SCH	%
Basidiomycota;Agaricomycetes	363.8	313.6	332.4	68.1	87.5	61	68	47
Ascomycota;Leotiomycetes	49.2	75.6	93.5	14.7	25.2	33.8	37.6	21
unidentified;unidentified	36.8	28	15.9	5.4	17.9	15.9	14.7	10.5
Ascomycota;Pezizomycetes	14.9	51.6	21.3	5.9	7	8.9	6.2	4.8
Ascomycota;Dothideomycetes	12.9	8.4	9.4	2.1	10.1	8.6	8.6	5.9
Ascomycota;Sordariomycetes	5.6	5.8	2.9	1	5.5	6.7	3.7	3.5
Zygomycota;Incertaesedis	4.8	4.9	2.2	0.8	4.3	4.6	3.5	2.7
Ascomycota;unidentified	1.9	1.4	10	0.9	1.8	2.2	3.3	1.6
Ascomycota;Eurotiomycetes	1.3	0.7	3.2	0.3	1.5	1.3	3.2	1.3

c) Order

	Mean number of sequences per order per plot				Mean number of OTUs per order per plot			
	ALB	HAI	SCH	%	ALB	HAI	SCH	%
Basidiomycota;Agaricomycetes;Russulales	158.7	204.6	186.2	37.1	26.7	24.1	31	17.8
Basidiomycota;Agaricomycetes;Agaricales	107.4	59.2	72	16.1	28.9	17.8	17.5	13.9
Ascomycota;Leotiomycetes;Helotiales	43.8	62.2	76.1	12.3	21.3	25.3	28.6	16.3
unidentified;unidentified;unidentified	36.8	28	15.9	5.4	17.9	15.9	14.7	10.5
Ascomycota;Pezizomycetes;Pezizales	14.9	51.6	21.3	5.9	7	8.9	6.1	4.8
Basidiomycota;Agaricomycetes;Thelephorales	22.2	21.2	19.5	4.2	9.9	6.8	5.1	4.7
Basidiomycota;Agaricomycetes;Sebacinales	35.6	13.1	1.5	3.4	7	3.7	0.7	2.5
Basidiomycota;Agaricomycetes;Atheliales	24.2	8.1	6.2	2.6	8.7	3.9	2	3.2
Ascomycota;Leotiomycetes;unidentified	1.3	8.1	15.4	1.7	0.8	4.6	6.4	2.6
Basidiomycota;Agaricomycetes;Boletales	2.1	2.2	30.2	2.3	0.7	1.1	5.6	1.6
Zygomycota;Incertaesedis;Mortierellales	4.7	3.7	1.7	0.7	4.1	4.2	2.5	2.4
Ascomycota;Leotiomycetes;Incertaesedis	3.6	4.9	1.3	0.7	2.9	3.6	2	1.9
Ascomycota;unidentified;unidentified	1.9	1.4	10	0.9	1.8	2.2	3.3	1.6
Ascomycota;Dothideomycetes;Incertaesedis	2.6	1.2	3.7	0.5	2.5	2.2	3.6	1.8
Ascomycota;Dothideomycetes;Hysteriales	4.5	1.5	3.9	0.7	2.8	1.4	3.3	1.6
Ascomycota;Sordariomycetes;Hypocreales	2	3.6	0.3	0.4	2.9	3.5	1.3	1.7
Basidiomycota;Agaricomycetes;Trechisporales	2.3	0.3	9	0.8	1.3	0.6	2.9	1.1
Ascomycota;Dothideomycetes;unidentified	3.3	2.2	0.7	0.4	2.8	2.1	0.8	1.3
Basidiomycota;Agaricomycetes;Cantharellales	4.1	4	0.7	0.6	1.6	1.5	0.6	0.8
Ascomycota;Dothideomycetes;Pleosporales	1.7	3.2	1.1	0.4	1.4	2.3	0.7	1
Basidiomycota;Agaricomycetes;unidentified	0.6	0.2	4.8	0.4	1	0.4	1.7	0.7
Ascomycota;Eurotiomycetes;Eurotiales	0.3	0.2	2.7	0.2	0.6	0.5	2.7	0.8

d) Family

	Mean number of sequences per family per plot				Mean number of OTUs per family per plot			
	ALB	HAI	SCH	%	ALB	HAI	SCH	%
Basidiomycota;Agaricomycetes;Russulales;Russulaceae	158.7	204.6	186.1	37.1	26.6	24.1	30.9	17.7
unidentified;unidentified;unidentified;unidentified	36.8	28	15.9	5.4	17.9	15.9	14.7	10.5
Basidiomycota;Agaricomycetes;Agaricales;Mycenaceae	28.5	13.9	54.3	6.5	5.2	4.4	8.9	4
Ascomycota;Leotiomycetes;Helotiales;unidentified	17.3	18.7	18	3.6	8.4	9.7	10.7	6.3
Basidiomycota;Agaricomycetes;Thelephorales;Thelephoraceae	22.1	21.2	19.5	4.2	9.8	6.8	5.1	4.7
Ascomycota;Pezizomycetes;Pezizales;Pyronemataceae	10.8	47.6	14	4.9	4.5	7	3.7	3.3
Ascomycota;Leotiomycetes;Helotiales;Helotiaceae	7.5	12	33.2	3.6	3	4.2	8.9	3.5
Basidiomycota;Agaricomycetes;Sebacinales;Sebacinaceae	35.6	13.1	1.5	3.4	7	3.7	0.7	2.5
Basidiomycota;Agaricomycetes;Atheliales;Atheliaceae	22.8	7.8	6.2	2.5	7.5	3.5	2	2.8
Basidiomycota;Agaricomycetes;Agaricales;Tricholomataceae	15.5	6.4	6.7	1.9	5.6	3.4	3.4	2.7
Ascomycota;Leotiomycetes;Helotiales;Incertaesedis	6.7	21.1	1.1	2	3.2	6.8	1.5	2.5
Basidiomycota;Agaricomycetes;Agaricales;Hygrophoraceae	24	12.3	1.4	2.5	5.6	1.9	0.5	1.7
Ascomycota;Leotiomycetes;unidentified;unidentified	1.3	8.1	15.4	1.7	0.8	4.6	6.4	2.6
Basidiomycota;Agaricomycetes;Boletales;Boletaceae	2	2	26.7	2.1	0.6	1	5	1.4
Basidiomycota;Agaricomycetes;Agaricales;Inocybaceae	14.8	7.7	0.7	1.6	4.7	2.5	0.8	1.7
Basidiomycota;Agaricomycetes;Agaricales;Cortinariaceae	13.1	8.5	2.1	1.6	3.9	2.4	1	1.6
Ascomycota;Leotiomycetes;Helotiales;Vibrisseaceae	9.3	1.3	8.3	1.3	3.7	1	3.3	1.8
Zygomycota;Incertaesedis;Mortierellales;Mortierellaceae	4.5	3.7	1.6	0.7	3.9	4.1	2.3	2.2
Ascomycota;Leotiomycetes;Incertaesedis;Incertaesedis	3.6	4.9	1.3	0.7	2.9	3.6	2	1.9
Ascomycota;unidentified;unidentified;unidentified	1.9	1.4	10	0.9	1.8	2.2	3.3	1.6
Ascomycota;Dothideomycetes;Hysteriales;Gloniaceae	4.5	1.5	3.9	0.7	2.8	1.4	3.3	1.6
Ascomycota;Leotiomycetes;Helotiales;Dermateaceae	1.9	3.9	5.5	0.8	2.4	2.7	2.1	1.6
Ascomycota;Leotiomycetes;Helotiales;Hyaloscyphaceae	1.1	5.2	10	1.1	0.5	0.9	2.1	0.8
Ascomycota;Dothideomycetes;unidentified;unidentified	3.3	2.2	0.7	0.4	2.8	2.1	0.8	1.3
Ascomycota;Dothideomycetes;Incertaesedis;Myxotrichaceae	0.8	0.5	3.3	0.3	1	0.9	3.2	1.1
Basidiomycota;Agaricomycetes;Cantharellales;Clavulinaceae	2.7	3.9	0.6	0.5	1.3	1.2	0.5	0.7
Basidiomycota;Agaricomycetes;Agaricales;Strophariaceae	2	3.9	0.8	0.5	0.9	1.6	0.4	0.6

	Mean number of sequences per family per plot				Mean number of OTUs per family per plot			
	ALB	HAI	SCH	%	ALB	HAI	SCH	%
Basidiomycota;Agaricomycetes;unidentified;unidentified	0.6	0.2	4.8	0.4	1	0.4	1.7	0.7
Basidiomycota;Agaricomycetes;Trechisporales;Hydnodontaceae	1.2	0	8.1	0.6	0.3	0.1	1.4	0.4
Ascomycota;Pezizomycetes;Pezizales;Tuberaceae	2.3	2.4	1.5	0.4	1.4	0.9	0.4	0.6

e) Genus

	Mean number of sequences per genus per plot				Mean number of OTUs per genus per plot			
	ALB	HAI	SCH	%	ALB	HAI	SCH	%
Basidiomycota;Agaricomycetes;Russulales;Russulaceae;unidentified (!with uncultured *Lactarius*)	78.8	168.7	94	23	10.4	13.7	12.7	8
Basidiomycota;Agaricomycetes;Russulales;Russulaceae;*Russula*	69	19.6	49.1	9.3	14.1	6.8	12.7	7.3
unidentified;unidentified;unidentified;unidentified;unidentified	36.8	28	15.9	5.4	17.9	15.9	14.7	10.5
Ascomycota;Leotiomycetes;Helotiales;unidentified;unidentified	17.3	18.7	18	3.6	8.4	9.7	10.7	6.3
Ascomycota;Pezizomycetes;Pezizales;Pyronemataceae;unidentified	8.9	42.9	12.9	4.4	3.6	5.8	3.1	2.7
Basidiomycota;Agaricomycetes;Thelephorales;Thelephoraceae; unidentified	16.4	19.3	14.8	3.4	7.5	5.7	3.7	3.7
Basidiomycota;Agaricomycetes;Russulales;Russulaceae;*Lactarius*	10.9	15.6	38.8	4.4	2.1	3.4	5.1	2.3
Ascomycota;Leotiomycetes;Helotiales;Helotiaceae;unidentified	7.1	8.5	33	3.3	2.6	2.8	8.5	3
Basidiomycota;Agaricomycetes;Agaricales;Mycenaceae;*Mycena*	24.5	12.6	13.1	3.4	4.1	3.7	3.9	2.5
Basidiomycota;Agaricomycetes;Agaricales;Mycenaceae;unidentified	3.9	1.2	41.2	3.1	1.1	0.7	5	1.5
Basidiomycota;Agaricomycetes;Sebacinales;Sebacinaceae;*Sebacina*	30.5	10.8	1.4	2.9	4.8	2.5	0.5	1.7
Ascomycota;Leotiomycetes;unidentified;unidentified;unidentified	1.3	8.1	15.4	1.7	0.8	4.6	6.4	2.6
Basidiomycota;Agaricomycetes;Agaricales;Hygrophoraceae; *Hygrophorus*	21.4	11.5	1.4	2.3	4.4	1.4	0.5	1.4
Basidiomycota;Agaricomycetes;Atheliales;Atheliaceae;unidentified	13.9	6	5.6	1.7	4.3	2.6	1.5	1.8
Basidiomycota;Agaricomycetes;Agaricales;Tricholomataceae; unidentified	8.7	6	5.9	1.4	3.7	2.8	3.2	2.1

	Mean number of sequences per genus per plot				Mean number of OTUs per genus per plot			
	ALB	HAI	SCH	%	ALB	HAI	SCH	%
Ascomycota;Leotiomycetes;Helotiales;Incertaesedis;*Tetracladium*	5.5	18.3	0.1	1.6	2.1	4.6	0.3	1.5
Basidiomycota;Agaricomycetes;Agaricales;Inocybaceae;*Inocybe*	12.9	7.3	0.5	1.4	3.7	2.1	0.3	1.3
Basidiomycota;Agaricomycetes;Agaricales;Cortinariaceae; unidentified	12	8.4	0.4	1.4	3.1	2.2	0.4	1.2
Ascomycota;unidentified;unidentified;unidentified;unidentified	1.9	1.4	10	0.9	1.8	2.2	3.3	1.6
Ascomycota;Leotiomycetes;Helotiales;Vibrisseaceae;*Phialocephala*	8.9	1.2	5.7	1.1	3.3	0.8	2.4	1.4
Ascomycota;Dothideomycetes;Hysteriales;Gloniaceae;*Cenococcum*	4.5	1.5	3.9	0.7	2.8	1.4	3.3	1.6
Zygomycota;Incertaesedis;Mortierellales;Mortierellaceae; *Mortierella*	2.5	2.1	1.4	0.4	3	2.8	1.9	1.7
Basidiomycota;Agaricomycetes;Boletales;Boletaceae;*Xerocomus*	1.7	1.2	19	1.5	0.4	0.3	1.7	0.5
Ascomycota;Leotiomycetes;Incertaesedis;Incertaesedis; Meliniomyces	3.1	4.4	1.1	0.6	2.2	2.6	1.5	1.4
Ascomycota;Dothideomycetes;unidentified;unidentified; unidentified	3.3	2.2	0.7	0.4	2.8	2.1	0.8	1.3
Ascomycota;Leotiomycetes;Helotiales;Hyaloscyphaceae;*Lachnum*	1	5	9.1	1	0.4	0.5	1.8	0.6
Ascomycota;Leotiomycetes;Helotiales;Dermateaceae; *Cryptosporiopsis*	1.4	2.5	0.9	0.3	1.8	2.1	0.7	1
Basidiomycota;Agaricomycetes;Sebacinales;Sebacinaceae; unidentified	5.1	2.3	0.1	0.5	2.2	1.3	0.2	0.8
Basidiomycota;Agaricomycetes;Boletales;Boletaceae;unidentified	0.3	0.7	6.2	0.5	0.2	0.5	2.6	0.7
Ascomycota;Dothideomycetes;Incertaesedis;Myxotrichaceae; *Oidiodendron*	0.7	0.4	2.6	0.3	0.9	0.8	2.4	0.9
Basidiomycota;Agaricomycetes;unidentified;unidentified; unidentified	0.6	0.2	4.8	0.4	1	0.4	1.7	0.7
Basidiomycota;Agaricomycetes;Trechisporales; Hydnodontaceae;*Trechispora*	1.2	0	8.1	0.6	0.3	0.1	1.4	0.4
Ascomycota;Pezizomycetes;Pezizales;Tuberaceae;*Tuber*	2.3	2.4	1.5	0.4	1.4	0.9	0.4	0.6

f) Species

	Mean number of sequences per species per plot				Mean number of OTUs per species per plot			
	ALB	HAI	SCH	%	ALB	HAI	SCH	%
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; uncultured *Lactarius*	67.3	166.1	75	20.8	6.1	11.3	6.4	5.2
unidentified; unidentified; unidentified; unidentified; uncultured fungus	34.5	27.1	14.9	5.2	16.3	14.7	13.4	9.6
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Russula ochroleuca*	7	9.3	35.3	3.5	3.3	3.8	8.5	3.4
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae; Pyronemataceae sp	4.2	41.7	12.7	4	1.9	5.1	3	2.2
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Russula firmula*	48.2	7	0.2	3.7	6.2	1.3	0.2	1.7
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Helotiales sp 3 BB 2010	4.7	8.2	27	2.7	1.3	2.4	6	2.1
Basidiomycota; Agaricomycetes; Agaricales; Mycenaceae; Mycenaceae sp.	3.9	1.2	41.2	3.1	1.1	0.7	5	1.5
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Lactarius quietus*	0.2	9.4	35.4	3	0.3	2	4.2	1.4
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; uncultured *Russula*	7.4	2	13	1.5	2.9	1.8	5.4	2.2
Ascomycota; Leotiomycetes; Helotiales; unidentified; uncultured Hyaloscyphaceae	8.6	10.8	3.7	1.6	2.8	4.9	2.2	2.1
Ascomycota; Leotiomycetes; unidentified; unidentified; uncultured Leotiomycetes	0.5	8	10.8	1.3	0.5	4.5	5.3	2.2
Basidiomycota; Agaricomycetes; Agaricales; Tricholomataceae; uncultured *Mycena*	7.3	5.6	3.6	1.1	3.3	2.4	2.5	1.8
Basidiomycota; Agaricomycetes; Sebacinales; Sebacinaceae; *Sebacina* sp.	22.6	3.4	0.2	1.8	3.2	1.4	0.3	1.1
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; uncultured *Tomentella*	8.2	5.9	4.5	1.3	2.8	2.4	1.8	1.5
Basidiomycota; Agaricomycetes; Agaricales; Mycenaceae; *Mycena* sp. 1 KO 2013	18.4	6.9	2.9	1.9	1.7	1.1	0.8	0.8
Ascomycota; Leotiomycetes; Helotiales; Incertaesedis; *Tetracladium* sp.	4.9	16.9	0.1	1.5	1.7	3.4	0.3	1.2
Ascomycota; Leotiomycetes; Helotiales; unidentified; Helotiales sp	4.4	3.7	2.2	0.7	2.7	2.4	2.6	1.7
Basidiomycota; Agaricomycetes; Agaricales; Hygrophoraceae; *Hygrophorus pustulatus*	13.8	8.5	0	1.5	2.5	0.7	0.1	0.7

	Mean number of sequences per species per plot				Mean number of OTUs per species per plot			
	ALB	HAI	SCH	%	ALB	HAI	SCH	%
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; uncultured Thelephoraceae	2.6	9.5	6.7	1.3	1.3	1.8	1.1	0.9
Basidiomycota; Agaricomycetes; Agaricales; Cortinariaceae; uncultured *Inocybe*	10.3	6.6	0.1	1.1	2.3	1.8	0.2	0.9
Basidiomycota; Agaricomycetes; Boletales; Boletaceae; *Xerocomus* sp.	1.7	1.2	19	1.5	0.4	0.3	1.7	0.5
Ascomycota; Dothideomycetes; Hysteriales; Gloniaceae; uncultured *Cenococcum*	3.9	1.3	3.7	0.6	2.2	1.1	2.7	1.3
Basidiomycota; Agaricomycetes; Agaricales; Mycenaceae; *Mycena* sp.	2.3	3.2	8.1	0.9	1	1.4	1.8	0.9
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; Thelephoraceae sp.	4.4	3	1.2	0.6	2.7	0.9	0.4	0.9
Basidiomycota; Agaricomycetes; Atheliales; Atheliaceae; Atheliaceae sp.	2.4	5	2	0.6	1.1	1.7	0.6	0.8
Ascomycota; Leotiomycetes; Helotiales; Vibrisseaceae; *Phialocephala* sp .KO 2013	5.1	0.6	5.2	0.7	1.3	0.3	1.2	0.6
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; Russulaceae sp.	4.1	0.5	6	0.7	1.4	0.4	0.8	0.6
Ascomycota; Dothideomycetes; unidentified; unidentified; Dothideomycetes sp.	3.1	1.6	0.5	0.4	2.5	1.1	0.7	0.9
Ascomycota; Leotiomycetes; Helotiales; unidentified; uncultured Helotiales	0.6	1.6	1.7	0.3	1	1.5	1.8	0.9
Ascomycota; Leotiomycetes; Incertaesedis; Incertaesedis; Meliniomyces sp.	2	2.1	0.2	0.3	1.4	2.1	0.6	0.9
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Russula integra*	8.7	0	1.1	0.7	1.8	0	0.3	0.5
Basidiomycota; Agaricomycetes; Atheliales; Atheliaceae; uncultured *Tylospora*	9.3	0.1	0	0.6	1.7	0.2	0	0.4
Ascomycota; unidentified; unidentified; unidentified; *Ascomycota* sp.6; RB; 2011	0.5	0.1	8.9	0.6	0.2	0.2	1.5	0.4
Basidiomycota; Agaricomycetes; Trechisporales; Hydnodontaceae; *Trechispora* sp.	1.2	0	8.1	0.6	0.3	0.1	1.4	0.4
Basidiomycota; Agaricomycetes; Boletales; Boletaceae; uncultured *Xerocomus*	0.3	0.3	5.9	0.4	0.2	0.4	2	0.5

3.1.3.3 Richness and Diversity within the three Exploratories

The total number of OTUs and diversity estimators are dependent on the number of sequences used for their calculation, as the OTU rarefaction curves still increase with increasing number of plots (Figure 3.5). To avoid bias due to different sequence abundances the number of sequences per plots was limited to 494 sequences or accordingly for lifestyle lower, for richness and diversity calculation as described above (Table 3.1). On this basis the numbers of OTUs per Exploratory/dominant tree species were compared. Lifestyles other than EM or saprophytic fungi were too rare to calculate reliable richness estimators. Therefore further analysis address all fungi, EM, saprophytes and unknown fungi.

Species richness was calculated applying the Michaelis Menten Fit (MMF) in addition to Shannon diversity index, which is based on species abundance. Remarkably the comparison of morphotyping based abundance of five fungal species did not correlate with OTU sequence abundance of the same fungi, except for *Russula integra* (p=0.015, rho=0.414). *Amphinema byssoide*s (not found as OTU), *Cenococcum geophilum, Hymenogaster griseus* (not found as OTU), *Inocybe geophylla, Lactarius subsericatus* (not found as OTU), *Lactarius subdulcis, Russula acrifolia* and *Tricholoma orirubens* (not found as OTU) were sequenced on species level from morphotyping and were therefore candidates for the comparison to OTU abundance, however their abundances did not correlate. Therefore the abundance based Shannon Index needs to be regarded with suspicion.

The results show that the richness of all fungi is significantly higher in Alb than in Hainich (Figure 3.5 and Figure 3.6). The rarefaction curves, especially that one of Alb plots still show an increase in species richness but are already levelling off. The diversity of all fungi is significantly higher in Alb plots in comparison to the other two Exploratories. Furthermore within the Alb plots EM richness and diversity are significantly higher compared to Hainich or Schorfheide. The richness and diversity of saprophytes is significantly lower in Schorfheide. Unknown fungi richness and diversity is significantly higher in Alb in comparison to Schorfheide (Table 3.6).

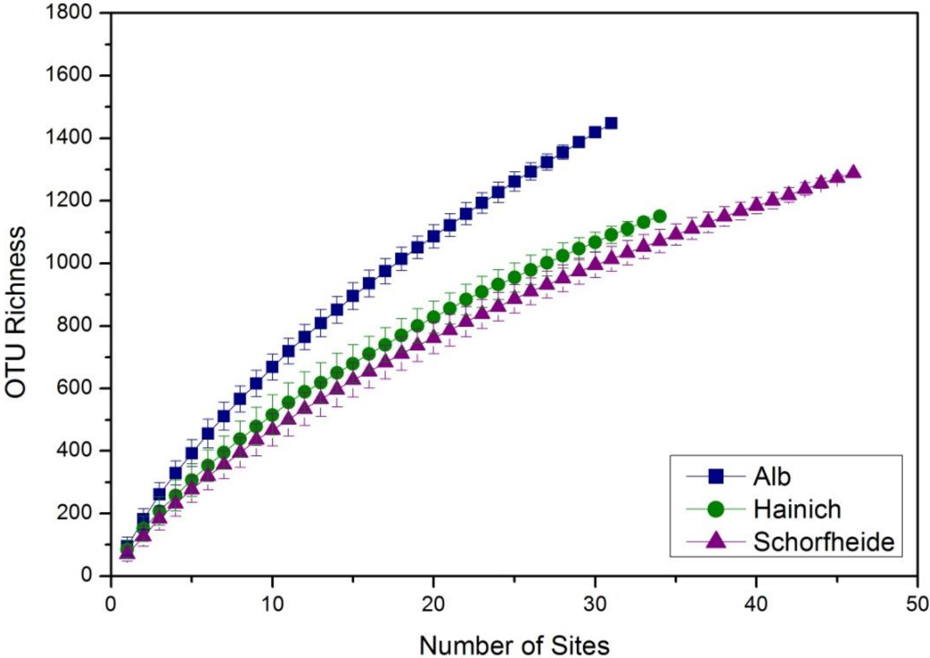


Figure 3.5: Rarefaction curve by Michaelis Menten for the three Exploratories with a normalized abundance matrix (Alb n=31; Hainich n=34, Schorfheide n=46)

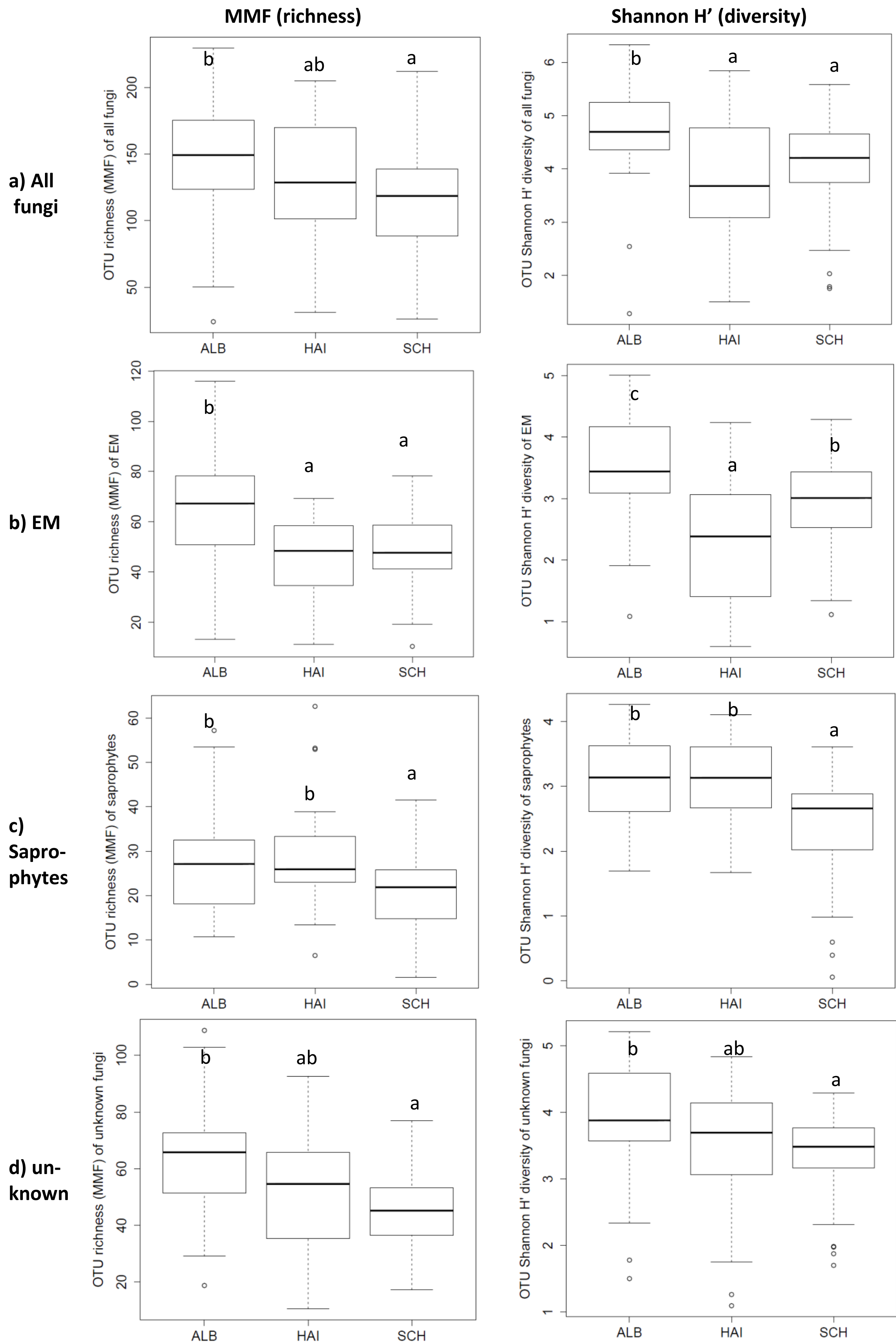

MMF (richness)
Shannon H' (diversity)
a) All fungi
b) EM
c) Sapro-phytes
d) un-known
OTU richness (MMF) of all fungi
OTU Shannon H' diversity of all fungi
OTU richness (MMF) of EM
OTU Shannon H' diversity of EM
OTU richness (MMF) of saprophytes
OTU Shannon H' diversity of saprophytes
OTU richness (MMF) of unknown fungi
OTU Shannon H' diversity of unknown fungi
ALB
HAI
SCH

Figure 3.6: Rarefied OTU richness and diversity per plot for a) all fungi, b) EM, c) Saprophytes and d) unknown fungi, separated by Exploratory. For corresponding numbers of plots and sequences see Table 3.1 and for p-values see Table 3.3 (n= c.f. Table 3.1)

Table 3.3: Comparisons of richness, represented by MMF from all fungi, EM, Saprophytes and unknown fungi, separated by Exploratory (p-vales from Anova (global) & TukeyHSD). Corresponding graphs see Figure 3.6.

	MMF				Shannon			
	All fungi	EM	Saprophytes	unknown	All fungi	EM	Saprophytes	unknown
global	**0.002**	**<0.001**	**0.003**	0.475	**<0.001**	**<0.001**	**<0.001**	**0.011**
ALB-HAI	0.127	**<0.001**	0.789	0.111	**<0.001**	**<0.001**	0.994	0.209
ALB-SCH	**<0.001**	**<0.001**	**0.033**	**0.002**	**0.003**	**0.016**	**0.002**	**0.007**
SCH-HAI	0.228	0.930	**0.003**	0.285	0.811	**0.014**	**<0.001**	0.361

When comparing the estimated number of OTUs (richness by MMF) to the observed number of OTUs for all fungi, in general over 60% of the estimated OTUs were detected within the samples (Table 3.4). For EM it was a little more than for all fungi, reaching nearly 70% within Schorfheide. Saprophytic fungi also reached over 60% in all three Exploratories, whereas unknown fungi did not fully reach 60% and more than 43% of them remained undetected (Table 3.4).

Table 3.4: Mean ± SE from the percentage of OTUs found in comparison to OTUs estimated by MMF per Exploratory

	All fungi		EM		Saprophytic		unknown	
Alb	63.56	±5.36	67.35	±5.93	63.94	±9.59	54.10	±7.59
Hainich	60.79	±6.31	64.05	±7.89	61.36	±9.67	57.36	±9.21
Schorfheide	65.16	±5.17	69.27	±7.58	64.74	±9.93	58.33	±6.86

3.1.3.4 Comparison of the fungal community structure between the three Exploratories

Comparing the fungal communities of the three Exploratories, based on an abundance matrix with equal (494) number of sequences per plot, the community of all fungi differed significantly between the three Exploratories (Figure 3.7 a, Table 3.5). The difference is highest between Alb and Schorfheide. The fungal community within the Hainich is only a little more different to Schorfheide than to Alb. Within the three Exploratories also the communities of EM fungi were significant different (Figure 3.7 b, Table 3.5). But EM fungal communities were less different between the Exploratories than the whole fungal communities were. The EM communities within Hainich and Schorfheide were more similar than both in comparison to Alb. Alb and Schorfheide had the highest R value (R=0.343) and were therefore more separated than Alb and Hainich. The three Exploratories differed in their saprophytic fungal communities, whereas Alb and Hainich were most similar (Figure 3.7 c, Table 3.5). Unknown fungi show a similar pattern than all or EM fungi (Figure 3.7 d, Table 3.5).

Table 3.5: Global and pair wise R and p-values of ANOSIM comparisons of the different lifestyles for similarities between Exploratories (Bray-Curtis with Bonferroni correction; n= c.f. Table 3.1).

	All		**EM**		**Saprophytes**		**unknown**	
	R	**p**	**R**	**p**	**R**	**p**	**R**	**p**
Exploratory global	0.331	**<0.001**	0.220	**<0.001**	0.352	**<0.001**	0.278	**<0.001**
ALB - HAI	0.245	**<0.001**	0.203	**<0.001**	0.080	**0.005**	0.230	**<0.001**
ALB - SCH	0.470	**<0.001**	0.343	**<0.001**	0.490	**<0.001**	0.365	**<0.001**
HAI - SCH	0.287	**<0.001**	0.138	**0.002**	0.431	**<0.001**	0.238	**<0.001**

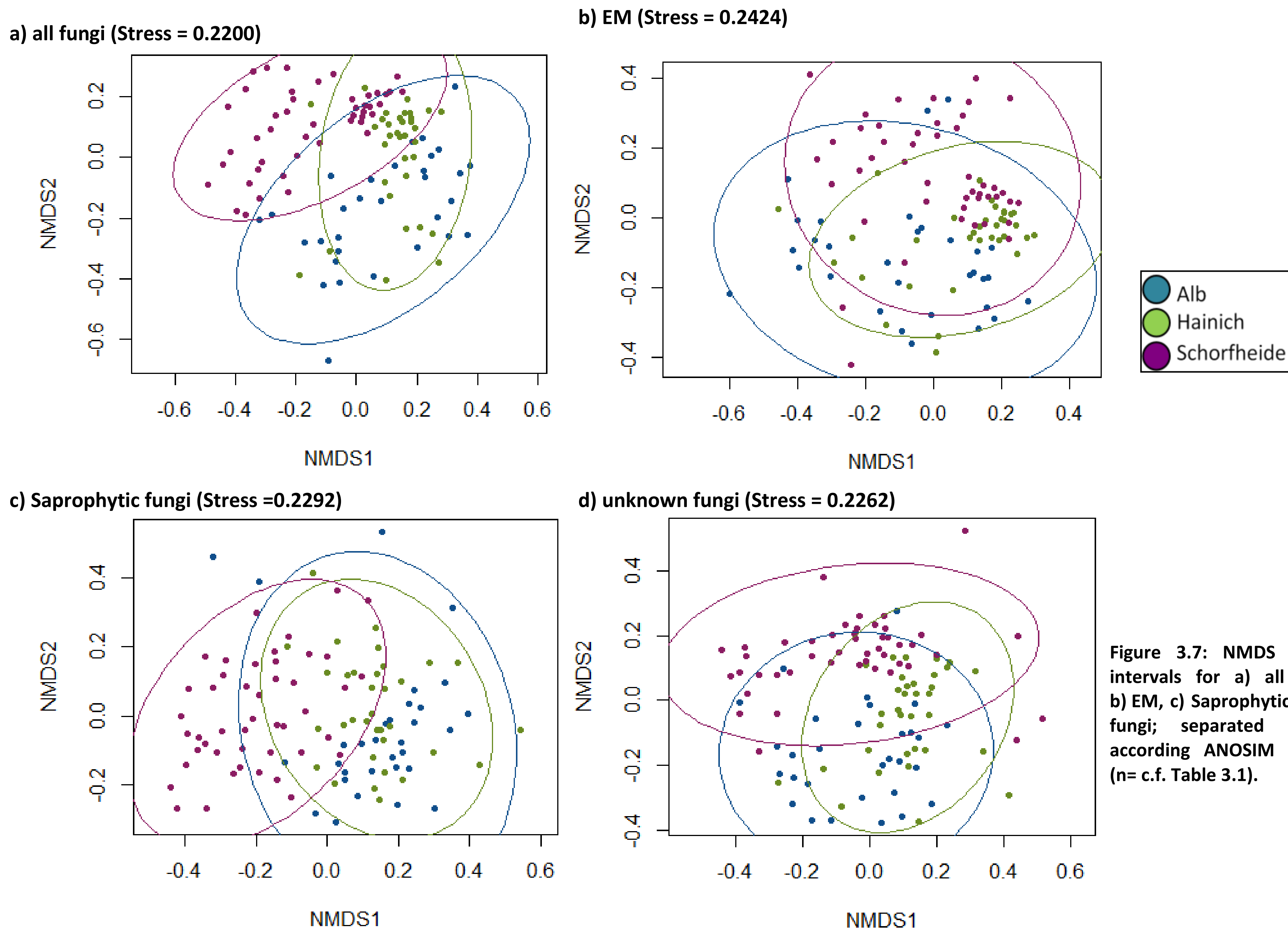


Figure 3.7: NMDS with 95% confidence intervals for a) all root-associated fungi b) EM, c) Saprophytic fungi and d) unknown fungi; separated by Exploratory. For according ANOSIM results see Table 3.5 (n= c.f. Table 3.1).

3.1.4 Root-associated fungi on plots, dominated by different tree species

3.1.4.1 Overlap of OTUs between plots, dominated by different tree species

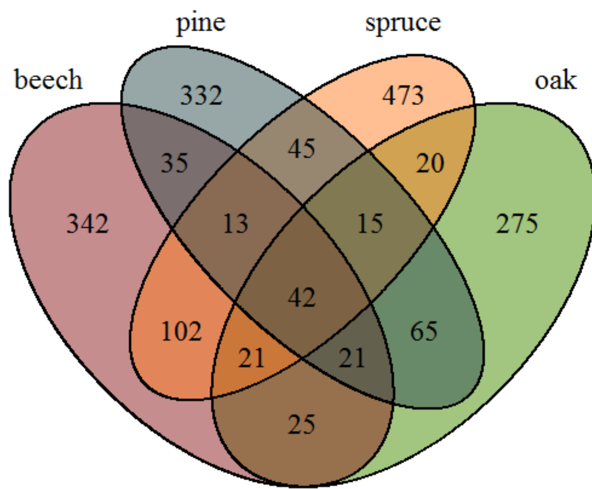
When considering only five randomly selected plots per dominant tree species (the maximum of oak plots) 42 OTUs were shared between beech, oak, spruce and pine. Spruce and Beech with 178 and oak and pine with 143 OTUs shared most fungi (Figure 3.8 a). If 15 plots, the maximum of spruce plots, were considered for beech, spruce and pine over 200 OTUs were shared between all tree species (Figure 3.8 b)). Spruce shared nearly the same amount of OTUs with beech and pine, around 430 OTUs, whereas beech and pine shared a little less with around 400 OTUs.

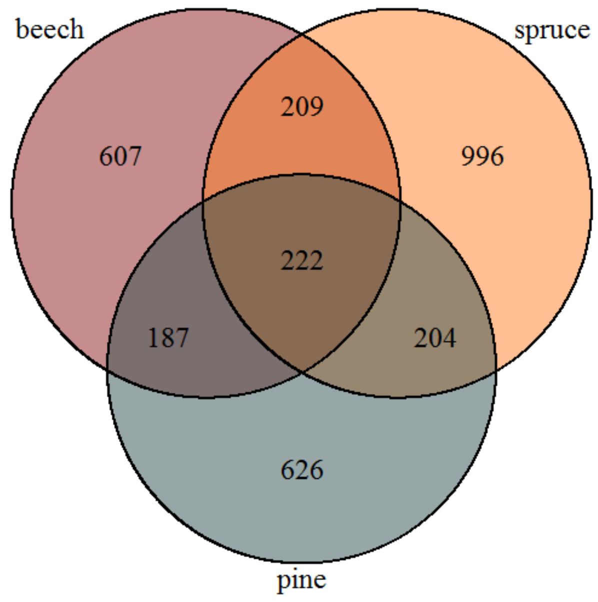
When grouping the fungi according to their lifestyle, 82 EM OTUs were shared between beech, spruce and pine plots. Pine plots shared twice as many OTUs with ectomycorrhizal lifestyle with beech than with spruce (Figure 3.8 b). With 91 OTUs a little less EM-OTUs were shared between beech and spruce than between beech and pine. The number of saprophytic OTUs shared between the different tree species was very similar (Figure 3.8 c). Reardeles which combination of beech spruce and pine was considered, they always shared around 60 OTUs, but the coniferes showed more saprophytic species than beech plots. Most unknown fungi were shared between spruce and pine plots (178 unknown OTUs), followd by beech and spruce plots (159 unknown OTUs; Figure 3.8 d). Less unknown fungi were common between beech and pine (141 unknown OTUs). For the comparison of the unknown fungi only 14 plots per tree species could be considered, because one spruce plot, which limited the plotstelection to 15, had no OTUs with unknown lifestyle.

For four tree species (5 plots)

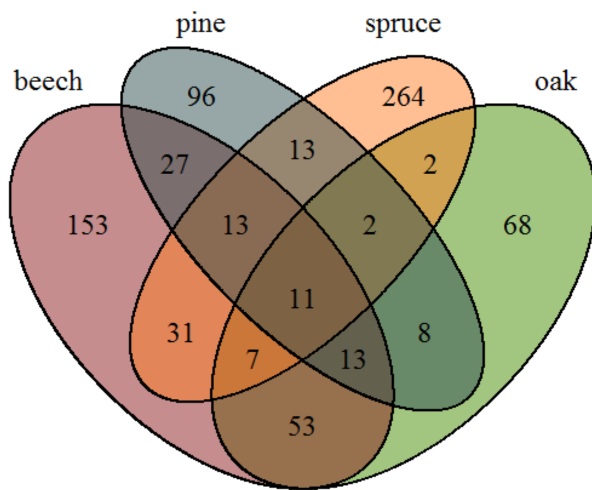
For three tree species (14/15 plots)

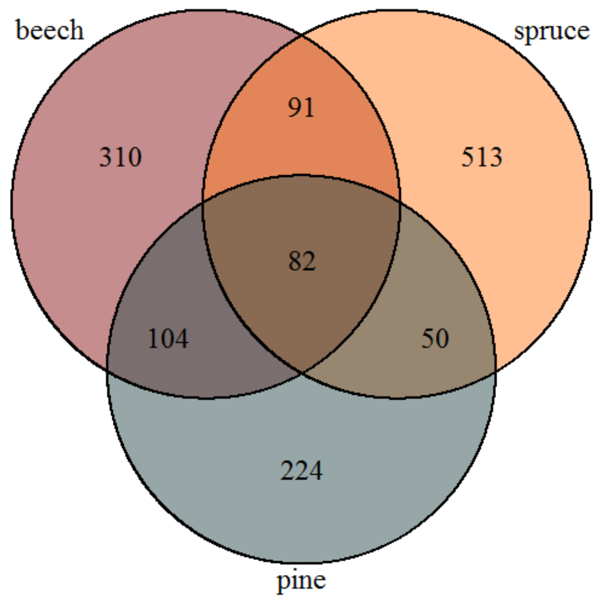
a) All



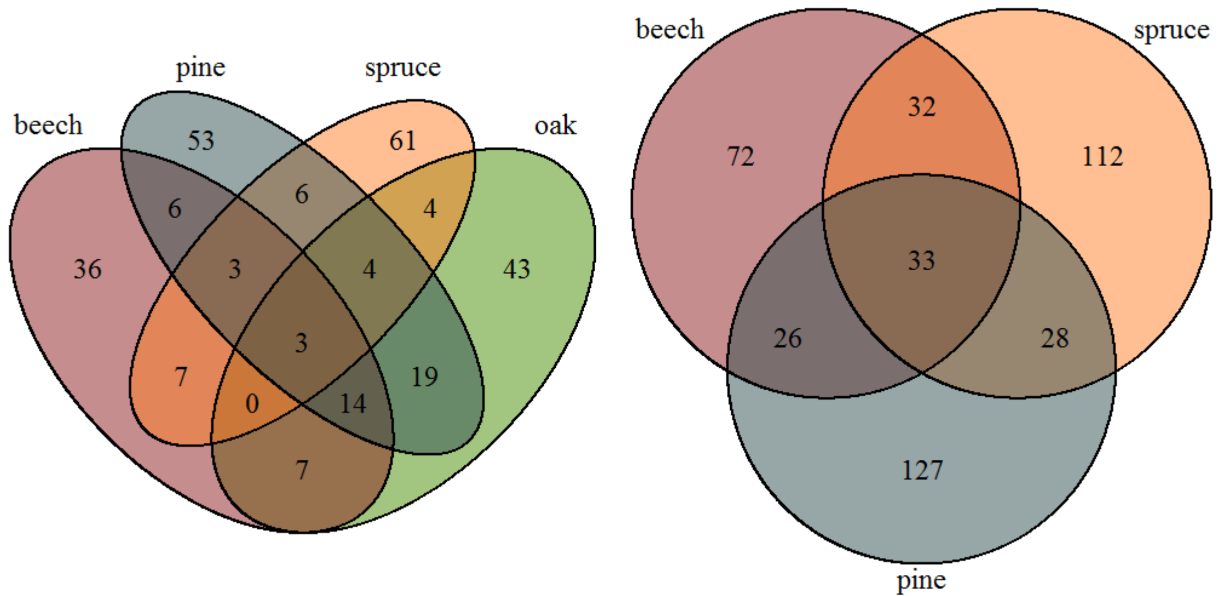


b) EM





c) Saprophytes



d) Unknown

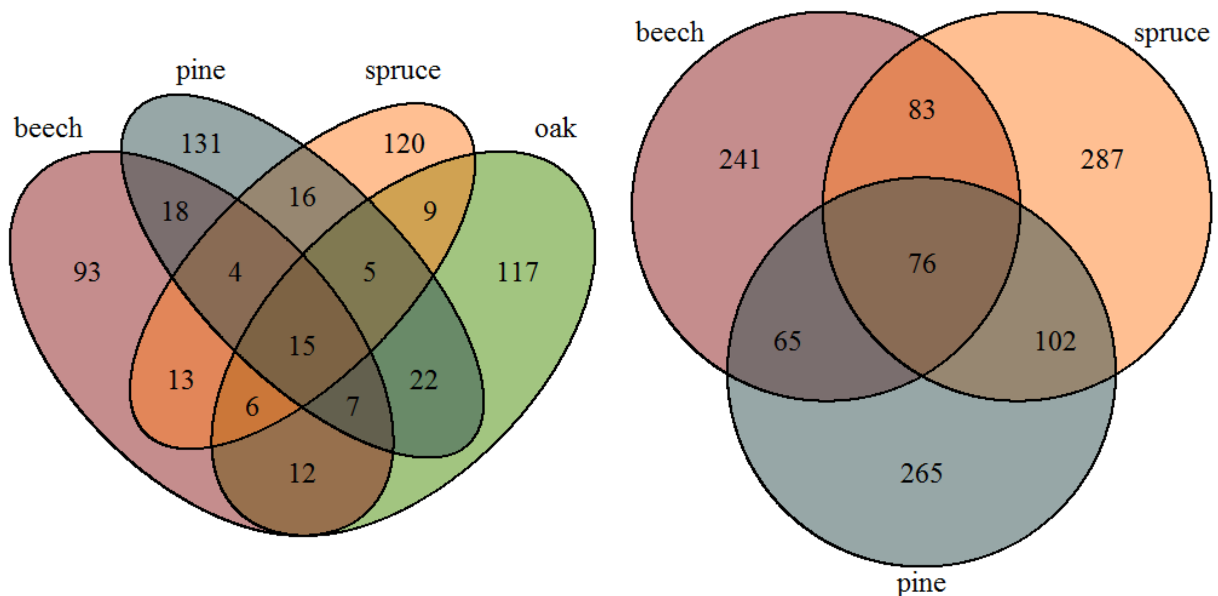


Figure 3.8: Venn diagrams representing the number and overlap of OTUs, categorised by dominant tree species on the plot. For five plots, the maximum for oak plots and randomly chosen plots from the other tree species (n=5) and for 15 plots from the other tree species (n=15, for unkown lifestyle n=14). A) all fungi, b) EM, c) saprophytes and d) unknown lifestyle.

3.1.4.2 Taxonomic distribution on plots, dominated by different tree species

This paragraph is about the fungal taxonomic composition, which differs between the different dominant tree species. Oak plots with an average of 411 sequences per plot had the highest mean number of sequences for Basidiomycota, whereas spruce had 10% less, beech 18% less and pine 27% less Basidiomycota sequences per plot (Table 3.6 a)). In contrast to this, when regarding the number of OTUs and not the number of sequences, spruce dominated plots had up to 40% more OTUs (105

OTUs) from Basidiomycota than plots dominated by other tree species. Oak plots had the lowest mean number of sequences per plot for Ascomycota. Spruce dominated plots had ~50% more Ascomycota than oak, beech twice as much sequences than oak plots and pine plots nearly three times more Ascomycota sequences than oak ones. The mean number of OTUs per oak plot was less for Ascomycota. Beech plots with an average of 56 OTUs per plot had ~20% more Ascomycota than oak. Around 70 Ascomycota OTUs were found on conifer dominated plots. Zygomycota with 2.7-4.3 mean number of sequences and 3.7-4.8 OTUs per plot were very rare and Glomeromycota accounted only for 0.02% of all sequences and 0.1% of all OTUs.

The genus *Lactarius*, especially with *Lacatrius quietus* on oak plots and several uncultured *Lactarius* species on beech plots was the most dominant genus on plots with deciduous trees (Table 3.6 f). However *Russula* species like *Russula firmula* or *Russula integra* on spruce plots and *Russula ochroleuca* on both conifer dominated plots form the most abundant group here. *Hygrophorus pustulatus*, also one of the most dominant species, was mainly detected on spruce dominated plots. *Tomentella* species were most abundant in their mean number of sequences on beech plots, but did not differ remarkably between beech, oak and spruce dominated plots with an average of around 2.2-2.5 OTUs, whereas pine plots had a little less *Tomentella* species with 1.3 OTUs (please beware, that “uncultures *Tomentella*” will not be listed as *Tomentella* within the genera overview, based on the counting by names). *Cenococcum* species were present on plots of all tree species, but were more abundant on pine dominated plots than on others. *Xerocomus* species were most abundant on beech and pine plots, mainly within the Schorfheide (Table 3.2 e).

Table 3.6: Distribution of sequences and number of OTUs according to taxonomic levels separated by dominant tree species (based on standardized abundance matrix with 494 sequences per plot) according to different taxonomic levels: a) Phylum, b) Class, c) Order, d) Family, e) Genus, f)Species. All taxa with a total abundance (sum of the percentage from mean number of sequences and mean number of OTus) with more than 1% are shown (for more details see Supplementary S4.2.).

0 50% highest value respectively

a) Phylum

	Mean number of sequences per phylum per plot					Mean number of OTUs per phylum per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
Basidiomycota	337.7	410.8	365.4	298.6	71.5	69.4	64.8	105.4	61.7	47.6
Ascomycota	125.4	63.8	95.2	171.5	23.1	56	45.6	70.3	70.6	38.3
unidentified	26.3	16.3	30.7	21	4.8	13	14.6	24.3	20.1	11.4
Zygomycota	4.3	3.1	2.7	2.8	0.7	4	4.8	4.5	3.7	2.7
Glomeromycota	0.3	0	0	0.1	0.02	0.1	0	0.1	0.1	0.1

b) Class

	Mean number of sequences per class per plot					Mean number of OTUs per class per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
Basidiomycota; Agaricomycetes	336.3	410.2	364.5	293.9	71.1	68.5	63.4	103.2	59.9	46.6
Ascomycota; Leotiomycetes	70.6	34.5	54.4	117.3	14	30.3	22.6	34.3	43.3	20.6
unidentified; unidentified	26.3	16.3	30.7	21	4.8	13	14.6	24.3	20.1	11.4
Ascomycota; Dothideomycetes	7.9	5.8	14.1	15.6	2.2	7.4	6.8	14.5	10.9	6.3
Ascomycota; Pezizomycetes	37.6	3.3	16.4	14.3	3.6	8.5	3.4	7.4	4	3.7
Ascomycota; Sordariomycetes	5.1	2.3	2.7	4.7	0.8	5.2	5.8	6.3	4	3.4
Ascomycota; unidentified	1.8	15.9	2	15.8	1.8	2	3.6	2.6	4.1	1.9
Zygomycota; Incertaesedis	4.3	3.1	2.7	2.8	0.7	4	4.8	4.5	3.7	2.7
Ascomycota; Eurotiomycetes	1.8	1.7	0.8	3.1	0.4	2	2.4	1.5	3	1.4

c) Order

	Mean number of sequences per order per plot					Mean number of OTUs per order per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
Basidiomycota; Agaricomycetes; Russulales	200.6	232.7	177.6	122.3	37.1	28	27.4	36.5	20.2	17.7
Basidiomycota; Agaricomycetes; Agaricales	67.7	138.4	84.5	93.3	19.4	20.2	18.2	25.5	20	13.2
Ascomycota; Leotiomycetes; Helotiales	56.3	29.4	50.4	101.5	12	22.1	17	30.3	35.8	16.6
unidentified; unidentified; unidentified	26.3	16.3	30.7	21	4.8	13	14.6	24.3	20.1	11.4
Basidiomycota; Agaricomycetes; Thelephorales	21.8	32	19.1	16	4.5	6.5	9.4	11.6	4.5	5.1
Ascomycota; Pezizomycetes; Pezizales	37.6	3.3	16.4	14.3	3.6	8.5	3.4	7.4	3.8	3.6
Basidiomycota; Agaricomycetes; Atheliales	7.7	0.4	40.7	7.3	2.8	3.1	0.6	15.5	2	3.3
Basidiomycota; Agaricomycetes; Sebacinales	17.1	0.3	24	2.7	2.2	4	0.6	4.9	0.9	1.7
Basidiomycota; Agaricomycetes; Boletales	14.4	3.9	0.1	23.9	2.1	3	3	0.2	4.3	1.7
Ascomycota; unidentified; unidentified	1.8	15.9	2	15.8	1.8	2	3.6	2.6	4.1	1.9
Ascomycota; Leotiomycetes; unidentified	10.1	4.2	2.1	12.7	1.5	5	3	1.3	4.4	2.2
Zygomycota; Incertaesedis; Mortierellales	3.6	2.7	2.7	2.2	0.6	3.6	3.8	4.1	2.6	2.2
Basidiomycota; Agaricomycetes; Trechisporales	1.6	2.2	4	14.9	1.1	1	3.2	2.2	3.6	1.6
Ascomycota; Dothideomycetes; Incertaesedis	1.6	0.5	2.9	6.4	0.6	2.1	1.6	3.9	5.1	2
Ascomycota; Dothideomycetes; Hysteriales	2.6	1.6	3.5	6.3	0.7	2.3	1.4	2.7	3.8	1.6
Ascomycota; Leotiomycetes; Incertaesedis	3.9	0.6	1.8	1.9	0.4	3	1.8	2.5	2.3	1.5
Ascomycota; Dothideomycetes; unidentified	1.4	1.5	5.6	1.1	0.5	1.3	0.8	4.9	1.3	1.3
Ascomycota; Sordariomycetes; Hypocreales	2.3	0.6	1.7	0.4	0.3	2.4	2	3.8	1.5	1.5
Ascomycota; Dothideomycetes; Pleosporales	2.1	2.2	1.2	1.7	0.4	1.3	3	2.1	0.7	1.1
Basidiomycota; Agaricomycetes; unidentified	0.8	0	0.9	8.3	0.5	0.5	0.2	2.1	2.6	0.9
Ascomycota; Eurotiomycetes; Eurotiales	1.2	1.2	0.1	2.4	0.2	1.3	2.2	0.5	2.5	1
Basidiomycota; Agaricomycetes; Cantharellales	2.9	0	5.9	0.2	0.5	1.3	0.2	2.1	0.2	0.6

d) Family

Family	Mean number of sequences per family per plot					Mean number of OTUs per family per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
Basidiomycota; Agaricomycetes; Russulales; Russulaceae	200.6	232.7	177.5	122.2	37.1	28	27.4	36.5	20.1	17.7
Basidiomycota; Agaricomycetes; Agaricales; Mycenaceae	18.4	111.7	20.9	80.6	11.7	5.1	10.4	5.3	11.1	5
unidentified; unidentified; unidentified; unidentified	26.3	16.3	30.7	21	4.8	13	14.6	24.3	20.1	11.4
Ascomycota; Leotiomycetes; Helotiales; unidentified	15.2	6.6	21.8	27.4	3.6	8.4	7.8	12.5	13	6.6
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae	21.8	32	18.9	16	4.5	6.5	9.4	11.4	4.5	5
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae	19.8	1.5	6.1	32.7	3	5.9	2	3.1	8.4	3.1
Basidiomycota; Agaricomycetes; Atheliales; Atheliaceae	7.6	0.4	38	7.3	2.7	2.9	0.6	12.7	2	2.9
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae	32.7	1.6	13.9	4.5	2.7	6.1	0.8	4.9	2	2.2
Ascomycota; Leotiomycetes; Helotiales; Vibrisseaceae	1.2	0.1	17.3	17.5	1.8	0.6	0.2	8.4	6.4	2.5
Basidiomycota; Agaricomycetes; Agaricales; Tricholomataceae	11.2	16.9	3.5	3.9	1.8	4.3	4.2	3.2	3.5	2.4
Basidiomycota; Agaricomycetes; Agaricales; Hygrophoraceae	8.1	0	42.7	0.9	2.6	2.1	0.2	6.5	0.7	1.5
Basidiomycota; Agaricomycetes; Sebacinales; Sebacinaceae	17.1	0.3	24	2.7	2.2	4	0.6	4.9	0.9	1.7
Ascomycota; unidentified; unidentified; unidentified	1.8	15.9	2	15.8	1.8	2	3.6	2.6	4.1	1.9
Ascomycota; Leotiomycetes; unidentified; unidentified	10.1	4.2	2.1	12.7	1.5	5	3	1.3	4.4	2.2
Basidiomycota; Agaricomycetes; Boletales; Boletaceae	12.4	1.2	0.1	23.1	1.9	2.7	2	0.2	3.8	1.4
Ascomycota; Leotiomycetes; Helotiales; Dermateaceae	4.4	16.5	1.2	1.7	1.2	2.4	4	2.7	1.7	1.7
Ascomycota; Leotiomycetes; Helotiales; Incertaesedis	12.8	1.3	2.6	1.7	0.9	4.2	1.6	2.9	2.4	1.8
Zygomycota; Incertaesedis; Mortierellales; Mortierellaceae	3.5	2.6	2.7	1.9	0.5	3.5	3.4	4.1	2.3	2.1
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae	2.9	3.3	1.4	20.5	1.4	0.6	1.2	0.7	4	1
Ascomycota; Dothideomycetes; Hysteriales; Gloniaceae	2.6	1.6	3.5	6.3	0.7	2.3	1.4	2.7	3.8	1.6
Basidiomycota; Agaricomycetes; Agaricales; Inocybaceae	9.5	0	5.2	0.6	0.8	2.7	0	3.8	0.9	1.2
Ascomycota; Leotiomycetes; Incertaesedis; Incertaesedis	3.9	0.6	1.8	1.9	0.4	3	1.8	2.5	2.3	1.5
Basidiomycota; Agaricomycetes; Agaricales; Cortinariaceae	10.2	0.1	2.9	1.5	0.7	2.6	0.6	2.6	1.3	1.1
Ascomycota; Dothideomycetes; unidentified; unidentified	1.4	1.5	5.6	1.1	0.5	1.3	0.8	4.9	1.3	1.3
Ascomycota; Dothideomycetes; Incertaesedis; Myxotrichaceae	0.7	0.3	1.3	5.9	0.4	1.1	0.8	1.9	4.7	1.4
Basidiomycota; Agaricomycetes; Trechisporales; Hydnodontaceae	1.1	0.5	2.4	13.9	0.9	0.4	0.8	0.7	1.7	0.6

Family	Mean number of sequences per family per plot					Mean number of OTUs per family per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
Basidiomycota; Agaricomycetes; unidentified; unidentified	0.8	0	0.9	8.3	0.5	0.5	0.2	2.1	2.6	0.9
Ascomycota; Pezizomycetes; Pezizales; Discinaceae	0.7	1.2	0.1	9.7	0.6	0.7	2	0.1	1.4	0.7
Basidiomycota; Agaricomycetes; Trechisporales; unidentified	0.4	1.6	1.6	1	0.2	0.6	2.4	1.5	1.9	1
Basidiomycota; Agaricomycetes; Agaricales; Strophariaceae	2.6	6.7	1	0.1	0.5	1.1	1.2	0.6	0.3	0.5

e) Genus

Genus	Mean number of sequences per genus per plot					Mean number of OTUs per genus per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Russula*	26.9	26.2	144.8	41.9	12.1	8.9	6	27.5	9.1	8.1
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; unidentified	157.9	2.1	16	57.2	11.8	15.5	2.8	7.1	8	5.3
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Lactarius*	13.7	204.3	16.8	20	12.9	3.4	18.6	1.9	2.6	4.2
unidentified; unidentified; unidentified; unidentified; unidentified	26.3	16.3	30.7	21	4.8	13	14.6	24.3	20.1	11.4
Basidiomycota; Agaricomycetes; Agaricales; Mycenaceae; unidentified	3.3	104.9	5	58.5	8.7	1.6	5.6	1.2	6.2	2.3
Ascomycota; Leotiomycetes; Helotiales; unidentified; unidentified	15.2	6.6	21.8	27.4	3.6	8.4	7.8	12.5	13	6.6
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; unidentified	18.4	21.2	12	13.2	3.3	5.3	7.6	8.2	3	3.8
Basidiomycota; Agaricomycetes; Agaricales; Mycenaceae; *Mycena*	15.1	6.7	15.9	22.2	3	3.5	4.8	4.1	5	2.7
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; unidentified	18.3	1.4	4.1	32.3	2.8	5	1.6	2.7	7.9	2.7
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae; unidentified	29	0.1	13.5	4.3	2.4	5	0.4	4.5	1.6	1.8
Basidiomycota; Agaricomycetes; Agaricales; Hygrophoraceae; *Hygrophorus*	6.6	0	42.7	0.9	2.5	1.3	0.2	6.4	0.7	1.4
Ascomycota; unidentified; unidentified; unidentified; unidentified	1.8	15.9	2	15.8	1.8	2	3.6	2.6	4.1	1.9
Basidiomycota; Agaricomycetes; Atheliales; Atheliaceae; unidentified	5.6	0.2	23.6	7.1	1.8	2.1	0.4	7.3	1.6	1.8
Ascomycota; Leotiomycetes; unidentified; unidentified; unidentified	10.1	4.2	2.1	12.7	1.5	5	3	1.3	4.4	2.2
Basidiomycota; Agaricomycetes; Agaricales; Tricholomataceae; unidentified	8	16.8	3.2	2.7	1.5	3.3	4	2.6	3.2	2.1
Ascomycota; Leotiomycetes; Helotiales; Vibrisseaceae; *Phialocephala*	1.2	0.1	16.6	12	1.5	0.5	0.2	7.3	4.4	2

Genus	Mean number of sequences per genus per plot					Mean number of OTUs per genus per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
Basidiomycota; Agaricomycetes; Sebacinales; Sebacinaceae; *Sebacina*	14.7	0.3	19.4	2.6	1.9	2.8	0.6	2.9	0.7	1.1
Ascomycota; Dothideomycetes; Hysteriales; Gloniaceae; *Cenococcum*	2.5	1.6	3.5	6.3	0.7	2.3	1.4	2.7	3.8	1.6
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; *Lachnum*	2.8	3.2	1.3	18.6	1.3	0.4	1	0.4	3.6	0.8
Zygomycota; Incertaesedis; Mortierellales; Mortierellaceae; Mortierella	2	2.2	1.6	1.7	0.4	2.5	2.6	3.1	2	1.6
Ascomycota; Dothideomycetes; unidentified; unidentified; unidentified	1.4	1.5	5.6	1.1	0.5	1.3	0.8	4.9	1.3	1.3
Basidiomycota; Agaricomycetes; Boletales; Boletaceae; *Xerocomus*	8.9	0	0	16.6	1.3	1	0	0	1.3	0.4
Basidiomycota; Agaricomycetes; Agaricales; Inocybaceae; *Inocybe*	8.5	0	4.9	0.1	0.7	2.1	0	3.1	0.2	0.9
Ascomycota; Leotiomycetes; Helotiales; Dermateaceae; unidentified	2.5	14.4	0.4	0.8	0.9	0.9	1.8	0.7	0.6	0.6
Basidiomycota; Agaricomycetes; Trechisporales; Hydnodontaceae; *Trechispora*	1.1	0.5	2.4	13.9	0.9	0.4	0.8	0.7	1.7	0.6
Ascomycota; Dothideomycetes; Incertaesedis; Myxotrichaceae; *Oidiodendron*	0.5	0.3	1.1	4.9	0.3	0.8	0.8	1.7	3.8	1.1
Ascomycota; Leotiomycetes; Incertaesedis; Incertaesedis; Meliniomyces	3.3	0.2	1.6	1.8	0.3	2.1	1	1.9	2	1.1
Ascomycota; Leotiomycetes; Helotiales; Incertaesedis; *Tetracladium*	11.2	0.2	0.8	0.1	0.6	3	0.6	1	0.2	0.8
Basidiomycota; Agaricomycetes; unidentified; unidentified; unidentified	0.8	0	0.9	8.3	0.5	0.5	0.2	2.1	2.6	0.9
Basidiomycota; Agaricomycetes; Agaricales; Cortinariaceae; unidentified	9	0.1	2.2	0.6	0.6	2.2	0.4	1.7	0.5	0.8
Ascomycota; Pezizomycetes; Pezizales; Discinaceae; *Hydnotrya*	0.7	1.2	0.1	9.7	0.6	0.7	2	0.1	1.4	0.7
Basidiomycota; Agaricomycetes; Trechisporales; unidentified; unidentified	0.4	1.6	1.6	1	0.2	0.6	2.4	1.5	1.9	1
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; *Thelephora*	2.1	9	1.4	2	0.7	0.6	0.8	1	0.8	0.5
Ascomycota; Leotiomycetes; Helotiales; Dermateaceae; *Cryptosporiopsis*	1.9	1.8	0.7	0.9	0.3	1.5	1.4	1.9	0.9	0.9
Basidiomycota; Agaricomycetes; Boletales; Boletaceae; unidentified	3.3	0.6	0	4.2	0.4	1.5	1.2	0.1	1.6	0.7
Basidiomycota; Agaricomycetes; Atheliales; Atheliaceae; *Tylospora*	0.2	0.2	11	0.1	0.6	0.1	0.2	2.4	0.2	0.5
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; *Tomentella*	0.9	1.9	4.6	0.2	0.4	0.6	1	1.9	0.5	0.6

f) Species

Species	Mean number of sequences per species per plot					Mean number of OTUs per species per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Lactarius quietus*	9.3	204.3	0	13.2	11.5	1.9	18.6	0.2	1.9	3.6
unidentified; unidentified; unidentified; unidentified; uncultured; fungus	25.1	16	28.9	19	4.5	11.9	13.4	22.9	18	10.5
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; uncultured; *Lactarius*	150.6	1.1	5	26.5	9.3	11.5	1.6	1.5	1.6	2.6
Basidiomycota; Agaricomycetes; Agaricales; Mycenaceae; Mycenaceae sp	3.3	104.9	5	58.5	8.7	1.6	5.6	1.2	6.2	2.3
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Russula firmula*	1.2	0	110.3	0.1	5.6	0.5	0.2	14.1	0.1	2.4
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Russula ochroleuca*	17.6	17.7	14.1	29.8	4	5.6	2.4	6.5	5.8	3.2
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; uncultured *Russula*	5.1	0.7	5.6	21.5	1.7	3.4	1	3.1	5.3	2
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Helotiales sp.3 BB 2010	17.5	0.2	0.1	20.9	2	4.6	0.4	0.4	3.5	1.4
Ascomycota; Leotiomycetes; Helotiales; unidentified; Helotiales sp.	1.3	3.4	12.4	3.3	1	1.5	3.8	5.7	3.6	2.3
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae; Pyronemataceae; sp.	28.6	0.1	2.8	4	1.8	4.5	0.4	1.7	1.4	1.3
Basidiomycota; Agaricomycetes; Agaricales; Hygrophoraceae; *Hygrophorus pustulatus*	1.8	0	39.4	0	2.1	0.3	0.2	5.4	0.1	1
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; uncultured Thelephoraceae	6.8	16.9	2.6	5.3	1.6	1.3	3.6	1.5	0.9	1.1
Ascomycota; Leotiomycetes; unidentified; unidentified; uncultured Leotiomycetes	10	4	0.5	2.6	0.9	4.9	2.4	0.9	2	1.6
Basidiomycota; Agaricomycetes; Agaricales; Tricholomataceae; uncultured *Mycena*	6.8	4.3	2.6	2.3	0.8	2.7	2.4	2.3	2.8	1.6
Basidiomycota; Agaricomycetes; Agaricales; Mycenaceae; *Mycena sp.*	3.1	4.2	0.2	14.8	1.1	1.3	3	0.9	2	1.1
Basidiomycota; Agaricomycetes; Agaricales; Mycenaceae; *Mycena* sp. 1 KO 2013	9.6	2.4	12.5	3	1.4	1	1.4	2	0.8	0.8
Ascomycota; unidentified; unidentified; unidentified; Ascomycota	0.5	14.3	0.9	14.5	1.5	0.4	1.2	0.3	2.1	0.6

Species	Mean number of sequences per species per plot					Mean number of OTUs per species per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
sp. 6; RB 2011										
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; uncultured *Tomentella*	7.8	2.2	2.7	3.4	0.8	2.5	2.2	2.3	1.3	1.3
Ascomycota; Leotiomycetes; Helotiales; Vibrisseaceae; *Phialocephala* sp. KO 2013	0.5	0.1	10.1	11	1.1	0.3	0.2	2.7	2	0.8
Ascomycota; Dothideomycetes; Hysteriales; Gloniaceae; uncultured *Cenococcum*	2.2	1.6	3.3	6	0.7	1.9	1.2	1.9	3	1.3
Ascomycota; Leotiomycetes; Helotiales; unidentified; uncultured Hyaloscyphaceae	10.7	1.1	2.1	0.7	0.7	4.6	0.6	1.3	0.6	1.1
Basidiomycota; Agaricomycetes; Boletales; Boletaceae; *Xerocomus* sp.	8.9	0	0	16.6	1.3	1	0	0	1.3	0.4
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Russula integra*	0.8	0	14.5	2.3	0.9	0.1	0	3.3	0.6	0.6
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; Thelephoraceae sp.	2.6	1.3	6.2	0.6	0.5	0.9	1	3.9	0.4	1
Ascomycota; Dothideomycetes; unidentified; unidentified; Dothideomycetes sp.	1	1.3	5.3	0.8	0.4	0.7	0.6	4.4	1.2	1.1
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; Russulaceae sp	2.1	0.4	5.2	9.1	0.9	0.6	0.2	2.3	1	0.6
Basidiomycota; Agaricomycetes; Atheliales; Atheliaceae; uncultured *Tylospora*	0.5	0	17.4	0	0.9	0.2	0	3.4	0	0.6
Ascomycota; Leotiomycetes; Helotiales; unidentified; Helotiales sp. 1 MV 2011	0	0.5	5.1	13.4	1	0	0.6	0.6	1.9	0.5
Basidiomycota; Agaricomycetes; Trechisporales; Hydnodontaceae; *Trechispora* sp.	1.1	0.4	2.4	13.9	0.9	0.4	0.6	0.7	1.7	0.5
Ascomycota; Leotiomycetes; Helotiales; Dermateaceae; Dermateaceae sp. KO 2013	2.3	14.4	0.1	0.4	0.9	0.7	1.8	0.2	0.6	0.5
Basidiomycota; Agaricomycetes; Sebacinales; Sebacinaceae; *Sebacina* sp.	10.9	0	3.6	0.3	0.8	1.9	0.2	1.3	0.4	0.6
Basidiomycota; Agaricomycetes; Atheliales; Atheliaceae; uncultured *Amphinema*	0.4	0	4.2	7	0.6	0.2	0	2.4	1.5	0.6
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae *Thelephora castanea*	2.1	9	1.3	2	0.7	0.6	0.8	0.9	0.7	0.5
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; *Lachnum*	0.3	0.5	0	10.4	0.6	0.1	0.8	0.1	2.8	0.6

Species	Mean number of sequences per species per plot					Mean number of OTUs per species per plot				
	Beech	Oak	Spruce	Pine	%	Beech	Oak	Spruce	Pine	%
sp.										
Ascomycota; Leotiomycetes; Helotiales; Incertaesedis; *Tetracladium* sp.	10.2	0.2	0.7	0.1	0.6	2.3	0.6	0.7	0.2	0.6
Basidiomycota; Agaricomycetes; unidentified; unidentified; uncultured; Agaricomycetes	0.7	0	0.7	7.8	0.5	0.4	0.2	1.7	2.1	0.7
Ascomycota; Leotiomycetes; unidentified; unidentified; Leotiomycetes sp.	0	0.2	1.6	10.2	0.6	0.1	0.6	0.4	2.3	0.5
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; uncultured; Helotiaceae	0.5	0	3.2	7.6	0.6	0.2	0	1.2	2.1	0.6
Basidiomycota; Agaricomycetes; Russulales; Russulaceae; *Russula* cf *aeruginea* E00186067	3.2	4.7	0	4.8	0.6	0.7	1.2	0.1	1	0.5
Basidiomycota; Agaricomycetes; Agaricales; Tricholomataceae; uncultured; Laccaria	0.9	12.4	0.6	0.3	0.7	0.5	1.2	0.3	0.4	0.4
Ascomycota; Leotiomycetes; Helotiales; unidentified; uncultured; Helotiales	1.5	0.2	0.5	1.8	0.2	1.4	0.2	1.8	1.9	0.8
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae; uncultured; Wilcoxina	0	0	10.2	0.4	0.5	0.1	0	2.7	0.2	0.5
Basidiomycota; Agaricomycetes; Atheliales; Atheliaceae; Tylospora; asterophora	0.2	0.2	10.9	0	0.6	0.1	0.2	2.2	0	0.4
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; sp; ECHh	2.4	2.8	1.3	8.1	0.7	0.2	0.2	0.3	0.6	0.2

3.1.4.3 Richness and diversity on plots dominated by different tree species

The curve progression of the rarefaction curves of OTU richness did not differ remarkably between the different tree species (Figure 3.9). This is supported by the results of the richness (MMF) comparison if considering all fungi there were no significant differences between neither the richness nor the diversity between plots with different dominant tree species (Figure 3.9, Figure 3.10 a). Only the richness from spruce plots, which had the highest richness, showed a trend in comparison to the lowest richness on oak dominated plots. Spruce plots also had the highest richness from EM, but only to pine this difference was significant (Figure 3.9, Figure 3.10 b). The diversity of the EM did not differ between the different tree species. In contrast the estimated number of saprophytes and their diversity were significantly higher on beech than on oak or pine plots (Figure 3.9, Figure 3.10 c). Neither the richness nor the diversity of unknown fungi differed between plots dominated by different tree species (Figure 3.9, Figure 3.10 d).

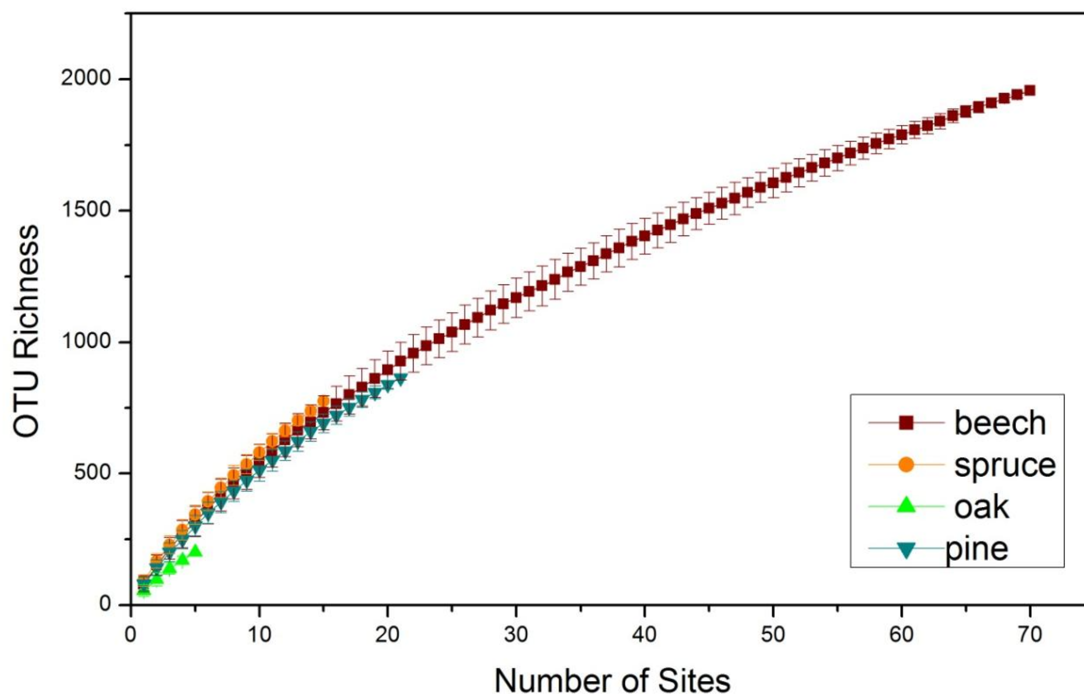


Figure 3.9: Rarefaction curve by Michaelis Menten for the different dominant tree species for normalized abundance (beech n=70, oak n=5, spruce n=15, pine n=21)

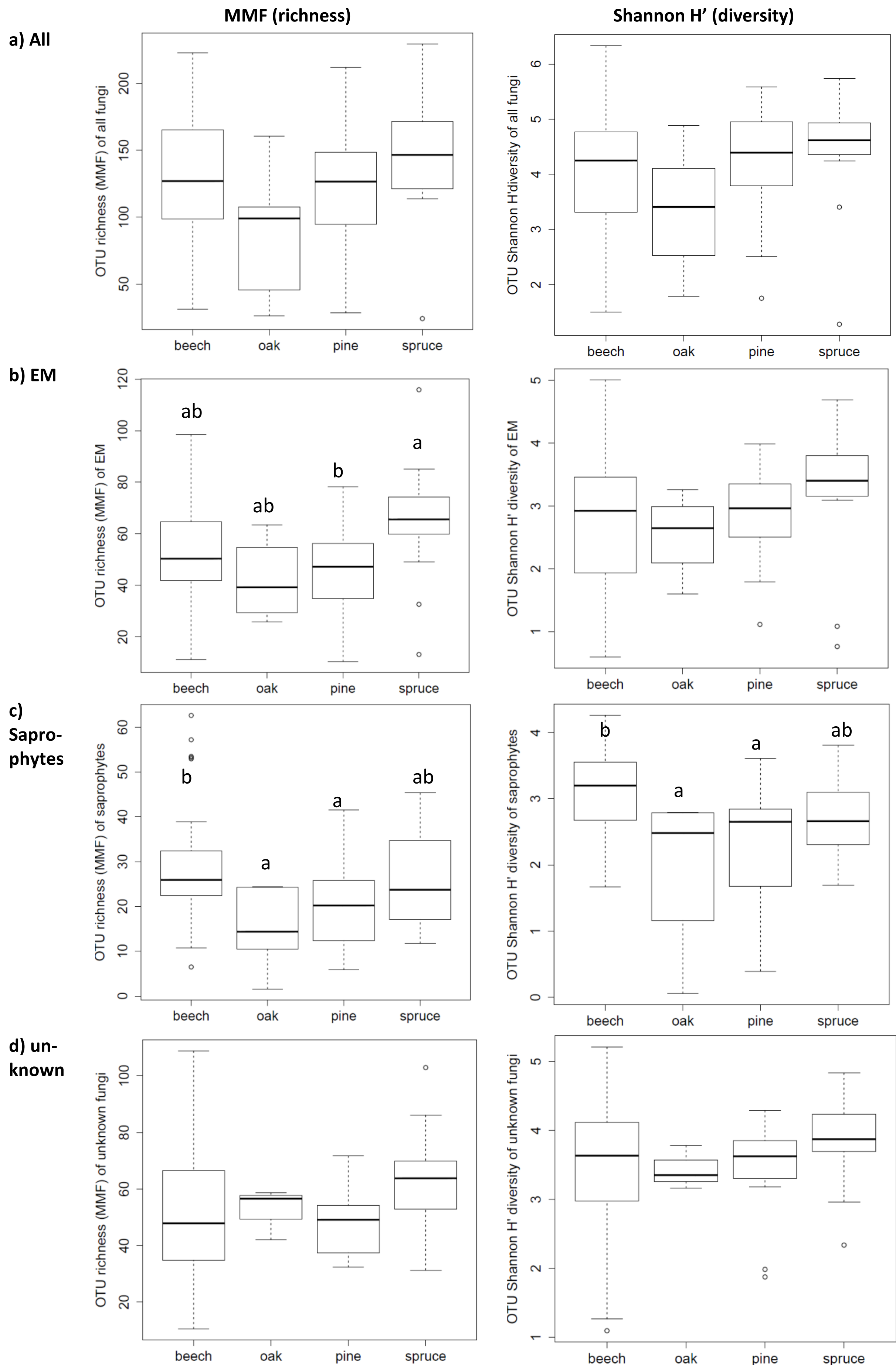

MMF (richness)
Shannon H' (diversity)
a) All
b) EM
c) Sapro-phytes
d) un-known
OTU richness (MMF) of all fungi
OTU Shannon H'diversity of all fungi
OTU richness (MMF) of EM
OTU Shannon H' diversity of EM
OTU richness (MMF) of saprophytes
OTU Shannon H' diversity of saprophytes
OTU richness (MMF) of unknown fungi
OTU Shannon H' diversity of unknown fungi
beech
oak
pine
spruce
ab
ab
b
a
b
a
a
ab
b
a
a
ab

Figure 3.10: OTU richness and diversity per plot for a) all fungi, b) EM, c) Saprophytes and d) unknown fungi, separated by main tree species on the plot respectively. For exact p-values see Table 3.7(beech n=70, oak n=5, spruce n=15, pine n=21)

Table 3.7: Comparisons of richness, represented by MMF from all fungi, EM, Saprophytes and unknown fungi, separated by main tree species on the plot respectively (p-vales from Anova (global) & TukeyHSD, n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05). Corresponding graphs see Figure 3.6.

	MMF				Shannon			
	All fungi	**EM**	**Saprophytes**	**unknown**	**All fungi**	**EM**	**Saprophytes**	**unknown**
global	0.069	**0.019**	**0.001**	n.s.	n.s.	n.s.	**<0.001**	n.s.
Beech-Oak	n.s.	n.s.	**0.009**				**0.003**	
Beech-Pine	n.s.	n.s.	**0.013**				**<0.001**	
Beech-Spruce	n.s.	n.s.	n.s.				n.s.	
Pine-Oak	n.s.	n.s.	n.s.				n.s.	
Spruce-Oak	0.059°	n.s.	n.s.				n.s.	
Pine-Spruce	n.s.	**0.019**	n.s.				n.s.	

When comparing the estimated number of OTUs (richness by MMF) to the observed number of OTUs on oak plots nearly 70% of the estimated OTUs were detected (Table 3.8). On plots dominated by other tree species this values were around 62%. For EM fungi the rate of detection was a little better, over 70% on oak and pine plots and around 66% for beech and spruce plots. Saprophytic fungi had a similar detection rate like all fungi, whereas from unknown fungi on average only around 55% were detected.

Table 3.8: Mean ± SE from the percentage of OTUs found in comparison to OTUs estimated by MMF per dominant tree species

	All fungi		EM		Saprophytic		unknown	
beech	62.73	±5.82	66.04	±7.05	62.56	±9.49	57.77	±8.59
oak	69.38	±9.10	70.23	±4.64	72.01	±15.47	53.17	±5.29
spruce	62.04	±4.87	65.55	±7.30	62.00	±9.42	52.97	±5.97
pine	65.06	±4.78	71.39	±8.56	64.76	±8.70	57.21	±6.55

3.1.4.4 Comparison of the fungal community structure between plots dominated by different tree species

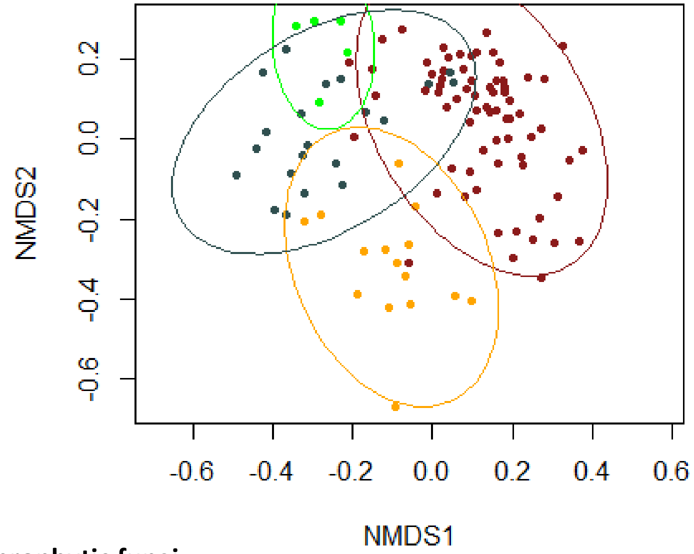
The fungal community from all, EM or unknown were more distinct between main tree species than compared to Exploratories, whereas this effect could not be detected for saprophytic fungal community (Figure 3.7; Figure 3.11; Table 3.9 and Table 3.5). All tree species differed significantly in their overall fungal community structure despite pine and oak (Figure 3.11 a, Table 3.9). Even when regarding only the EM community these two were not different (Figure 3.11 b, Table 3.9) Again in EM

communities there were more differences between the tree species than between the Exploratories. Overall the saprophytic fungal communities were less different between the different tree species than EM fungal communities (Figure 3.11 c, Table 3.9). Between the different tree species oak is not different from spruce or pine, whereas all other tree species differ for saprophytic fungi. Unknown fungal community showed similar similarity comparisons like EM fungal community (Figure 3.11 d, Table 3.9).

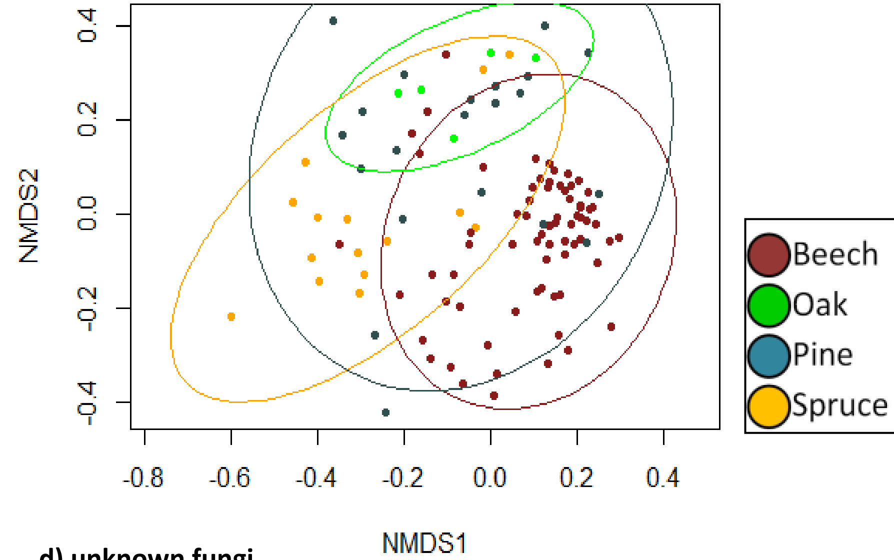
Table 3.9: Global and pair-wise R and p-values of ANOSIM comparisons of the different lifestyles for similarities between Exploratories and main tree species (Bray-Curtis with Bonferroni correction). (beech n=70, oak n=5, spruce n=15, pine n=21, n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05).)

	All		**EM**		**Saprophytes**		**unknown**	
	R	**p**	**R**	**p**	**R**	**p**	**R**	**p**
global	0.620	**<0.001**	0.539	**<0.001**	0.385	**<0.001**	0.534	**<0.001**
spruce - beech	0.692	**<0.001**	0.661	**<0.001**	0.250	**0.010**	0.582	**<0.001**
spruce - pine	0.620	**<0.001**	0.465	**<0.001**	0.518	**<0.001**	0.375	**<0.001**
spruce - oak	0.799	**0.001**	0.751	**<0.001**	0.242	n.s.	0.797	**<0.001**
beech - pine	0.568	**<0.001**	0.442	**<0.001**	0.487	**<0.001**	0.375	**<0.001**
beech - oak	0.667	**<0.001**	0.552	**0.004**	0.401	**0.011**	0.567	**<0.001**
oak - pine	0.263	n.s.	0.148	n.s.	-0.030	n.s.	0.446	**0.046**

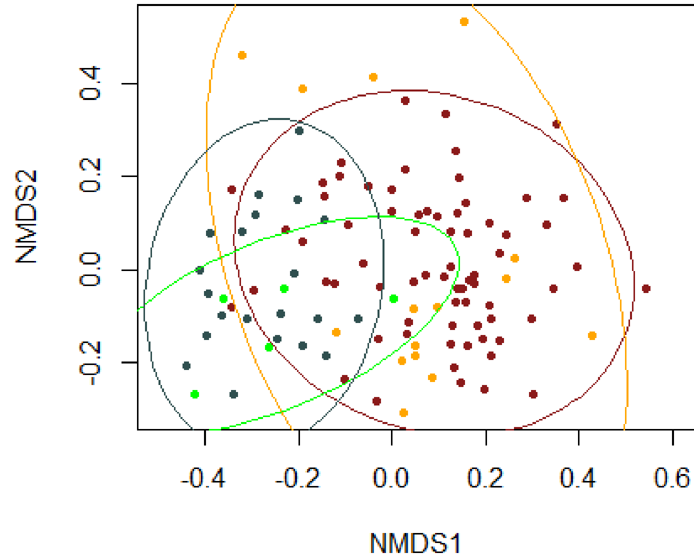
a) all fungi



b) EM



c) Saprophytic fungi



d) unknown fungi

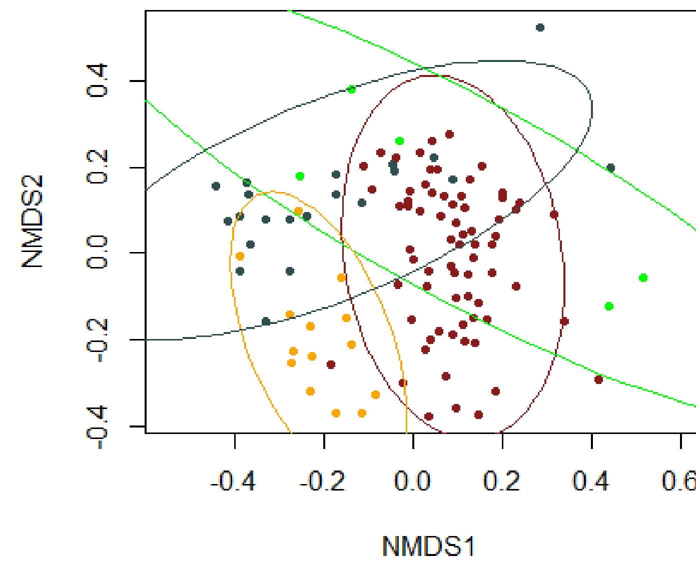


Figure 3.11: NMDS with 95% confidence intervals for a) all root-associated fungi b) EM, c) Saprophytic fungi and d) unknown fungi; separated by main tree species on the plot. For according ANOSIM results see Fehler! erweisquelle konnte nicht gefunden werden.. **(n= c.f. Table 3.1).**

3.1.5 Relationship of fungal richness and diversity to different environmental variables

Generalized linear models with quasipoisson distribution were calculated for several biotic or abiotic environmental variables, like pH value, forest management indices, root element or carbohydrate concentration, to test for the relation on the richness or diversity of root-associated fungi. Additionally correlations via spearman rank correlation were calculated as some variables were not normal distributed (the results of the models and the correlations for all fungi and for the different lifestyles described within this section are summarized in Table 3.10 and Table 3.11, for correlations of all variables see Supplementary Table S5).

The richness of all root-associated fungi depended mainly on root nitrogen (N). The more nitrogen the less root-associated fungi were detected. Concentration of carbon and other elements like Al, Ca, Fe, Mg, Mn, Na and P showed a correlation with the richness of all Root-associated fungi but were not important in the overall model. The diversity of all root-associated fungi also mainly depended on soil and root nitrogen concentration. Remarkably the soil and the root N concentration showed a negative correlation. An increase in soil N concentration leaded to an increase in fungi, whereas root N concentration is correlated with a decrease in root-associated fungal richness and diversity (Table 3.11). Additionally the C concentration of the mineral soil and the root Na concentration were significant variables in the overall model.

As more than half of all root-associated fungi were classified as EM their MMF did also negatively depend on root nitrogen concentration. Additionally Ca in a positive way and root K concentration in a negative way correlated to the richness of EM. Remarkably pH also seemed to be weakly linked with the richness of EM, as it was the last variable removed from the model and it also showed a significant positive correlation. So with increasing pH more EM were detected. Also some other elements within the roots showed significant correlation with the richness of EM, for example Al, Fe, Mg, Mn and S.

The richness of EM fungi correlated positively with forest management index SMI, but both management indices were significant within the overall model (SMI and ForMI). As the ForMI can be separated in different variables whereof one is the amount of harvested biomass (Iharv; Kahl and Bauhus (2014)) it was shown that the amount of harvested biomass was positively associated with the richness of EM. Both management indices and the amount of harvested biomass showed a significant negative correlation to root N concentration (SMI: rho =-0.392, p<0.001; ForMI rho= -0.326, p<0.001; Iharv(harvested biomass): rho: -0.239 p=0.004; Supplementary Table S5).

For the diversity of the EM soil C and N concentration were significant within the model. Furthermore root glucose and root Fe concentration were significantly within the model and root glucose showed a strong positive correlation with the diversity of EMs.

The richness of saprophytic fungi mainly depended on the carbon concentration of the roots. They positively correlated on the amount of carbohydrates, mainly fructose, within the roots. The management index ForMI is negatively correlated with the richness of saprophytic root-associated fungi. ForMI and root fructose concentration were also associated with the diversity of the root-associated saprophytic fungi. Additionally root Mg, Na and S concentrations were significant within the diversity model for saprophytic fungi.

The group of fungi with unknown lifestyle depended again mainly positive on N in the roots. Additionally Ca and Mg have a positive effect on the richness of the unknown fungi. Some other root elements also showed significant correlations with the richness of unknown fungi, but they were not relevant within the model. As well as within the model for richness and within the model for the diversity of the unknown fungi the number of tree species per plot was significant. Additionally root C and N concentration were significantly important for the diversity of unknown fungi. Further on root Fe and Mg concentration cohered with the diversity of the unknown fungi significantly.

Table 3.10: Results from generalized linear models with quasipoisson distribution(wrp= was removed from the model at … position) a) for All and EM fungi; b) for saprophytes and unknown fungi (n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05).. For a correlation matrix of all variables see Supplementary Table S5.

a)

	All fungi				EM			
	Shannon		MMF		Shannon		MMF	
	wrp	p	wrp	p	wrp	p	wrp	p
Mineral soil Total C		**0.019**	10	n.s.		**0.002**	12	n.s.
Mineral soil Total N		**0.031**	11	n.s.		**0.005**	13	n.s.
Mineral soil pH 1	7	n.s.	17	n.s.	14	n.s.	15	n.s.
ForMI	4	n.s.	14	n.s.	17	0.055		**0.025**
Iharv	15	n.s.	5	n.s.	15	n.s.	7	n.s.
SMI	3	n.s.	13	n.s.	11	n.s.		**0.008**
Number of tree species per plot	13	n.s.	2	n.s.	10	n.s.	6	n.s.
Fine Roots Biomass	9	n.s.	12	n.s.	2	n.s.	1	n.s.
Root glucose concentration	17	n.s.	8	n.s.		**0.034**	3	n.s.
Root fructose concentration	11	n.s.	16	n.s.	8	n.s.	0	n.s.
Root C concentration	1	n.s.	7	n.s.	7	n.s.	4	n.s.
Root N concentration		**0.001**		**<0.001**	0	n.s.		**0.014**
Root CN ratio	2	n.s.		**<0.001**	16	0.069		**0.027**
Root Al concentration	8	n.s.	9	n.s.	1	n.s.	2	n.s.
Root Ca concentration	6	n.s.	18	n.s.	13	n.s.		**0.042**
Root Fe concentration	16	n.s.	1	n.s.		**0.048**	11	n.s.
Root K concentration	14	n.s.	4	n.s.	4	n.s.		**0.011**
Root Mg concentration	5	n.s.	0	n.s.	5	n.s.	8	n.s.
Root Mn concentration	12	n.s.	6	n.s.	9	n.s.	9	n.s.
Root Na concentration		**<0.001**	19	n.s.	12	n.s.	14	n.s.
Root P concentration	0	n.s.	3	n.s.	3	n.s.	5	n.s.
Root S concentration	10	n.s.	15	n.s.	6	n.s.	10	n.s.

b)

	Saprophytes				unknown			
	Shannon		MMF		Shannon		MMF	
	wrp	**p**	**wrp**	**p**	**wrp**	**p**	**wrp**	**p**
Mineral soil Total C	16	0.066	16	n.s.	0	n.s.	1	n.s.
Mineral soil Total N	8	n.s.	10	n.s.	12	n.s.	16	n.s.
Mineral soil pH 1	6	n.s.	11	n.s.	14	n.s.	0	n.s.
ForMI		**<0.001**		**0.002**	9	n.s.	14	0.053
Iharv	4	n.s.	0	n.s.	13	n.s.	15	n.s.
SMI	10	n.s.	17	n.s.	6	n.s.	7	n.s.
Number of tree species per plot	2	n.s.	8	n.s.		**0.019**		**0.022**
Fine Roots Biomass	1	n.s.	3	n.s.	4	n.s.	9	n.s.
Root glucose concentration	13	n.s.		**0.021**	8	n.s.	11	n.s.
Root fructose concentration		**0.005**		**0.001**	15	n.s.	13	n.s.
Root C concentration	9	n.s.		**0.002**		**0.003**	8	n.s.
Root N concentration	14	n.s.	1	n.s.		**0.013**		**<0.001**
Root CN ratio	12	n.s.	14	n.s.	3	n.s.		**0.020**
Root Al concentration	0	n.s.	6	n.s.	7	n.s.	5	n.s.
Root Ca concentration	5	n.s.	12	n.s.	2	n.s.	17	n.s.
Root Fe concentration	3	n.s.	7	n.s.		**0.014**	12	n.s.
Root K concentration	7	n.s.	2	n.s.	11	n.s.	3	n.s.
Root Mg concentration		**0.018**	4	n.s.		**0.021**	18	n.s.
Root Mn concentration	11	n.s.	15	n.s.	5	n.s.	6	n.s.
Root Na concentration		**0.003**	9	n.s.	1	n.s.	4	n.s.
Root P concentration	15	n.s.	13	n.s.	10	n.s.	2	n.s.
Root S concentration		**0.001**	5	n.s.	16	n.s.	10	n.s.

Table 3.11:Spearman rank correlations of Shannon and MMF richness estimators of all, EM, saprophytic and unknown fungi correlated with different variables (rho and p-values, n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05). a) for All and EM fungi; b) for saprophytes and unknown fungi. For correlations between variables see Supplementary Table S5.

a)

	All fungi				**EM**			
	Shannon		**MMF**		**Shannon**		**MMF**	
	rho	**p**	**rho**	**p**	**rho**	**p**	**rho**	**p**
Mineral soil Total C	0.253	**0.007**	0.300	**0.002**	0.240	**0.016**	0.330	**0.001**
Mineral soil Total N	0.219	**0.021**	0.291	**0.002**	0.182	0.070	0.311	**0.002**
Mineral soil pH 1	0.239	**0.012**	0.309	**0.001**	0.100	n.s.	0.218	**0.030**
ForMI	0.195	**0.042**	0.109	n.s.	0.167	n.s.	0.149	n.s.
Iharv	0.087	n.s.	0.119	n.s.	0.041	n.s.	0.209	**0.039**
SMI	0.261	**0.006**	0.173	0.069	0.267	**0.007**	0.291	**0.003**
Number of tree species per plot	0.184	0.053	0.201	**0.035**	0.170	n.s.	0.168	n.s.
Fine Roots Biomass	-0.125	n.s.	0.023	n.s.	-0.099	n.s.	0.041	n.s.
Coarse root biomass	-0.184	0.072	-0.055	n.s.	-0.367	**0.001**	-0.301	**0.005**
Root glucose concentration	0.244	**0.011**	0.082	n.s.	0.394	**<0.001**	0.242	**0.017**
Root fructose concentration	0.155	n.s.	0.155	n.s.	0.193	0.059	0.130	n.s.
Root C concentration	-0.224	**0.019**	-0.330	**0.001**	-0.053	n.s.	-0.162	n.s.
Root N concentration	-0.438	**<0.001**	-0.372	**<0.001**	-0.312	**0.002**	-0.271	**0.007**
Root CN ratio	0.365	**<0.001**	0.247	**0.009**	0.326	**0.001**	0.226	**0.025**
Root Al concentration	0.249	**0.010**	0.388	**<0.001**	0.107	n.s.	0.288	**0.005**
Root Ca concentration	0.224	**0.021**	0.324	**0.001**	0.151	n.s.	0.271	**0.008**
Root Fe concentration	0.249	**0.010**	0.308	**0.001**	0.195	0.057	0.236	**0.021**
Root K concentration	-0.094	n.s.	0.064	n.s.	-0.270	**0.008**	-0.196	0.056
Root Mg concentration	0.230	**0.018**	0.369	**<0.001**	0.076	n.s.	0.208	**0.042**
Root Mn concentration	0.181	0.063	0.164	n.s.	0.190	0.064	0.222	**0.030**
Root Na concentration	0.256	**0.008**	0.300	**0.002**	-0.025	n.s.	0.075	n.s.
Root P concentration	-0.137	n.s.	-0.234	**0.016**	0.008	n.s.	-0.123	n.s.
Root S concentration	-0.133	n.s.	-0.118	n.s.	-0.248	**0.015**	-0.268	**0.008**

b)

	Saprophytes				unknown			
	Shannon		MMF		Shannon		MMF	
	rho	p	rho	p	rho	p	rho	p
Mineral soil Total C	0.143	n.s.	0.120	n.s.	0.248	**0.013**	0.248	**0.013**
Mineral soil Total N	0.217	**0.030**	0.167	n.s.	0.266	**0.007**	0.259	**0.009**
Mineral soil pH 1	0.251	**0.012**	0.167	n.s.	0.314	**0.002**	0.323	**0.001**
ForMI	-0.295	**0.003**	-0.190	0.061	0.163	n.s.	0.131	n.s.
Iharv	-0.022	n.s.	0.021	n.s.	0.127	n.s.	0.091	n.s.
SMI	-0.117	n.s.	-0.008	n.s.	0.226	**0.024**	0.146	n.s.
Number of tree species per plot	-0.020	n.s.	0.029	n.s.	0.316	**0.001**	0.341	**0.001**
Fine Roots Biomass	0.045	n.s.	0.046	n.s.	0.009	n.s.	-0.007	n.s.
Coarse root biomass	0.130	n.s.	0.157	n.s.	-0.146	n.s.	-0.176	n.s.
Root glucose concentration	0.017	n.s.	0.033	n.s.	0.057	n.s.	0.092	n.s.
Root fructose concentration	0.248	**0.013**	0.209	**0.038**	0.178	n.s.	0.157	n.s.
Root C concentration	-0.240	**0.016**	-0.216	**0.031**	-0.199	**0.048**	-0.290	**0.004**
Root N concentration	-0.220	**0.028**	-0.056	n.s.	-0.297	**0.003**	-0.378	**<0.001**
Root CN ratio	0.138	n.s.	-0.053	n.s.	0.215	**0.032**	0.260	**0.009**
Root Al concentration	0.288	**0.004**	0.212	**0.037**	0.271	**0.008**	0.333	**0.001**
Root Ca concentration	0.179	n.s.	0.144	n.s.	0.287	**0.005**	0.323	**0.002**
Root Fe concentration	0.098	n.s.	0.052	n.s.	0.111	n.s.	0.245	**0.017**
Root K concentration	0.207	**0.042**	0.223	**0.028**	0.002	n.s.	0.066	n.s.
Root Mg concentration	0.329	**0.001**	0.264	**0.009**	0.293	**0.004**	0.373	**<0.001**
Root Mn concentration	0.050	n.s.	0.020	n.s.	0.080	n.s.	0.077	n.s.
Root Na concentration	0.399	**<0.001**	0.199	0.051	0.060	n.s.	0.124	n.s.
Root P concentration	-0.234	**0.021**	-0.123	n.s.	-0.196	0.057	-0.248	**0.016**
Root S concentration	-0.166	n.s.	-0.133	n.s.	-0.132	n.s.	-0.180	n.s.

Another aspect of forest management is represented in the stand, or respectively inventory structure. The highest EM richness/diversity as well as the highest root glucose concentration is found at an average diameter at breast height (DBH) between 15 and 30 cm (Figure 3.12 a and b). Overall EM richness and root glucose concentration show a similar pattern in comparison between the different stand structures. This pattern could not be detected for saprophytic fungi which did not differ significantly between the different stand structures (Figure 3.12 c).

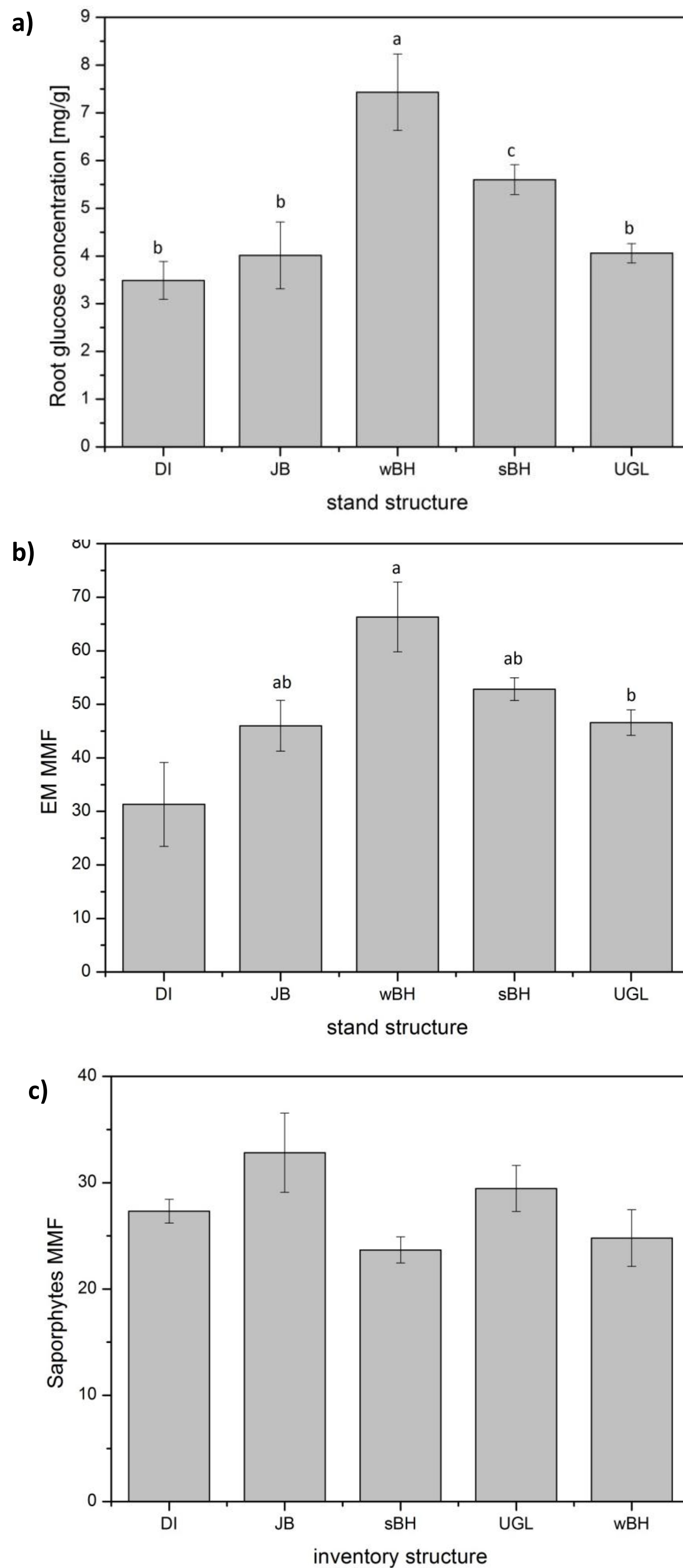


Figure 3.12: a) root glucose concentration, b) EM richness (MMF) and c) saprophytic richness of different stand structures. DI: thicket, taller than 2 m, but diameter at breast height (DBH) smaller than 7 cm, JB: pole stage forest, average DBH between 7 and 15 cm, wBH: average DBH between 15 and 30 cm, sBH: average DBH over 30 cm, UGL: great span of age and DBH. Different letters indicate for significant differences (for EM: DI n = 2 *therefore this was not used for statistical comparisons, JB n = 5, sBH n = 60, UGL n = 22, wBH n = 11; for glucose concentration: DI n = 13, JB n = 13, sBH n = 80, UGL n = 29, wBH n = 12; mean±SE).

3.1.6 Relationship of fungal community structure to different environmental variables

Because community structures of all fungi, EMs, saprophytes and fungi with unknown lifestyle were separated between the Exploratories and the dominant tree species on the plots, we were interested in identifying additional environmental drivers. Therefore a number of variables of soil parameters, management intensity and root carbohydrate and element concentrations were tested in the NMDS as explanatory variable. Comparisons of these variables (Table 3.12) between the Exploratories or the dominant tree species are listen in Table 3.13. Some important variables are shown within NMDS represented by contour lines or vectors (Figure 3.13, for all variables see Supplementary Figure S5). Those results are supported by the GCV score from the generalized adaptive models (gam; Table 3.14).

The pH value of the mineral soil was more important for the community structure of saprophytic fungi than for EM (Figure 3.13 a, Table 3.12, Table 3.13, Table 3.14). Even if the plots with different tree species differ in their pH value more important was the Exploratory. Alb had the highest pH value, followed by Hainich and lowest in Schorfheide. Soil C and N explain the community structure of saprophytic fungi better than that of EM (Figure 3.13 a, Table 3.12, Table 3.13, Table 3.14). The model for all fungi fitted better than for one of the lifestyle groups.

Forest Management had a little more influence on the community structure of EM than on that of saprophytic fungi. Coniferous plots have a higher management index than deciduous ones, independent from Exploratory. The model for all fungi fitted better than for one of the lifestyle groups. Both management indices (ForMI and SMI) resulted in comparable results (Figure 3.13 b, Table 3.12, Table 3.13, Table 3.14). The two single carbohydrates tested showed substantial differences in relation to the community structure of different lifestyles. Deciduous trees had less fructose in their roots than spruce, but more than pine. The root fructose concentration is independent from Exploratory. The models for the community structure of all different lifestyles tested (all, EM, saprophytic and unknown fungi) fitted with a very similar GCV score. In contrast to this the root glucose concentration, where the model for the community structure of all fungi fitted better than that one of EM (Table 3.14). The EM community structure fitted better to the glucose allocation than the saprophytic fungal community. This supports the result from the glm shown above, that the EM diversity, but not the richness, depended on the glucose concentration of the roots. In general spruce plots had significantly more glucose than beech or pine plots (Table 3.13). In addition tree roots from the Alb had significantly more glucose than those from Hainich or Schorfheide (Table 3.12). The vectors of Figure 3.13 b showed a stronger dependency of fructose than for glucose. Remarkably for saprophytes glucose had nearly no effect.

The Ca concentration within the roots was less in Schorfheide, whereas Alb and Hainich showed similar values. The Ca concentration of the soil was not dependent on the main tree species on the plot. The GCV score was lower for saprophytic than for EM community structure. In comparison to the richness and diversity as described above, the richness of EM was positively influenced by Ca, whereas the richness of saprophytes was not. For all fungi the Ca model fitted best in comparison to EM or saprophytes

One of the most important factors was root nitrogen. Figure 3.13 c shows the root CN ratio as contour lines, where no clear pattern in the direction of Exploratory is detected. Again there are differences between EM and saprophytic fungi, where as the saprophytes depended more on root C concentration and EM more on root N concentration. For all fungi a clear separation of higher CN values for conifers was detected (Figure 3.13 c, Table 3.13). Root N concentration was mainly independent from Exploratory, whereas the tree species played a major role. Spruce plots showed the lowest N root concentration whereas oak plots, together with some beech and pine plots the highest root N concentration for all fungi (Table 3.12, Table 3.14). The model fitted best for all fungi and better for EM community than for the saprophytic community. This leads to the assumption that EM fungal communities rely more on nitrogen than saprophytic communities. Root C concentration was less within the Schorfheide and significantly different to the other Exploratories. It was mainly independent from main tree species on the plot. For saprophytic fungi the model fits a little better than for EM fungi; consequently the community structure of EM was a little less dependent on root C concentration than the community structure of saprophytes. As described above also the richness and diversity of saprophytic fungi were influenced by root carbon concentration as the richness and diversity of EM were not.

When comparing the GCV scores of the different variables (z-scored variables in a gam with gaussian distribution, Table 3.14) we see that for all lifestyles pH is the variable which explained community distribution best. The second one is the Ca concentration of the root, which is not surprising as these two correlate highly significantly (rho= 0.921; $p<0.001$). The two management indices have a mean explanation to the community structures of all lifestyles, whereas the community structure of EMs is a little better explained than the one of saprophytes. Remarkably within the saprophytes the model for root N concentration fitted less than the model for root C concentration. Whereas for all fungi or EM it is the other way round that the model for root N concentration fitted better than the C root concentration model. When comparing the two single carbohydrates the model for root glucose concentration fitted better for all fungi and for EM, but not for saprophytes. For them the model for root fructose concentration fitted best.

Table 3.12: Variables used for Anova and NMDS-Ordisurf-gam analysis (mean ±SE) values within the three Exploratories and comparison of those variables between the Exploratories per Anova and Tukey posthoc test if residuals were normal distributed or wilkox test (than marked with "W", n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05).

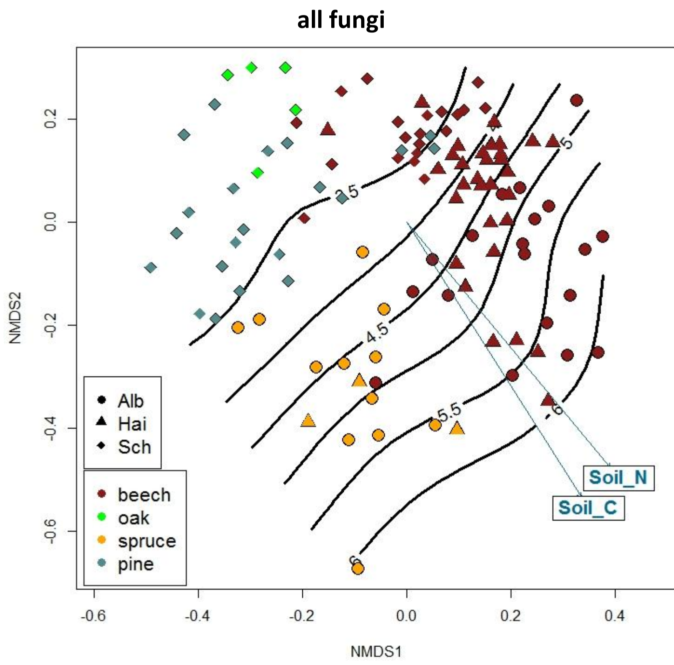
		ALB		HAI		SCH		Exploratory global	ALB - HAI	ALB - SCH	HAI - SCH
	unit	mean	SE	mean	SE	mean	SE	p	p	p	p
Mineral soil Inorganic C	[mg/g]	0.688	±0.217	0.262	±0.044	0.000	±0.000	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Mineral soil Organic C	[mg/g]	63.225	±2.756	34.268	±1.866	20.812	±0.766	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Mineral soil Total C	[mg/g]	63.910	±2.875	34.531	±1.902	20.812	±0.766	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Mineral soil Total N	[mg/g]	4.818	±0.219	2.575	±0.145	1.130	±0.045	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Mineral soil CN ratio		13.176	±0.189	13.443	±0.201	18.792	±0.413	W	n.s.	**<0.001**	**<0.001**
Mineral soil pH 1		5.089	±0.148	4.540	±0.115	3.371	±0.021	W	**0.007**	**<0.001**	**<0.001**
SMI		0.328	±0.034	0.201	±0.024	0.216	±0.015	**0.003**	**0.003**	**0.018**	0.637
Coarse Roots Biomass	[g/dm^3]	0.473	±0.159	4.800	±0.466	2.057	±0.282	W	**<0.001**	**<0.001**	**<0.001**
Fine Roots Biomass	[g/dm^3	2.037	±0.324	3.570	±0.471	1.830	±0.169	W	**0.004**	n.s.	**<0.001**
Root glucose concentration	[mg/g]	7.325	±0.702	4.412	±0.265	5.059	±0.276	W	**<0.001**	**0.016**	0.275
Root fructose concentration	[mg/g]	2.110	±0.139	2.279	±0.291	1.905	±0.124	W	n.s.	n.s.	n.s.
Root C concentration	[mg/g]	434.407	±5.406	451.316	±3.042	473.352	±3.689	**<0.001**	**0.015**	**<0.001**	**<0.001**
Root N concentration	[mg/g]	11.133	±0.325	12.832	±0.356	13.462	±0.405	**<0.001**	**0.011**	**<0.001**	n.s.
Root CN ratio		39.919	±1.188	36.011	±0.969	36.673	±1.160	0.051	0.065	n.s.	n.s.
Root Al concentration	[mg/g]	12.613	±0.712	8.198	±0.304	4.004	±0.172	W	**<0.001**	**<0.001**	**<0.001**
Root Ca concentration	[mg/g]	8.378	±0.520	5.173	±0.374	2.049	±0.099	W	**<0.001**	**<0.001**	**<0.001**
Root Fe concentration	[mg/g]	7.691	±0.455	5.572	±0.273	5.394	±0.393	W	**<0.001**	**<0.001**	n.s.
Root K concentration	[mg/g]	3.585	±0.249	5.201	±0.271	3.215	±0.118	W	**<0.001**	n.s.	**<0.001**
Root Mg concentration	[mg/g]	1.708	±0.078	1.649	±0.111	0.722	±0.026	W	n.s.	**<0.001**	**<0.001**
Root Mn concentration	[mg/g]	0.360	±0.021	0.378	±0.026	0.346	±0.026	W	n.s.	n.s.	n.s.
Root Na concentration	[mg/g]	0.307	±0.053	0.249	±0.026	0.166	±0.011	W	n.s.	**0.014**	**<0.001**
Root P concentration	[mg/g]	0.906	±0.063	0.802	±0.032	1.162	±0.037	W	n.s.	**<0.001**	**<0.001**
Root S concentration	[mg/g]	0.883	±0.034	1.089	±0.040	1.078	±0.022	W	**<0.001**	**<0.001**	1

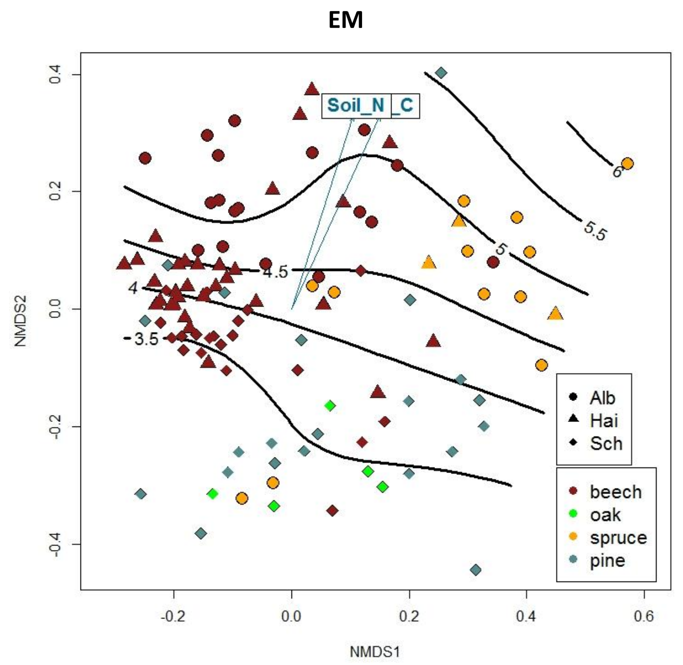
Table 3.13: Variables used for Anova and NMDS-Ordisurf-gam analysis (mean ±SE) values for the different dominant tree species and comparison of those variables between the tree species per Anova and Tukey posthoc test if residuals were normal distributed or wilkox test (than marked with "W", n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05).

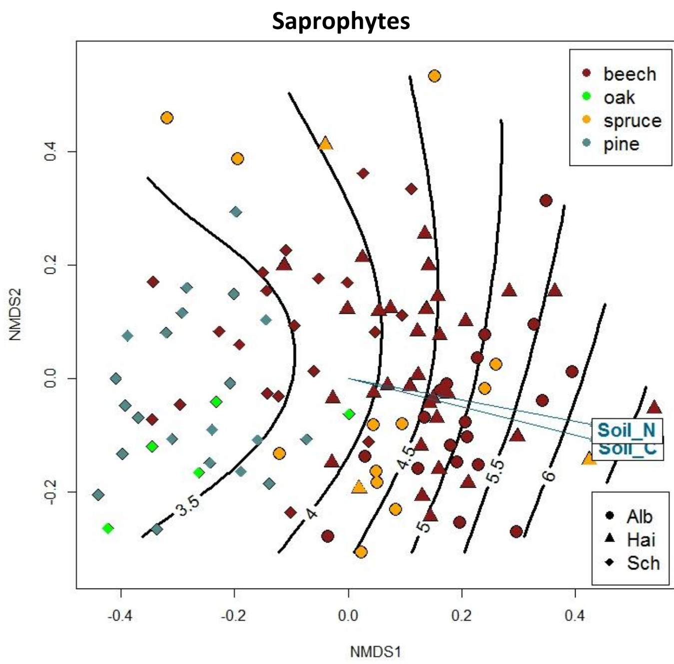
		beech		oak		spruce		pine	
	unit	mean	SE	mean	SE	mean	SE	mean	SE
Mineral soil Inorganic C	[mg/g]	0.244	±0.039	0.000	±0.000	0.848	±0.424	0.000	±0.000
Mineral soil Organic C	[mg/g]	36.971	±2.233	20.074	±1.180	62.320	±4.165	20.621	±1.477
Mineral soil Total C	[mg/g]	37.215	±2.261	20.074	±1.180	63.165	±4.463	20.621	±1.477
Mineral soil Total N	[mg/g]	2.750	±0.193	1.313	±0.089	4.455	±0.340	0.997	±0.075
Mineral soil CN ratio		14.310	±0.266	15.312	±0.196	14.148	±0.279	20.980	±0.554
Mineral soil pH 1		4.356	±0.104	3.406	±0.050	4.967	±0.249	3.334	±0.033
ForMI		0.840	±0.059	0.780	±0.123	2.070	±0.084	1.764	±0.061
SMI		0.185	±0.013	0.102	±0.023	0.509	±0.019	0.273	±0.018
Coarse Roots Biomass	[g/dm^3]	3.036	±0.293	0.854	±0.201	2.273	±1.145	2.523	±0.535
Fine Roots Biomass	[g/dm^3	2.826	±0.274	1.786	±0.322	2.253	±0.516	1.378	±0.209
Root glucose concentration	[mg/g]	5.021	±0.303	6.133	±0.685	8.190	±0.904	4.920	±0.406
Root fructose concentration	[mg/g]	2.065	±0.150	1.115	±0.418	2.410	±0.299	2.084	±0.181
Root C concentration	[mg/g]	453.215	±3.563	475.565	±8.467	443.772	±5.714	468.576	±6.315
Root N concentration	[mg/g]	12.946	±0.286	15.699	±0.902	11.038	±0.399	11.935	±0.571
Root CN ratio		36.025	±0.728	30.666	±1.657	41.020	±1.741	40.891	±1.811
Root Al concentration	[mg/g]	8.323	±0.514	4.455	±0.304	10.559	±1.095	4.397	±0.291
Root Ca concentration	[mg/g]	5.106	±0.337	2.388	±0.281	8.407	±0.893	1.576	±0.108
Root Fe concentration	[mg/g]	5.958	±0.274	4.933	±0.531	6.910	±0.617	6.269	±0.760
Root K concentration	[mg/g]	4.400	±0.188	3.871	±0.166	3.456	±0.345	2.760	±0.170
Root Mg concentration	[mg/g]	1.425	±0.080	0.929	±0.085	1.630	±0.116	0.658	±0.033
Root Mn concentration	[mg/g]	0.359	±0.016	0.482	±0.056	0.371	±0.035	0.329	±0.048
Root Na concentration	[mg/g]	0.261	±0.027	0.159	±0.029	0.171	±0.022	0.198	±0.021
Root P concentration	[mg/g]	0.930	±0.040	1.370	±0.113	0.973	±0.058	1.045	±0.044
Root S concentration	[mg/g]	1.044	±0.025	1.237	±0.081	0.845	±0.044	1.059	±0.031

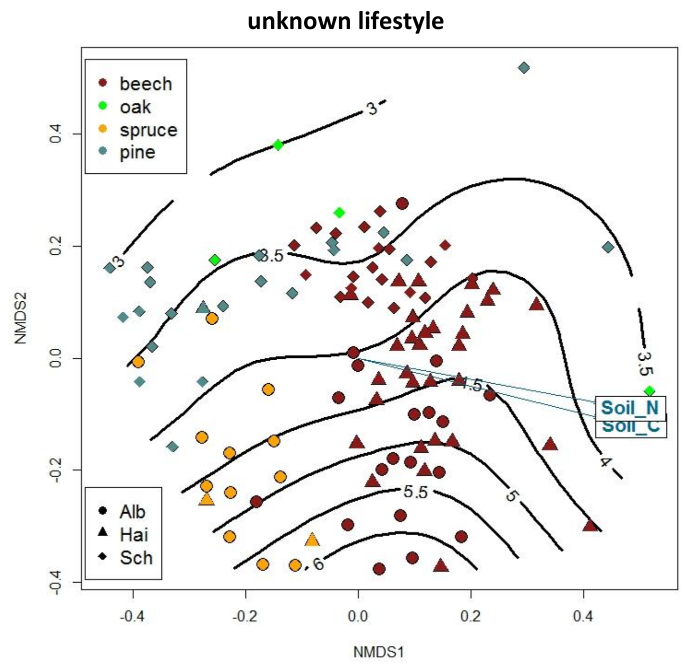
Environmental variable	Species global	Beech-Oak	Beech-Spruce	Beech-Pine	Spruce-Oak	Pine-Oak	Pine-Spruce
	p	p	p	p	p	p	p
Mineral soil Inorganic C	**0.002**	n.s.	**0.008**	n.s.	0.061	n.s.	**0.001**
Mineral soil Organic C	**W**	n.s.	**0.011**	**<0.001**	0.006	**<0.001**	**<0.001**
Mineral soil Total C	**<0.001**	0.068	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Mineral soil Total N	**W**	n.s.	**0.002**	**<0.001**	**<0.001**	n.s.	**<0.001**
Mineral soil CN ratio	**W**	n.s.	n.s.	**<0.001**	n.s.	**<0.001**	**<0.001**
Mineral soil pH 1	**<0.001**	**0.035**	**0.038**	**<0.001**	**<0.001**	n.s.	**<0.001**
ForMI	**W**	n.s.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.048**
SMI	**W**	n.s.	**<0.001**	**0.001**	**<0.001**	**0.016**	**<0.001**
Coarse Roots Biomass	**W**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fine Roots Biomass	**W**	n.s.	n.s.	**0.002**	n.s.	n.s.	n.s.
Root glucose concentration	**W**	n.s.	**<0.001**	n.s.	n.s.	n.s.	**0.016**
Root fructose concentration	**W**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Root C concentration	**0.024**	n.s.	n.s.	n.s.	n.s.	n.s.	0.055
Root N concentration	**<0.001**	0.056	**0.024**	n.s.	**<0.001**	**0.008**	n.s.
Root CN ratio	**<0.001**	n.s.	**0.041**	**0.026**	**0.011**	**0.011**	n.s.
Root Al concentration	**<0.001**	n.s.	n.s.	**<0.001**	**0.025**	n.s.	**<0.001**
Root Ca concentration	**<0.001**	n.s.	**<0.001**	**<0.001**	**<0.001**	n.s.	**<0.001**
Root Fe concentration	**W**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Root K concentration	**W**	n.s.	n.s.	**<0.001**	n.s.	**0.030**	n.s.
Root Mg concentration	**<0.001**	n.s.	n.s.	**<0.001**	n.s.	n.s.	**<0.001**
Root Mn concentration	**W**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Root Na concentration	**W**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Root P concentration	**W**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Root S concentration	**W**	n.s.	**0.001**	n.s.	**0.022**	n.s.	**0.003**

a) Soil parameter: pH as contour lines and soil C and N as vectors

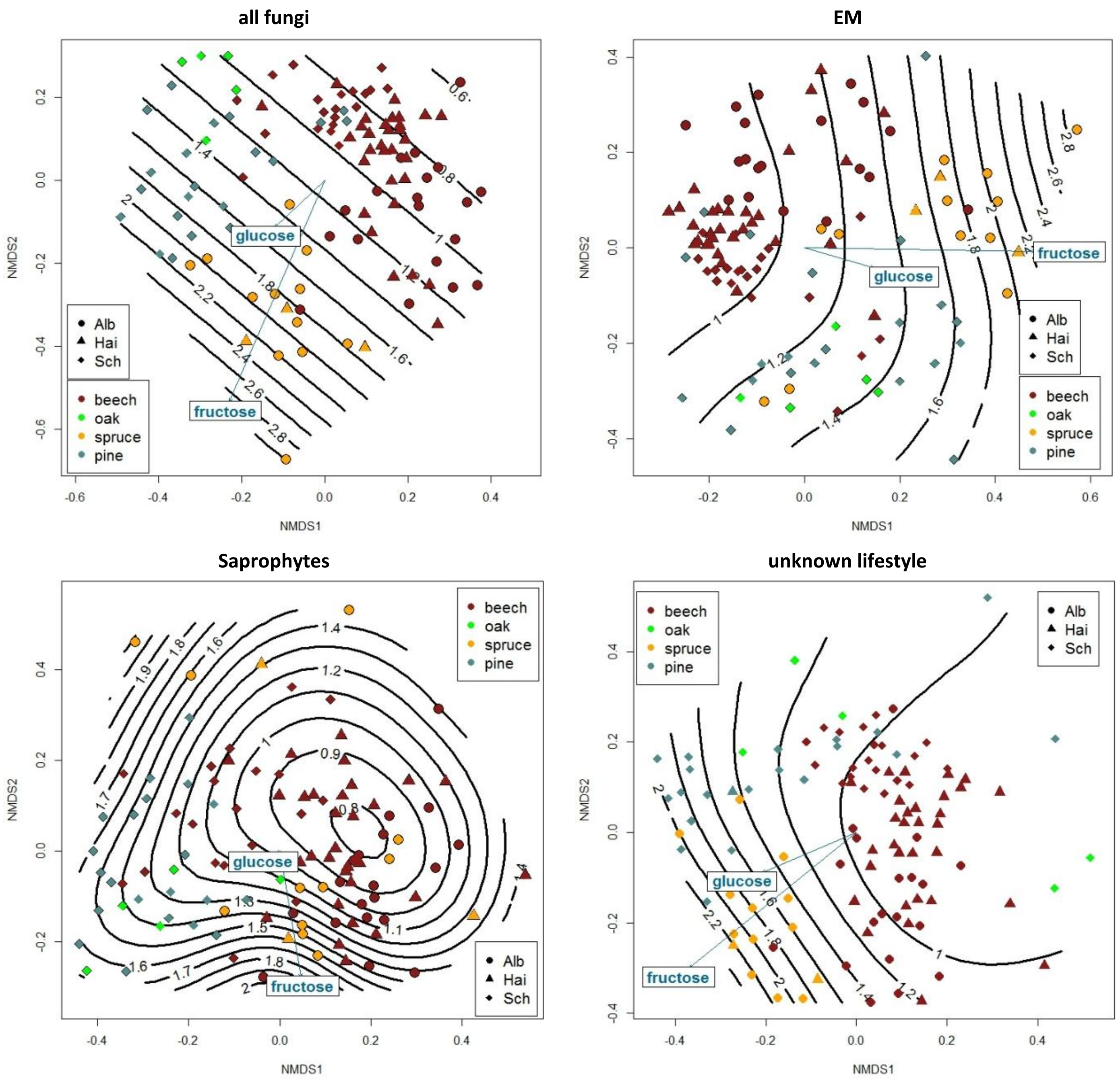








b) Management Index ForMI as contour lines and root single carbohydrate concentration as vectors



c) Root element concentrations: CN ratio as contour lines and root elements (C, N, Al, Ca, Fe, K, Mg, Na, P, S)

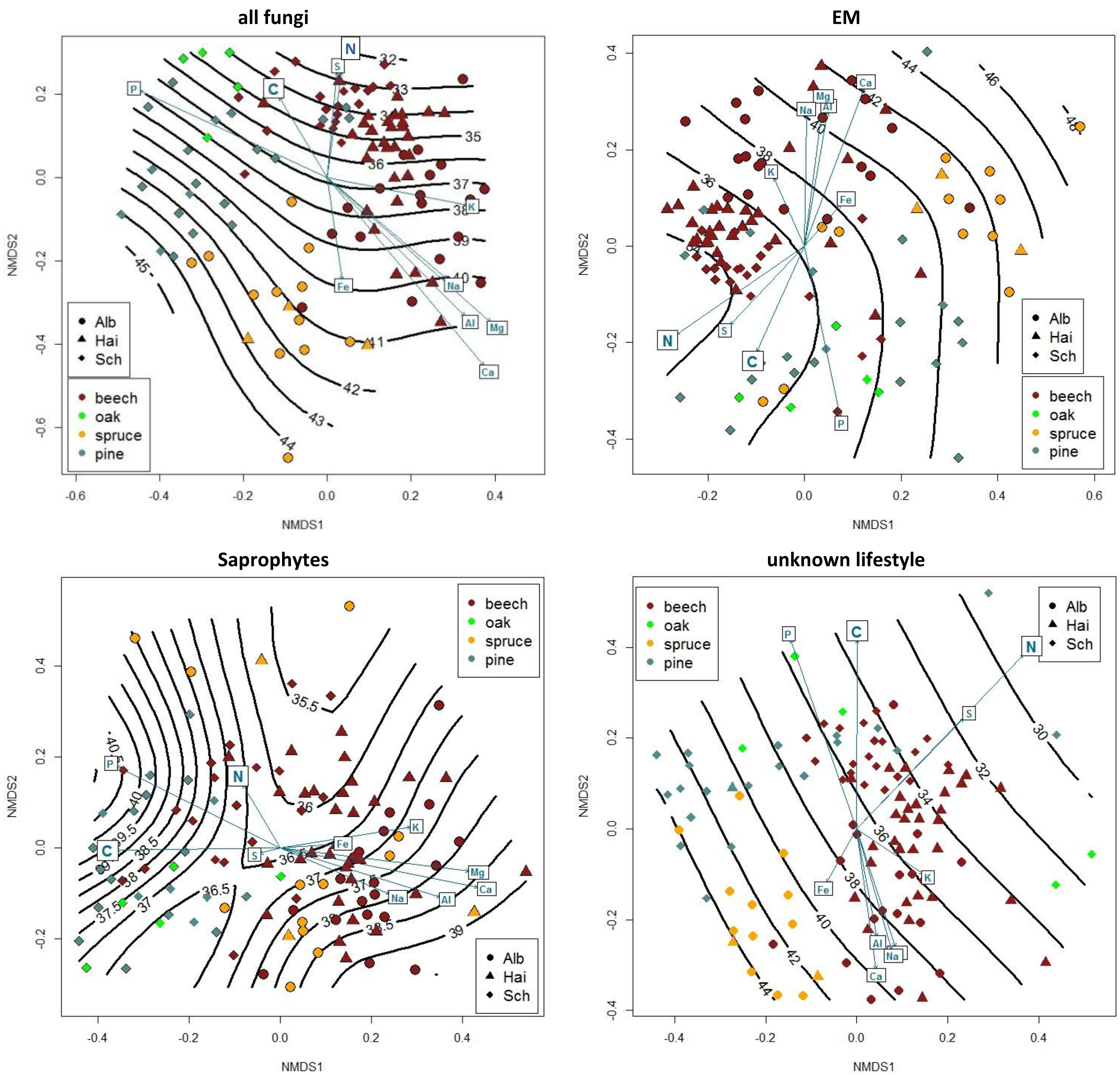


Figure 3.13: NMDS from all fungi, EMs and saprophytes for different variables represented by ordisurf contour lines or vectors. Different colours code for different main tree species on the plot and symbols for the three Exploratories. a) Soil parameter: pH as contour lines and soil C and N as vectors; b) Management Index ForMI as contour lines and root single carbohydrate concentration as vectors; c) Root element concentrations: CN ratio as contour lines and root elements (C, N, Al, Ca, Fe, K, Mg, Na, P, S). According GCV values are listen in Table 3.14. For NMDS-ordisurf plots for all variables see Supplementary S 6. (n= c.f. Table 3.1 and Table 2.3).

Table 3.14: GCV-scores (quasipoisson distribution in generalized additive models (gam) and gaussians distribution with z-scored variables in gams) of different biotic and abiotic variables influencing the community structure of all, EM, saprophytic or unknown fungi.

	All fungi				EM				Sparophytic Fungi				unknown fungi			
	quasi poisson		gaussian with z-scores		quasi poisson		gaussian with z-scores		quasi poisson		gaussian with z-scores		quasi poisson		gaussian with z-scores	
	DF	GCV	DF	GCV	DF	GCV	DF	GCV	DF	score	DF	GCV	DF	GCV	DF	GCV
Mineral soil Inorganic C	9.02	0.25	4.49	0.84	9.02	0.37	4.35	0.92	9.00	0.33	7.49	0.89	8.76	0.30	8.62	0.80
Mineral soil Organic C	8.34	3.88	7.89	0.42	8.34	6.08	7.33	0.61	8.58	4.75	7.72	0.50	7.58	5.04	6.64	0.49
Mineral soil Total C	8.3	3.97	7.81	0.42	8.30	6.23	7.30	0.61	8.60	4.88	7.74	0.50	7.58	5.13	6.66	0.48
Mineral soil Total N	8.87	0.33	8.51	0.35	8.87	0.59	7.21	0.57	8.61	0.40	7.67	0.43	7.60	0.50	6.56	0.48
Mineral soil CN ratio	8.76	0.26	8.70	0.42	8.76	0.53	5.37	0.79	7.61	0.27	6.88	0.42	7.38	0.36	5.44	0.55
Mineral soil pH 1	8.85	0.04	8.67	0.20	8.85	0.09	6.81	0.49	8.22	0.05	8.68	0.29	8.59	0.05	6.60	0.27
ForMI	2.94	0.31	5.42	0.55	2.94	0.36	6.85	0.67	5.28	0.39	6.07	0.78	4.97	0.34	6.21	0.62
SMI	6.81	0.05	7.23	0.41	6.81	0.06	5.98	0.60	5.44	0.08	6.66	0.88	4.62	0.05	5.67	0.52
Coarse Roots Biomass	7	2.18	6.85	0.95	7.00	2.22	5.59	0.96	5.58	2.12	3.53	0.92	4.31	2.32	6.93	1.00
Fine Roots Biomass	7.87	1.15	7.14	0.90	7.87	1.29	8.91	0.97	2.67	1.16	2.74	0.86	4.28	1.18	8.17	0.91
Root fructose content	1	0.58	1.00	1.01	1.00	0.58	1.00	1.01	1.33	0.58	1.96	1.01	2.08	0.57	1.00	1.00
Root glucose content	7.21	0.80	7.34	0.71	7.21	0.90	4.24	0.81	7.79	1.13	6.82	0.99	6.72	0.91	4.85	0.81
Root C content	5.88	1.41	5.96	0.73	5.88	1.64	4.26	0.86	6.78	1.55	7.70	0.81	7.67	1.47	4.25	0.77
Root N content	5.73	0.32	5.92	0.67	5.73	0.35	5.29	0.74	6.28	0.47	7.37	0.96	7.28	0.32	5.32	0.66
Root CN ratio	4.89	1.01	4.80	0.79	4.89	1.05	6.08	0.82	4.59	1.26	3.86	0.99	3.59	0.94	6.06	0.74
Root Al content	7.97	1.02	7.84	0.48	7.97	1.41	5.92	0.62	8.22	1.25	6.82	0.59	6.86	1.47	6.09	0.67
Root Ca content	9.07	0.43	8.24	0.27	9.07	0.99	5.64	0.54	8.29	0.77	8.46	0.44	8.80	0.65	6.32	0.33
Root Fe content	5.07	0.79	4.69	0.84	5.07	0.83	3.08	0.88	6.27	0.94	5.42	0.98	5.54	0.89	3.16	0.93
Root K content	2.89	0.41	2.87	0.81	2.89	0.47	2.88	0.92	4.25	0.40	4.74	0.80	5.00	0.42	2.90	0.84
Root Mg content	8.4	0.11	7.86	0.41	8.40	0.19	5.71	0.64	7.13	0.13	5.96	0.50	5.82	0.16	6.08	0.58
Root Mn content	1	0.06	1.00	1.01	1.00	0.06	2.92	0.96	4.56	0.06	6.23	1.01	6.32	0.06	3.87	0.99
Root Na content	6.39	0.08	5.56	0.87	6.39	0.08	6.40	0.85	6.44	0.08	6.27	0.90	7.27	0.08	6.90	0.90
Root P content	6.43	0.07	6.02	0.85	6.43	0.08	2.84	0.86	2.84	0.07	6.49	0.84	6.95	0.07	3.83	0.86
Root S content	4.45	0.04	4.20	0.97	4.45	0.04	2.07	0.99	3.74	0.04	3.72	1.01	4.17	0.04	2.13	0.94

3.2 Results of the disturbance root trenching experiment

The goal of this experimental approach was to investigate the belowground influence of forest management. As a surrogate for “management”, which includes tree removal and root severing, an experimental root severing was employed.

For this roots were cut belowground and degradation as well as recolonizing processes was observed for 18 months in “No Ingrowth” and “Ingrowth” cores. Undisturbed soil cores were harvested as “Controls”. The experiment was installed in April/May 2011, this was set to month=0.

First some general soil properties will be described. Differences in root biomass and the percentage of vital tips will be regarded. Mycorrhization rate and species abundance in the different treatments are shown, followed by the richness and diversity of the different treatments. Differences in the EM community and the similarity between Ingrowth and Control cores will be presented. Finally the functional diversity, represented by exploration types, is addressed.

3.2.1 Soil properties

Soil water content was significantly higher in No Ingrowth cores than in Control or Ingrowth cores, most probably caused by the plastic tube, which was used to prevent ingrowth of roots (Figure 3.14). The pH values did not differ significant between the treatments (Figure 3.15).

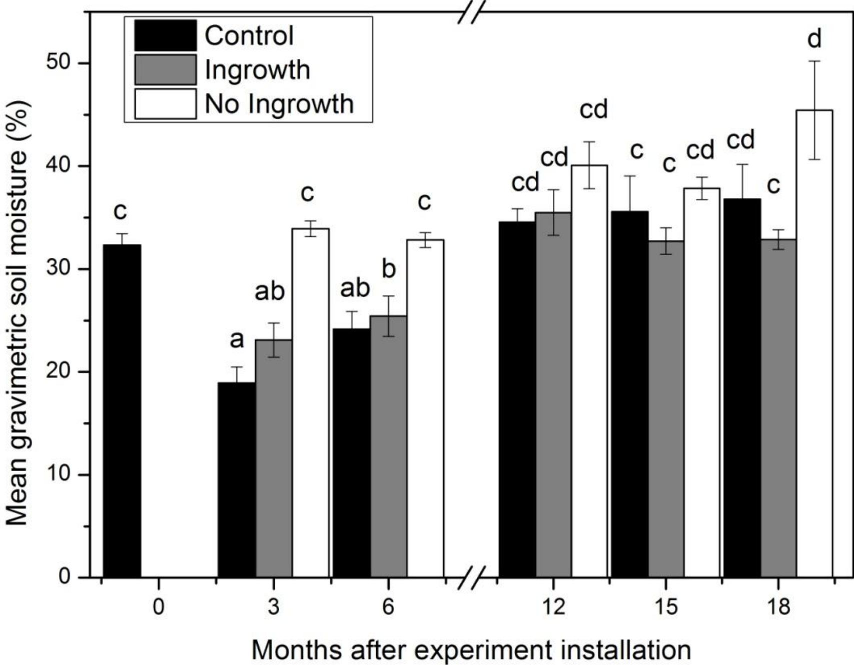


Figure 3.14: Gravimetric soil moisture for all harvests per treatment No Ingrowth, Ingrowth and Controls. Different letters indicate significant differences (mean ± SE, n= Material and Method Table 2.2).

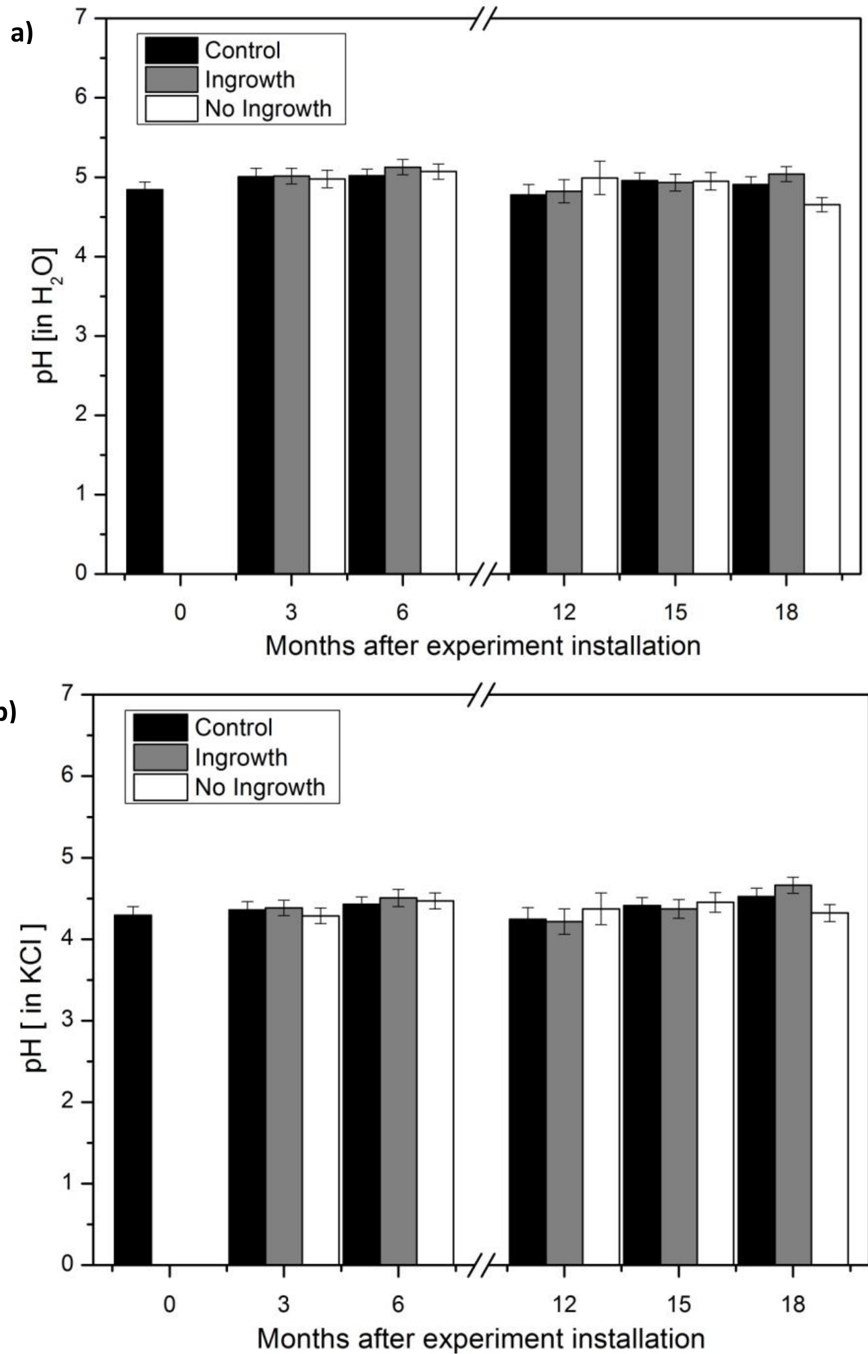


Figure 3.15: pH values within the soil cores for all harvests per treatment. A) measured in H_2O or b) in KCl. No significant differences were detected (mean ± SE, n= Material and Method Table 2.2).

3.2.2 Mean fine root biomass

Beech fine root dry biomass ranged between 0.19 ± 0.04 g at the 18 months harvest in No Ingrowth cores and 1.49 ± 0.19 g in Control cores at the beginning (Figure 3.16 a). The fine root biomass was significantly increased in Control cores at the beginning of the experiment in comparison to the other harvests. A significant mass loss in No Ingrowth cores in comparison to Ingrowth or Control cores after 18 months was detected. Roots in No Ingrowth cores degraded, whereas the fine root biomass within the Ingrowth cores did not differ significantly from Controls or over time (Figure 3.16 a). The coarse root biomass did not differ significantly between harvests or treatments and had an overall high variation between the soil cores, indicated by high standard errors (Figure 3.16 b) The coarse root biomasss is important for further interpretations.

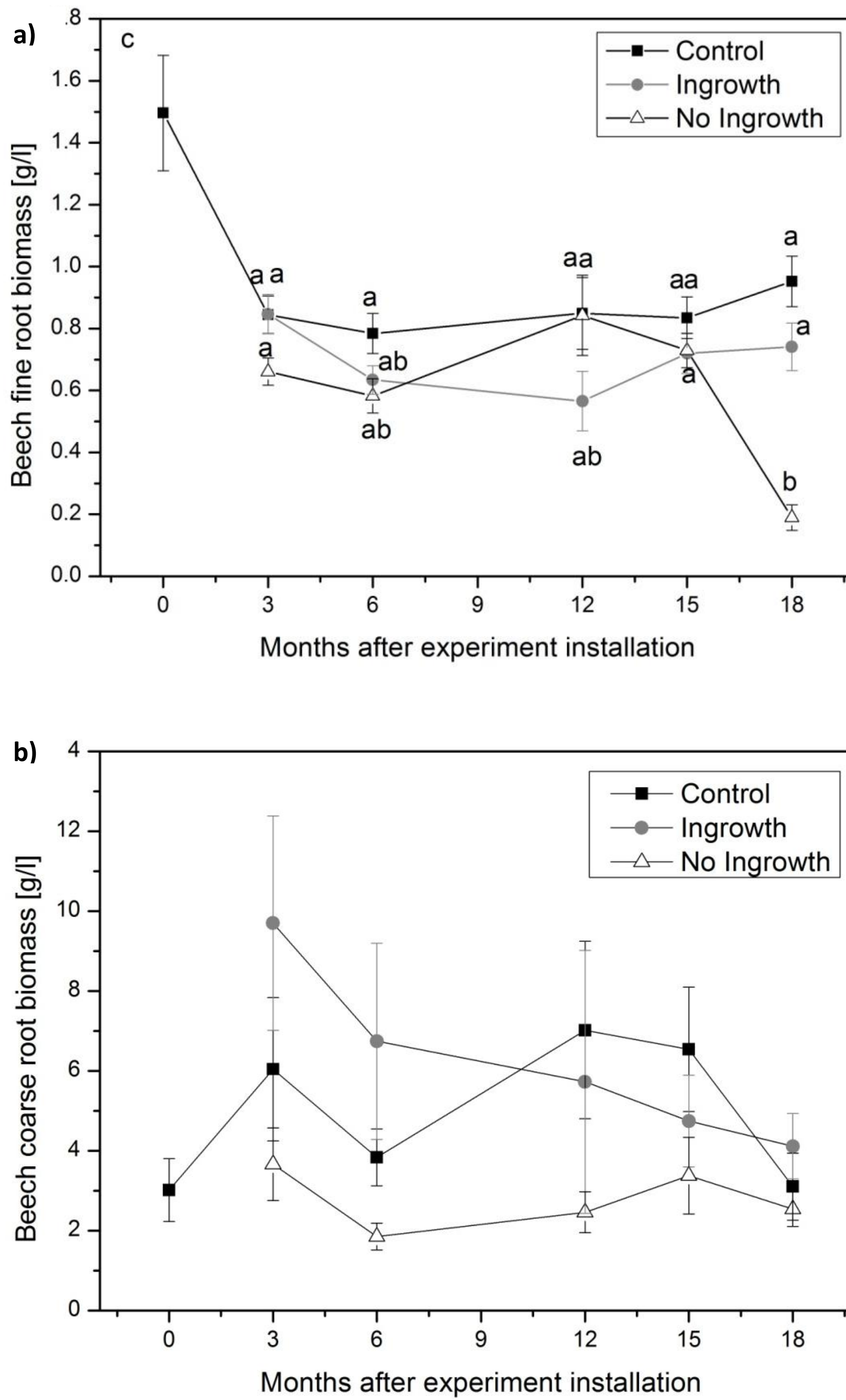


Figure 3.16: Biomass of beech roots within one soil core = 1 litre of soil a) Fine root and b) coarse root dry biomass of beech roots for all harvests and treatments. Different letters indicate significant differences. (mean ± SE, n= Material and Method Table 2.2).

3.2.3 Percentage of vital tips

The percentage of vital tips within the Control cores showed a high variation. At the beginning of the experiment around 30% of the roots tips were vital in May 2011 (Figure 3.17 a; Table 3.15). After six months the lowest level with 15% vital tips was reached (Figure 3.17 a; Table 3.15). The twelve months harvest revealed a high vitality rate over about 40% and decreases during the year again. Therefore, for a realistic evaluation of the recolonization process a comparison between Ingrowth to Control cores per harvest is essential. The percentage of vital looking tips within the No Ingrowth cores declined to nearly zero after six months. During the first three months the percentage of vital tips decreased in Ingrowth and No Ingrowth cores within the same ratio to around 10% of vital tips (Figure 3.17; Table 3.15). No new roots were detected within the Ingrowth cores after three months. The decrease of vital tips stopped in the Ingrowth cores after six months, whereas within the No Ingrowth cores nearly no vital looking tips were detected. After 15 months the percentage of vital tips within Ingrowth cores reached and slightly exceeded the percentage of the undisturbed Controls and they were not significant different any more (Figure 3.17 a; Table 3.15). The number of vital tips per soil core did not differ between the treatments within the first harvest (Figure 3.17 b, Table 3.16). After six and twelve months the total amount of vital tips was significantly higher in Control than in Ingrowth cores, but additionally significantly more in Ingrowth than in No Ingrowth cores. After 15 and 18 months the total amount if vital tips did not differ between Ingrowth and Control treatment any more. Remarkably, the total amount increased strongly within the 18 months harvest, for both, Ingrowth and Control cores (Figure 3.17 b, Table 3.16). The total amount of dry tips was not counted, as only for the first around 1000 tips also the dry ones were counted to calculate the percentage of vital tips. If more than 1000 tips were found within one core, only the vital tips were counted for more than 1000 tips.

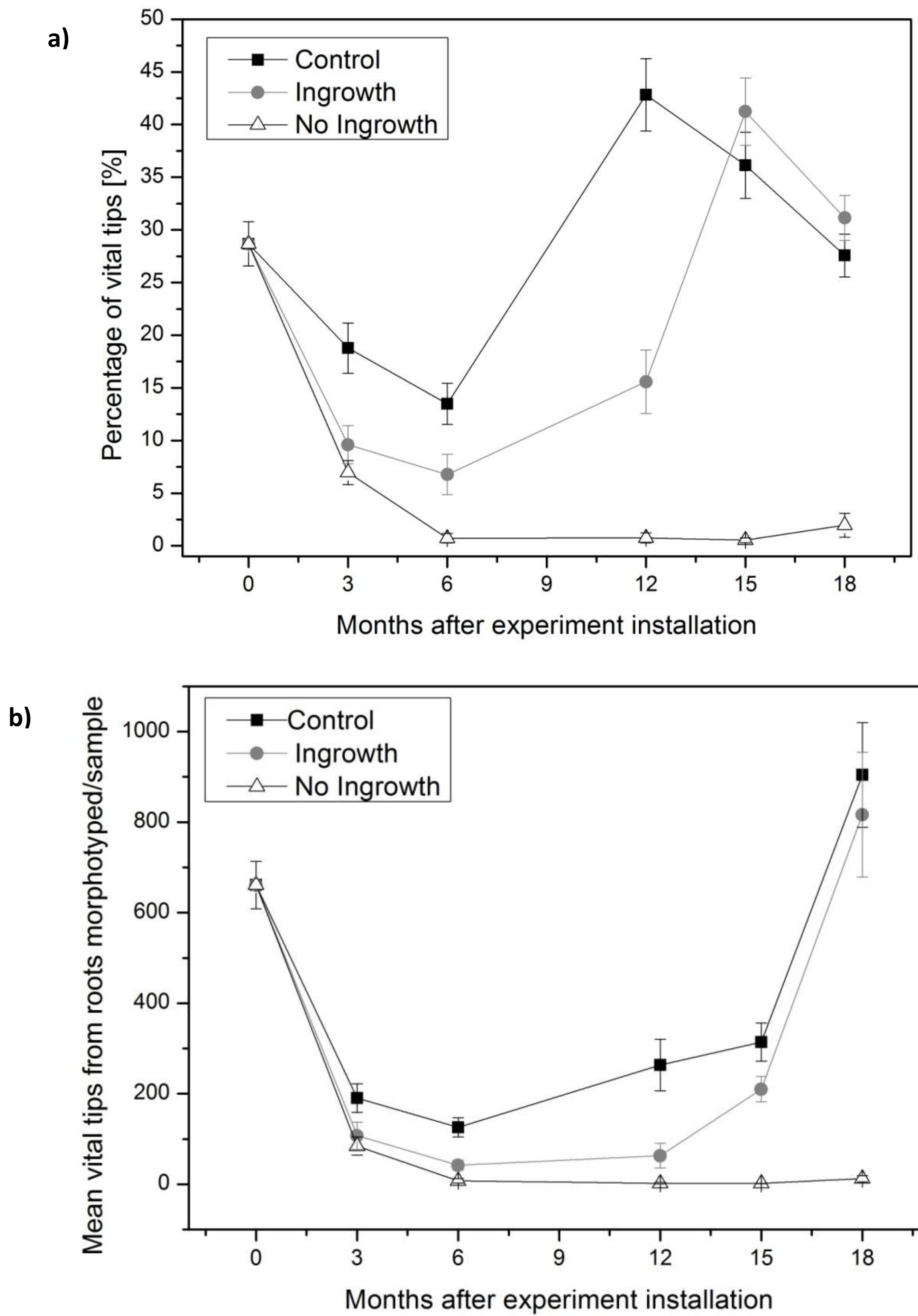


Figure 3.17: a) Percentage of vital tips per harvest and treatment. b) Vital dry tips per harvest and treatment (mean ± SE, n= Material and Method Table 2.2). For sig. differences see Table 3.15.

Table 3.15: P-values for vitality comparison of root tips (Figure 3.17 a, ANOVA; TukeyHSD). Signficant comparisons within one harvest are marked in grey. C= Control, In= Ingrowth and No In = No ingrowth. (n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05).

Months after installation		0	3			6			12			15			18	
	Treatment	C	C	In	No In	C	In	No In	C	In	No In	C	In	N oIn	C	In
3	C	n.s.														
	In	<0.001	0.012													
	No In	<0.001	<0.001	n.s.												
6	C	<0.001	n.s.	n.s.	n.s.											
	In	<0.001	<0.001	n.s.	n.s.	0.004										
	No In	<0.001	<0.001	<0.001	<0.001	<0.001	0.002									
12	C	n.s.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001								
	In	n.s.	n.s.	n.s.	n.s.	n.s.	0.023	<0.001	<0.001							
	No In	<0.001	<0.001	<0.001	0.012	<0.001	n.s.	n.s.	<0.001	<0.001						
15	C	n.s.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	n.s.	<0.001	<0.001					
	In	0.033	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	n.s.	<0.001	<0.001	n.s.				
	No In	<0.001	<0.001	<0.001	<0.001	<0.001	0.014	n.s.	<0.001	<0.001	n.s.	<0.001	<0.001			
18	C	n.s.	n.s.	<0.001	<0.001	<0.001	<0.001	<0.001	n.s.	n.s.	<0.001	n.s.	0.022	<0.001		
	In	n.s.	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	n.s.	0.010	<0.001	n.s.	n.s.	<0.001	n.s.	
	No In	<0.001	<0.001	<0.001	0.007	<0.001	n.s.	n.s.	<0.001	<0.001	n.s.	<0.001	<0.001	n.s.	<0.001	<0.001

Table 3.16: P-values for the number of vital tips per harvest and treatment (Figure 3.17 b, pairwise wilkox test). Comparisons within one harvest are highlighted in grey. C= Control, In= Ingrowth and No In = No ingrowth. (n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05).

Months after installation		0	3			6			12			15			18	
	Treatment	**C**	**C**	**In**	**No In**	**C**	**In**	**No In**	**C**	**In**	**No In**	**C**	**In**	**No In**	**C**	**In**
	C	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	**In**	**<0.001**	n.s.	-	-	-	-	-	-	-	-	-	-	-	-	-
	No In	**<0.001**	n.s.	n.s.	-	-	-	-	-	-	-	-	-	-	-	-
	C	**<0.001**	n.s.	n.s.	n.s.	-	-	-	-	-	-	-	-	-	-	-
6	**In**	**<0.001**	**0.001**	n.s.	n.s.	**0.004**	-	-	-	-	-	-	-	-	-	-
	No In	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	-	-	-	-	-	-	-	-	-
	C	**0.034**	n.s.	n.s.	n.s.	n.s.	**0.003**	**<0.001**	-	-	-	-	-	-	-	-
12	**In**	**0.001**	n.s.	n.s.	n.s.	n.s.	n.s.	**<0.001**	n.s.	-	-	-	-	-	-	-
	No In	**<0.001**	**<0.001**	**0.001**	**0.005**	**<0.001**	n.s.	n.s.	**0.005**	**0.017**	-	-	-	-	-	-
	C	**0.002**	n.s.	**<0.001**	**<0.001**	**0.007**	**<0.001**	**<0.001**	n.s.	**0.012**	<0.001	-	-	-	-	-
15	**In**	**<0.001**	n.s.	**0.034**	**0.008**	n.s.	**<0.001**	**<0.001**	n.s.	**0.040**	<0.001	1.000	-	-	-	-
	No In	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.010**	n.s.	**<0.001**	**0.001**	1.000	**<0.001**	**<0.001**	-	-	-
	C	n.s.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.010**	**<0.001**	<0.001	**0.001**	**<0.001**	**<0.001**	-	-
18	**In**	n.s.	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.	**<0.001**	<0.001	n.s.	**0.001**	**<0.001**	1.000	-
	No In	**<0.001**	**<0.001**	**0.001**	**0.007**	**<0.001**	n.s.	n.s.	**0.001**	0.09	n.s.	**<0.001**	**<0.001**	n.s.	**<0.001**	**<0.001**

3.2.4 Mycorrhization rate

Mycorrhization rate of roots within the Controls always ranged around 99% ±max 0.41%, whereas in Ingrowth cores it ranged between 97.24% ±0.48 (3month harvest) and 99.95% ±0.05 (15 months harvest). Controls from the 15 and 18 months harvest were significantly more mycorrhized than in the beginning of the experiment (p=0.017 and p<0.001 respectively). Within the first three months the mycorrhization rate even significantly increased in ingrowth cores (p=0.004), because no new roots were grown in. On decaying roots no non mycorrhizal vital tips were detected. However, as shown in Figure 3.17, the fraction of vital looking root tips ranged only between 1-2%. Roots in Ingrowth cores were less mycorrhized only at the 15 months harvest, even if this was a small difference of around 2.5% (p=0.001; Figure 3.18).

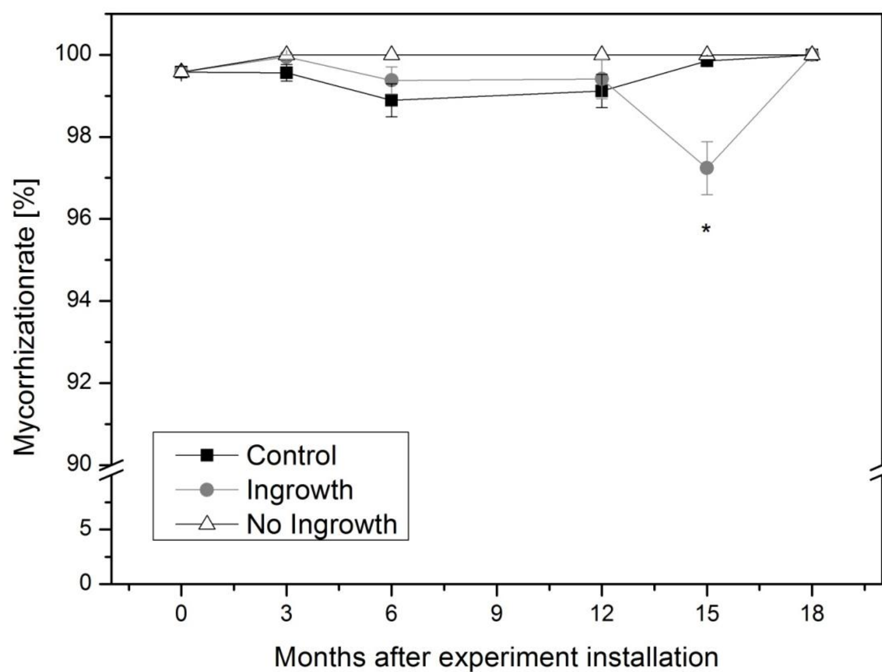


Figure 3.18: Mycorrhization rate (percentage) separated by treatment for each harvest. Significant differences between Control and Ingrowth cores within one harvest are marked with asterisks (Wilcox test; n= Material and Method Table 2.2).

3.2.5 Abundance of single species in the different treatments

In total 82 morphotypes were detected, whereof most (70) were successfully sequenced (for details see Supplementary Table S7, for relative abundances Supplementary Table S8). For 61% of the mycorrhizal root tips an EM species name could be assigned. Morphotypes with the same species name were compared by pictures which had been taken during morphotyping. If morphological structures were in accordance they were grouped together for further analysis as one species. The 82 morphotypes resulted in 55 different taxa and 17 morphotypes without a taxonomical name (further call MTH_*number*, Table 3.17). At the genus level 13% of the tips were identified. Nearly 18% of the root tips could not be identified (Table 3.17).

Table 3.17: Sum and percentage of the vital tips per taxonomical category of all vital mycorrhized root tips detected within the experiment.

Level of taxonomical Assignment	Number of taxa	Sum of vital tips per category	Percentages of the total amount of mycorrhized root tips counted
Species-Level	30	58695	60.9%
Genus-Level	16	12671	13.1%
Family-Level	4	3391	3.5%
Class-Level	1	1418	1.5%
Order-Level	4	2943	3.1%
None	17	17306	17.9%

Lactarius subdulcis was the most abundant species, colonizing more than 23% of all root tips (Figure 3.19). The second most abundant species *Xerocomus porosporus* only appeared at the last harvest (Figure 3.19, Figure 3.20). More than 4% of all mycorrhized root tips were colonized by MTH_29 and an *Amanita* species (Figure 3.19). Only a few species differed significantly between the Control and the Ingrowth cores. Overall the most abundant species which were present within the Control cores were also those species recolonizing the Ingrowth cores first (Figure 3.20). MTH_66 and *Xerocomus chrysenteron* were the only species, which were significantly more abundant on roots in Ingrowth

than in Control cores after 15 months. In the No Ingrowth cores *Cenococcum geophilum* and a *Heliotiales* sp. were those species which were vital looking for the longest time.

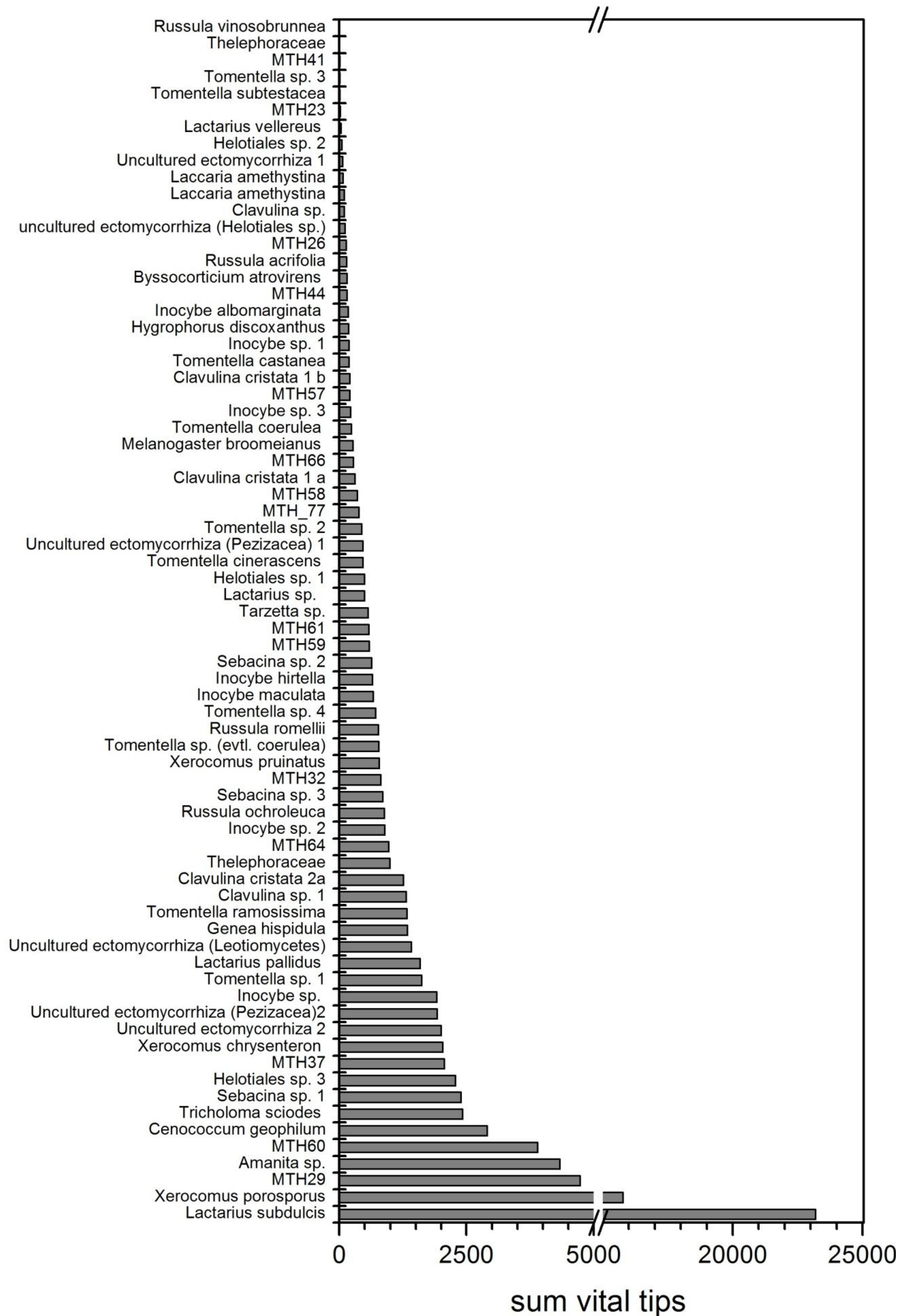


Figure 3.19: Sum vital tips per taxon, respectively morphotype, for all harvests and all treatments.

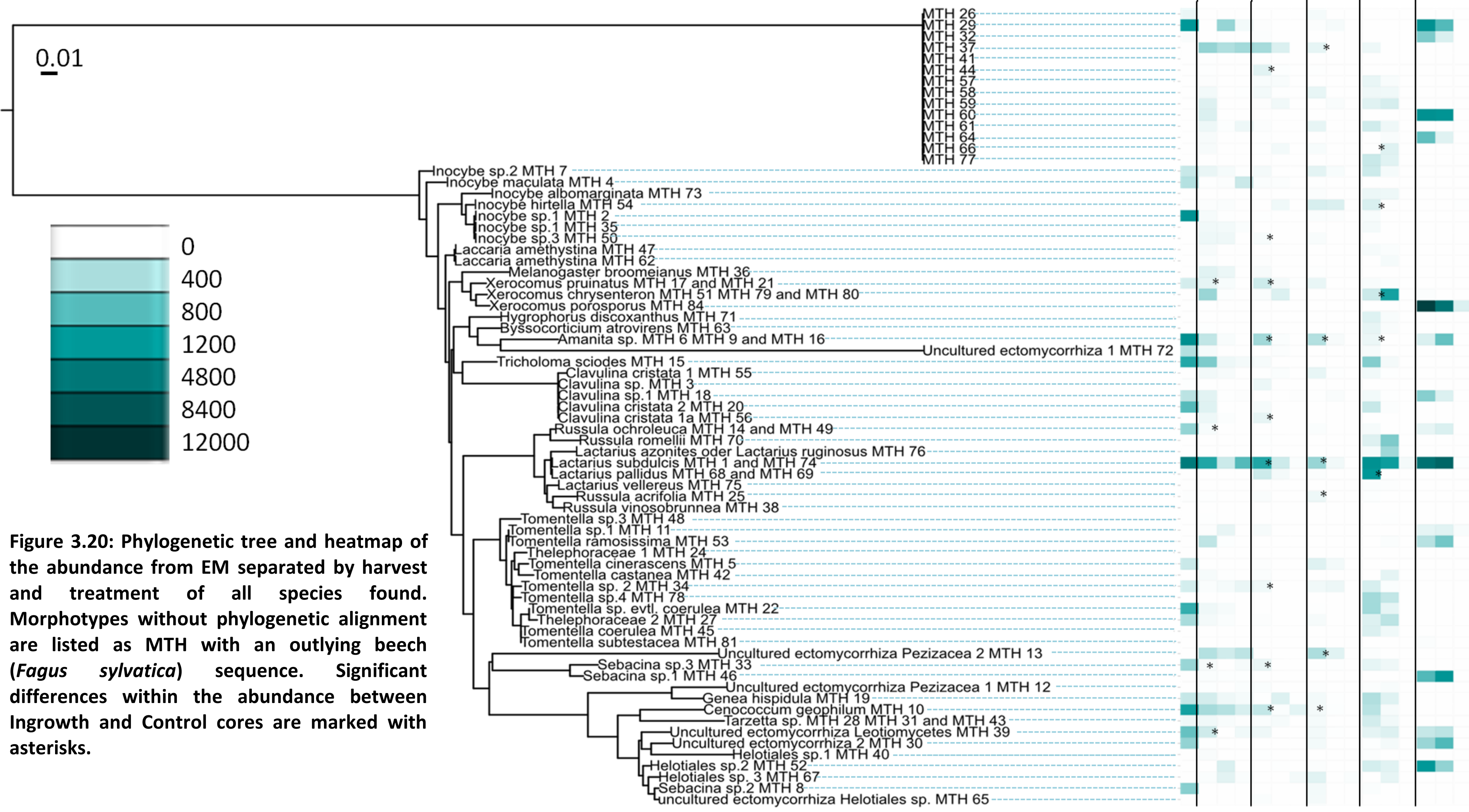


Figure 3.20: Phylogenetic tree and heatmap of the abundance from EM separated by harvest and treatment of all species found. Morphotypes without phylogenetic alignment are listed as MTH with an outlying beech (*Fagus sylvatica*) sequence. Significant differences within the abundance between Ingrowth and Control cores are marked with asterisks.

3.2.6 Richness and diversity of ingrowing and undisturbed roots

For each harvest for rarefaction curve a plateau was nearly reached for controls. For the harvests after 0 and 18 months with a lower richness but more individuals the saturation was reached. Ingrowth cores reached saturation after 15 months (Figure 3.21 a-f). After 15 months the richness and the diversity, represented by the Shannon Index, within the Ingrowth cores reached the level of Control cores (Figure 3.22; for p-values: Table 3.18 and Table 3.19).

After three months the richness within the Ingrowth and the No Ingrowth cores decreased significantly. But there only was a trend for the diversity in Ingrowth in comparison to Control cores to decrease. After six months, even if there already were some new vital mycorrhized tips, the richness of the Ingrowth and the No Ingrowth cores did not differ significantly. But they both differ to the Control cores. After one year, the diversity within the Ingrowth cores was significantly higher than that one in the No Ingrowth cores, but significantly lower in comparison to the Controls. After 15 months the richness and the diversity were not significantly different between the Control and the Ingrowth cores any more. The richness within the Ingrowth cores decreased again within the next three months. But also at the 18 months harvest there was no difference between the Control and the Ingrowth cores for richness or diversity. The fungal richness of the Control cores was significantly higher at the twelve months harvest in comparison to all other harvests, instead of the zero months harvest. So both May harvests had the highest richness. The Shannon Index of the control cores was significantly smaller within the 18 months harvest in comparison to all others. EM community of the twelve months harvest was more diverse than at the three months harvest in Control and Ingrowth cores. Within the Ingrowth cores the richness decreases significantly between the three and the six months harvest (for all p-values see Table 3.18 and Table 3.19).

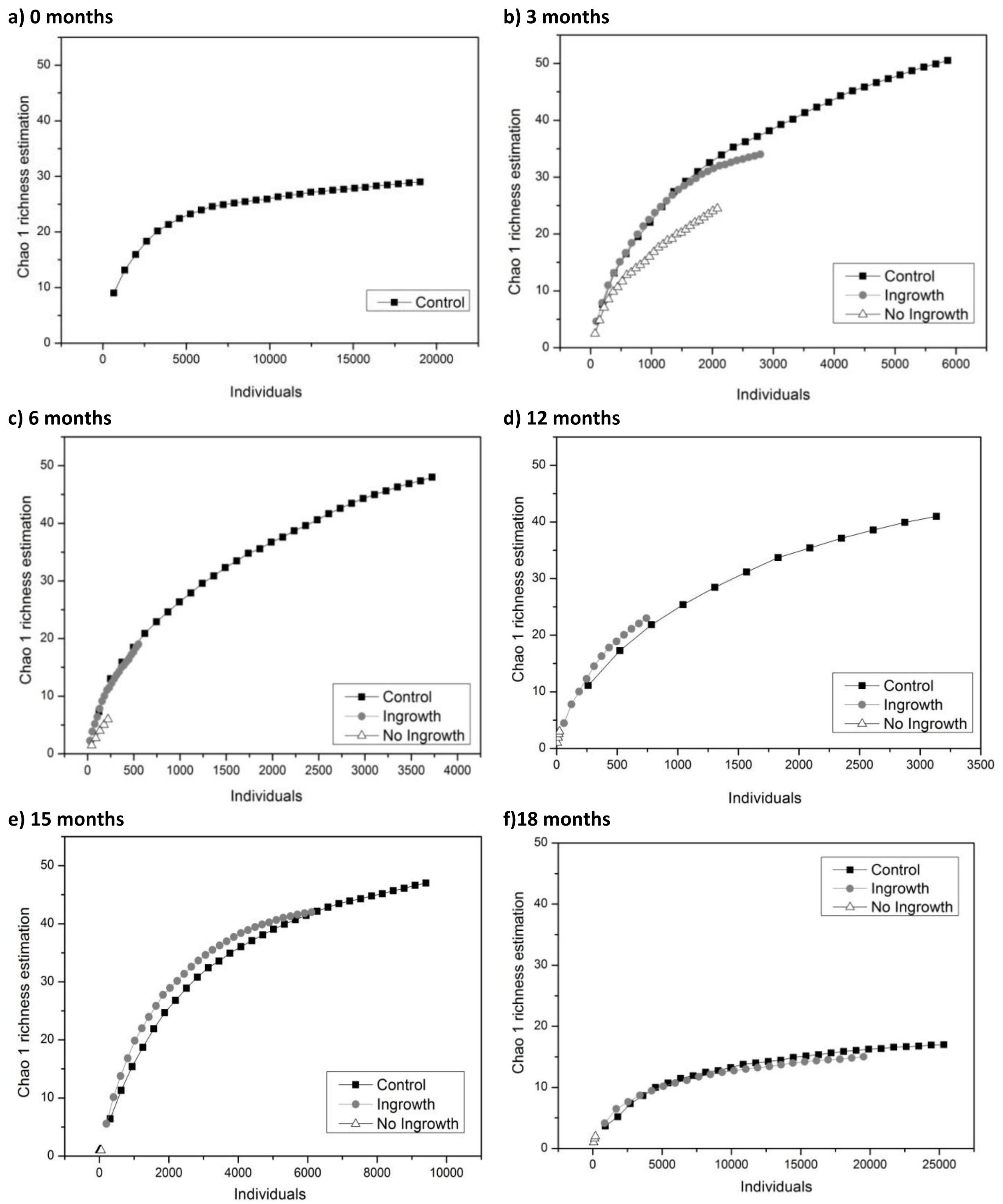


Figure 3.21: Rarefaction curves calculated with Chao 1 for all vital EM tips per soil core (= 1 l of soil) harvest and treatment. Separated by month after experiment installation: a) 0 months b) 3 months c) 6 months d) 12 months e) 15 months f) 18 months (n= Material and Method Table 2.2)

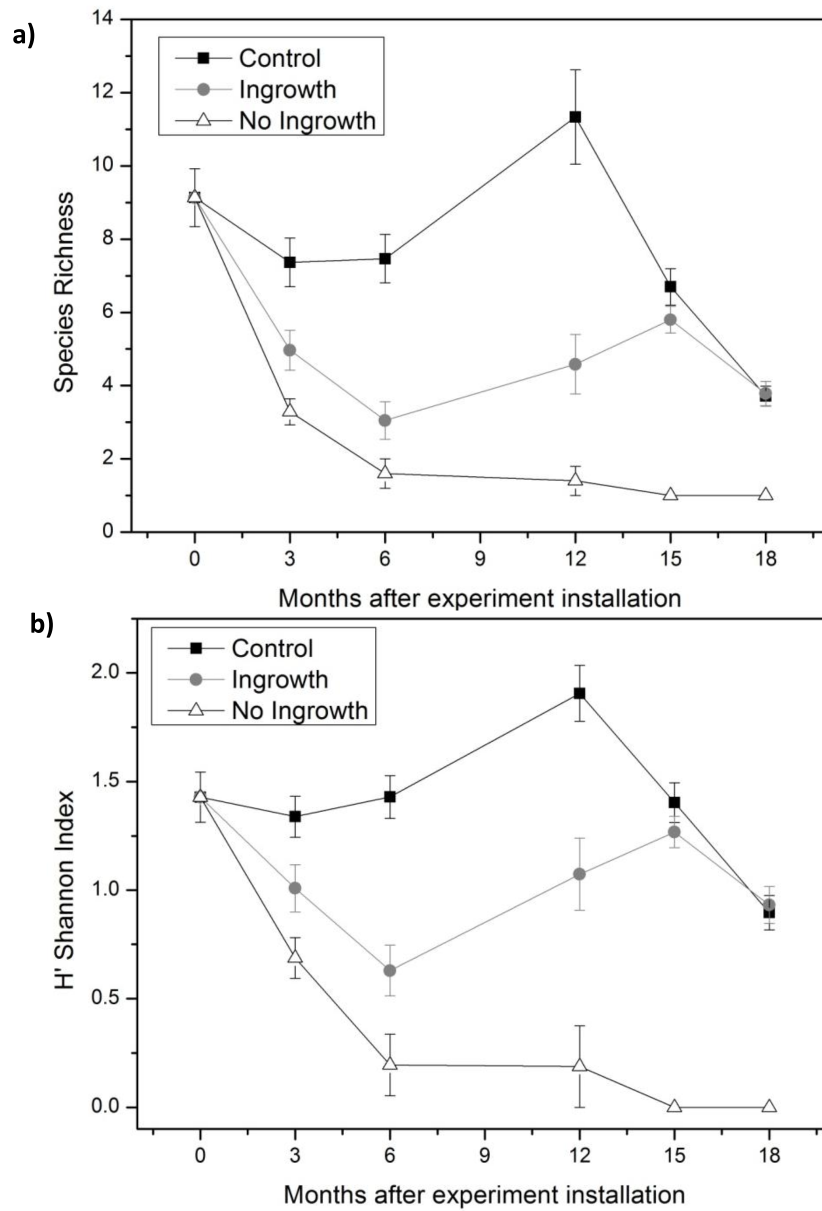


Figure 3.22: a) Species Richness and b) Shannon Index for each harvest and treatment (Mean ±SE). For statistics see Table 3.18 and Table 3.19. (n= Material and Method Table 2.2)

Table 3.18: P-values for comparison of treatments (Control, Ingrowth and No Ingrowth) within one harvest for Figure 3.22. P-values of the 18 months harvest were calculated with wilkox test, all other harvests with ANOVA. (n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05).

	Months after experiment installation	**3**		**6**		**12**		**15**		**18**	
	Treatment	**Ingrowth**	**No Ingrowth**	**Ingrowth**	**No Ingrowth**	**Ingrowth**	**No Ingrowth**	**Ingrowth**	**No Ingrowth**	**Ingrowth**	**No Ingrowth**
Richness	**Control**	**0.010**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.	**<0.001**	n.s.	**0.014**
	Ingrowth		0.060		n.s.		n.s.		**<0.001**		**0.016**
Shannon	**Control**	0.051	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**	n.s.	**<0.001**	n.s.	**0.017**
	Ingrowth		0.064		n.s.		**0.007**		**<0.001**		**0.019**

Table 3.19: P-values for comparison of harvests within one treatment (Control, Ingrowth and No Ingrowth) for Figure 3.22. P-values for No Ingrowth samples were calculated with wilkox test, control and Ingrowth samples with ANOVA. (n.s. = not significant = p-values above 0.007; significant differences are indicated by p -values <0.05).

Months after exp. install.	0	3	6	12	15
Richness					
Richness Control					
3	n.s.				
6	n.s.	n.s.			
12	n.s.	**0.029**	**0.040**		
15	n.s.	n.s.	n.s.	**0.008**	
18	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**
Richness Ingrowth					
6		**0.022**			
2		n.s.	n.s.		
15		n.s.	**<0.001**	n.s.	
18		n.s.	n.s.	n.s.	**0.023**
Richness No Ingrowth					
6		n.s.			
12		n.s.	n.s.		
15		**0.003**	n.s.	n.s.	
18		n.s.	n.s.	n.s.	-
Shannon					
Shannon Control					
3	n.s.				
6	n.s.	n.s.			
12	n.s.	**0.021**	n.s.		
15	0.060	n.s.	n.s.		
18	**0.002**	**0.019**	**0.001**	**<0.001**	**0.004**
Shannon Ingrowth					
6		0.065			
12		n.s.	n.s.		
15		n.s.	**<0.001**	n.s.	
18		n.s.	n.s.	n.s.	n.s.
Shannon No Ingrowth					
6		n.s.			
12		n.s.	n.s.		
15		**0.003**	n.s.	n.s.	
18		n.s.	n.s.	n.s.	-

3.2.7 EM community structure within different treatments

To investigate the EM community structures the dissimilarities between the communities of Ingrowth and Control cores were determined by Analysis of Similarities (ANOSIM). The community of the No Ingrowth cores were not considered as after six months not enough vital looking tips were detected as basis for Non Metric Multidimensional Scaling (NMDS) or ANOSIM analysis (for NMDS see Supplementary Figure S3). During the degradation process, observed after three months, no significant differences in the community structure were detected (Figure 3.23). After six months the EM community between the Ingrowth and the Control cores differed significantly. First new roots were observed in the Ingrowth cores after six months. After twelve months the EM community started to become more similar again, indicated by decline of the R values. The R value indicates dissimilarity between groups when it is close to 1 and similarity when it is close to 0. Finally, after 18 months the EM community did not differ between Ingrowth and Control cores any more (Figure 3.23).

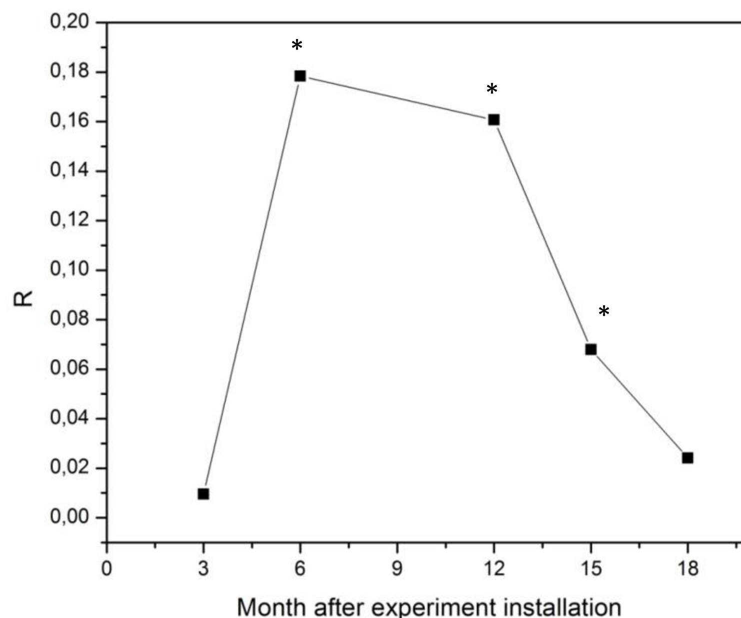


Figure 3.23: R values from ANOSIM between Ingrowth and Control EM fungal communities over time. Significant differences of the EM community between Control and Ingrowth cores within one harvest are marked with asterisks.

Overall, the Variation within the EM community in Control cores is higher than that within Ingrowth cores (Figure 3.24). The same species generally present on the plot recolonized the root free patches, as no species showed an outstanding position within the Principle Component Analysis (PCA). This confirms the result from the direct comparison of different species abundance that only two were significantly enriched in Ingrowth cores. The dying EM within the No Ingrowth cores only had a very small variation. The dissimilarity between the Ingrowth and Control communities is highest after six months when the first new roots grow into the cores and then decline rapidly after one year.

Figure 3.24: EM community for all harvests and all treatments. Principle component analysis (PCA) by species (z-standardized) with component 1 and component 2. 95% confidence ellipses represent the overall EM communities.

3.2.8 Similarity between Ingrowth and Control cores

Similarities between different EM communities are represented by the Morisita Horn Index. First Control and Ingrowth cores from the same plot and between different plots were compared (Figure 3.25 a, Table 3.20). After three months the EM community within the Control cores did not differ between or within plots. But the similarity of Ingrowth cores was decreased in comparison to Control cores. After six months the similarity between the Ingrowth cores from different plots were significantly decreased in comparison to Control cores from same or different plots or to Ingrowth cores from the same plot. This indicates that on each plot another EM community was important for ingrowth (Figure 3.25 a, Table 3.20). But the EM community within Ingrowth cores from the same plot had a slightly higher similarity to the Control cores on the same plot than to Control cores from other plots, even if those differences were not significant (Figure 3.25 b, Table 3.20). After twelve and 15 months no differences between Ingrowth and Control cores or between the same or different plots were detected (Figure 3.25 c, Table 3.20). After 18 months the EM community within Controls had a significantly higher similarity between different plots than that one from Ingrowth cores. This was, when, as shown above, the EM community in Ingrowth cores did not differ from those in Control cores anymore. This confirms that similar species like in the Control cores are important for the Ingrowth.

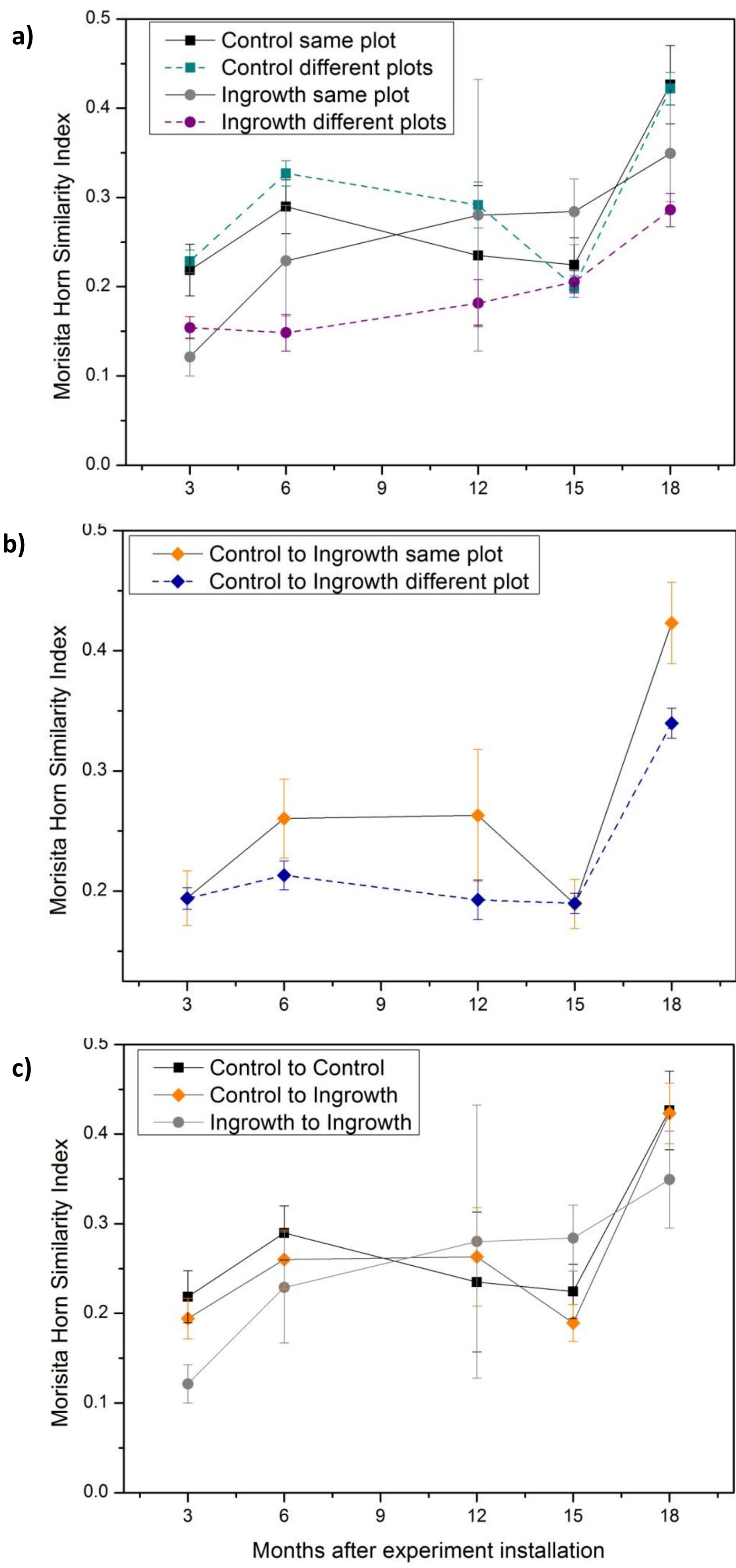


Figure 3.25: Morisita Horn similarity Index for the EM community of Ingrowth and Control cores on the same or on different plots. A) Control to Control and Ingrowth to Ingrowth comparisons on the same or on different plots, b) Control to Ingrowth comparisons on the same or on different plots and c) Control to Control, Ingrowth to Ingrowth and Control to Ingrowth cores on the same plot. For comparisons within one plot that Ingrowth and Control cores from one sampling tree were excluded from this analysis (Mean ±SE). For statistics see Table 3.20 (n= Material and Method Table 2.2).

Table 3.20: P-values of the Morisita Horn Similarity comparisons for Figure 3.25. Differences between Control and Ingrowth cores within the same or between different plots were analyzed. Months = months after experiment installation, C= EM community in Control cores, In= EM community in Ingrowth cores. (pairwise wilkox test, n.s. = not significant = p-values above 0.007; significant differences are indicated by p-values <0.05, n= Material and Method Table 2.2). Significant comparisons within one harvest are highlighted in grey.

months	Treatment comparison	dif. or same plot	3						6						12		
			C-C	C-C	C-In	C-In	In-In	In-In	C-C	C-C	C-In	C-In	In-In	In-In	C-C	C-C	C-In
			same plot	dif. plot	same plot	dif. plot	same plot	dif. plot	same plot	dif. plot	same plot	dif. plot	same plot	dif. plot	same plot	dif. plot	same plot
3	C-C	dif. plot	n.s.														
	C-In	same plot	n.s.	n.s.													
	C-In	dif. plot	n.s.	n.s.	n.s.												
	In-In	same plot	n.s.	n.s.	n.s.	n.s.											
	In-In	dif. plot	n.s.	**<0.001**	n.s.	**0.011**	n.s.										
6	C-C	same plot	n.s.	n.s.	n.s.	**0.017**	**0.001**	**<0.001**									
	C-C	dif. plot	n.s.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.								
	C-In	same plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.							
	C-In	dif. plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.045**	**<0.001**	n.s.						
	In-In	same plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.					
	In-In	dif. plot	**0.001**	**<0.001**	**0.003**	**<0.001**	n.s.	n.s.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.				
12	C-C	same plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			
	C-C	dif. plot	n.s.	n.s.	**0.049**	**0.001**	**<0.001**	**<0.001**	n.s.	n.s.	n.s.	**0.009**	n.s.	**<0.001**	n.s.		
	C-In	same plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.740	n.s.	n.s.	
	C-In	dif. plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**<0.001**	n.s.	n.s.	n.s.	**<0.001**	n.s.	n.s.	n.s.
	In-In	same plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	In-In	dif. plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.004**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
15	C-C	same plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.012**	n.s.	n.s.	n.s.
	C-C	dif. plot	n.s.	n.s.	n.s.	n.s.	n.s.	**0.020**	n.s.	**<0.001**	n.s.	n.s.	n.s.	**<0.001**	n.s.	**0.011**	n.s.
	C-In	same plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**<0.001**	n.s.	n.s.	n.s.	**<0.001**	n.s.	0.060	n.s.
	C-In	dif. plot	n.s.	n.s.	n.s.	n.s.	n.s.	**0.030**	**0.012**	**<0.001**	n.s.	n.s.	n.s.	**<0.001**	n.s.	**0.001**	n.s.
	In-In	same plot	n.s.	n.s.	n.s.	n.s.	n.s.	**0.044**	n.s.	n.s.	n.s.	n.s.	n.s.	**<0.001**	n.s.	n.s.	n.s.
	In-In	dif. plot	n.s.	n.s.	n.s.	n.s.	n.s.	**0.001**	n.s.	**<0.001**	n.s.	n.s.	n.s.	**<0.001**	n.s.	**0.037**	n.s.
18	C-C	same plot	n.s.	**0.004**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.	n.s.	n.s.	**<0.001**	n.s.	**<0.001**	n.s.	n.s.	n.s.
	C-C	dif. plot	**0.007**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.	n.s.	**0.012**	**<0.001**	n.s.	**<0.001**	n.s.	n.s.	n.s.
	C-In	same plot	n.s.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.	n.s.	n.s.	**<0.001**	n.s.	**<0.001**	n.s.	n.s.	n.s.
	C-In	dif. plot	n.s.	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	n.s.	n.s.	n.s.	**<0.001**	n.s.	**<0.001**	n.s.	n.s.	n.s.
	In-In	same plot	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.004**	n.s.	n.s.	n.s.
	In-In	dif. plot	n.s.	n.s.	n.s.	**0.002**	**0.010**	**<0.001**	n.s.	n.s.	n.s.	**0.003**	n.s.	**<0.001**	n.s.	n.s.	n.s.

months	Treatment comparison	dif. or same plot	12			15						18				
			C-In dif. plot	**In-In same plot**	**In-In dif. plot**	**C-C same plot**	**C-C dif. plot**	**C-In same plot**	**C-In dif. plot**	**In-In same plot**	**In-In dif. plot**	**C-C same plot**	**C-C dif. plot**	**C-In same plot**	**C-In dif. plot**	**In-In same plot**
3	**C-C**	**dif. plot**														
	C-In	**same plot**														
	C-In	**dif. plot**														
	In-In	**same plot**														
	In-In	**dif. plot**														
6	**C-C**	**same plot**														
	C-C	**dif. plot**														
	C-In	**same plot**														
	C-In	**dif. plot**														
	In-In	**same plot**														
	In-In	**dif. plot**														
12	**C-C**	**same plot**														
	C-C	**dif. plot**														
	C-In	**same plot**														
	C-In	**dif. plot**														
	In-In	**same plot**	n.s.													
	In-In	**dif. plot**	n.s.	n.s.												
15	**C-C**	**same plot**	n.s.	n.s.	n.s.											
	C-C	**dif. plot**	n.s.	n.s.	n.s.	n.s.										
	C-In	**same plot**	n.s.	n.s.	n.s.	n.s.	n.s.									
	C-In	**dif. plot**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.								
	In-In	**same plot**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.							
	In-In	**dif. plot**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.						
18	**C-C**	**same plot**	**0.002**	n.s.	**0.002**	n.s.	**<0.001**	**<0.001**	**<0.001**	n.s.	**<0.001**					
	C-C	**dif. plot**	**<0.001**	n.s.	**<0.001**	**0.004**	**<0.001**	**<0.001**	**<0.001**	n.s.	**<0.001**	n.s.				
	C-In	**same plot**	**<0.001**	n.s.	**0.001**	0.058	**<0.001**	**<0.001**	**<0.001**	n.s.	**<0.001**	n.s.	n.s.			
	C-In	**dif. plot**	**0.001**	n.s.	**0.008**	n.s.	**<0.001**	**<0.001**	**<0.001**	n.s.	**<0.001**	n.s.	n.s.	n.s.		
	In-In	**same plot**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	In-In	**dif. plot**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.001**	n.s.	n.s.	n.s.	**0.001**	n.s.	n.s.	n.s.

3.2.9 Functional diversity of EM community: exploration types

To test for functional differences EMs were separated into exploration types (for assignment see Supplementary Table S9). The exploration types differed in their proportion of the total EM community between Ingrowth and Control cores (Figure 3.26 a, Table 3.21). The proportion of the Contact type, the exploration type with the shortest emanating hyphae, differed after six and twelve months after experiment installation between Ingrowth and Control cores. Within the Ingrowth cores it was significantly reduced (Figure 3.26 a, Table 3.21). For the Short Distance type the proportion within the Ingrowth cores was higher than in the Control cores for one year, then the EM community within the Ingrowth cores has a rapid decline from over 40% short distance to under 20% of all root tips within the Ingrowth samples (Figure 3.26 b, Table 3.21). The percentages of vital tips belonging to the medium distance exploration type fluctuated between Ingrowth and Controls cores (Figure 3.26 c, Table 3.21). Tips belonging to the Long Distance exploration type also fluctuated in their proportion on the whole fungal community, resulting in the complete opposite pattern between Ingrowth or Control cores for the Medium Distance exploration type. After three months the Long Distance exploration type was less within the Ingrowth cores. After six months the long distance exploration type were more present within the control cores and after one year it again decreased in abundance. After 15 months the Ingrowth cores had a higher percentage of Long Distance exploration type tips being exceeded by the controls after 18 months (Figure 3.26 d, Table 3.21).

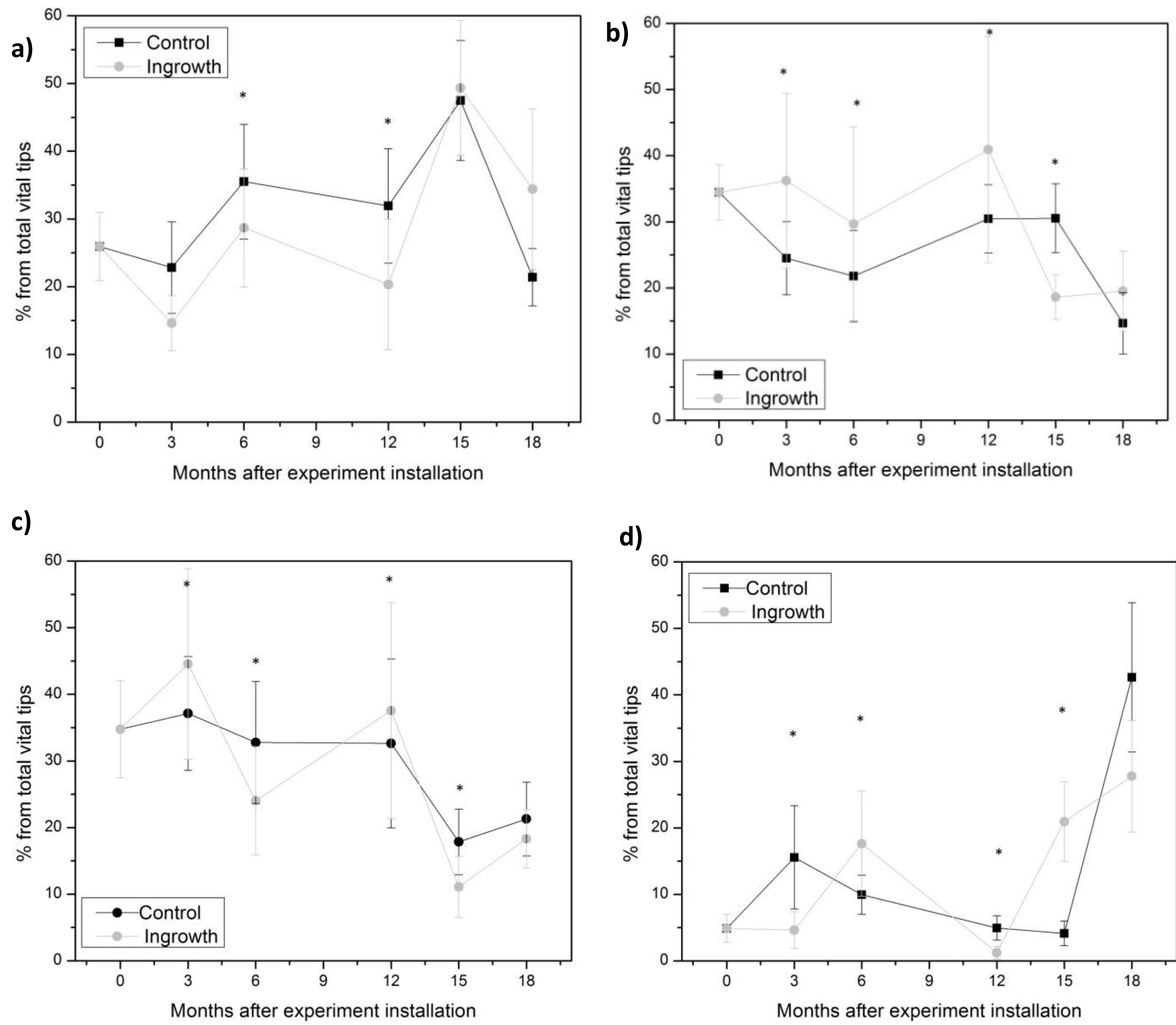


Figure 3.26: Fraction of EM vital tips from total vital tips per treatment and harvest classified by different exploration types: a) "Contact", b) "Short distance", c) Medium distance" and d) "Long distance". The characterization of the species and morphotypes to exploration types is listed in Supplementary Table S9. (Percentage ± SE). Significant differences between Control and Ingrowth cores within one harvest are marked with asterisks.

Table 3.21: exploration types: comparisons via kruskal wallis test between Ingrowth and Control cores per harvest.

Months after experiment installation	Contact		Short Distance		Medium Distance		Long Distance	
	chi-squared	p-value	chi-squared	p-value	chi-squared	p-value	chi-squared	p-value
3	2.330	n.s.	4.712	**0.030**	5.781	**0.016**	5.340	**0.0208**
6	16.821	**<0.001**	18.021	**<0.001**	13.015	**<0.001**	9.785	**0.002**
12	11.069	**<0.001**	5.993	**0.014**	3.963	**0.047**	7.428	**0.006**
15	2.078	n.s.	6.210	**0.013**	7.073	**0.008**	7.587	**0.006**
18	0.123	n.s.	1.283	n.s.	0.016	n.s.	1.539	n.s.

When regarding the functional differences between the EM communities of Control, Ingrowth and No Ingrowth cores, represented by exploration types, no differences in their variation was detected (Figure 3.27). All exploration types seemed to have similar influence on the community composition of Ingrowth, No Ingrowth and Control cores, when regarded over all harvests. The more Contact type root tips were present the more Long Distance type root tips were detected within the samples, too. Medium and Short Distance did not correlate with each other.

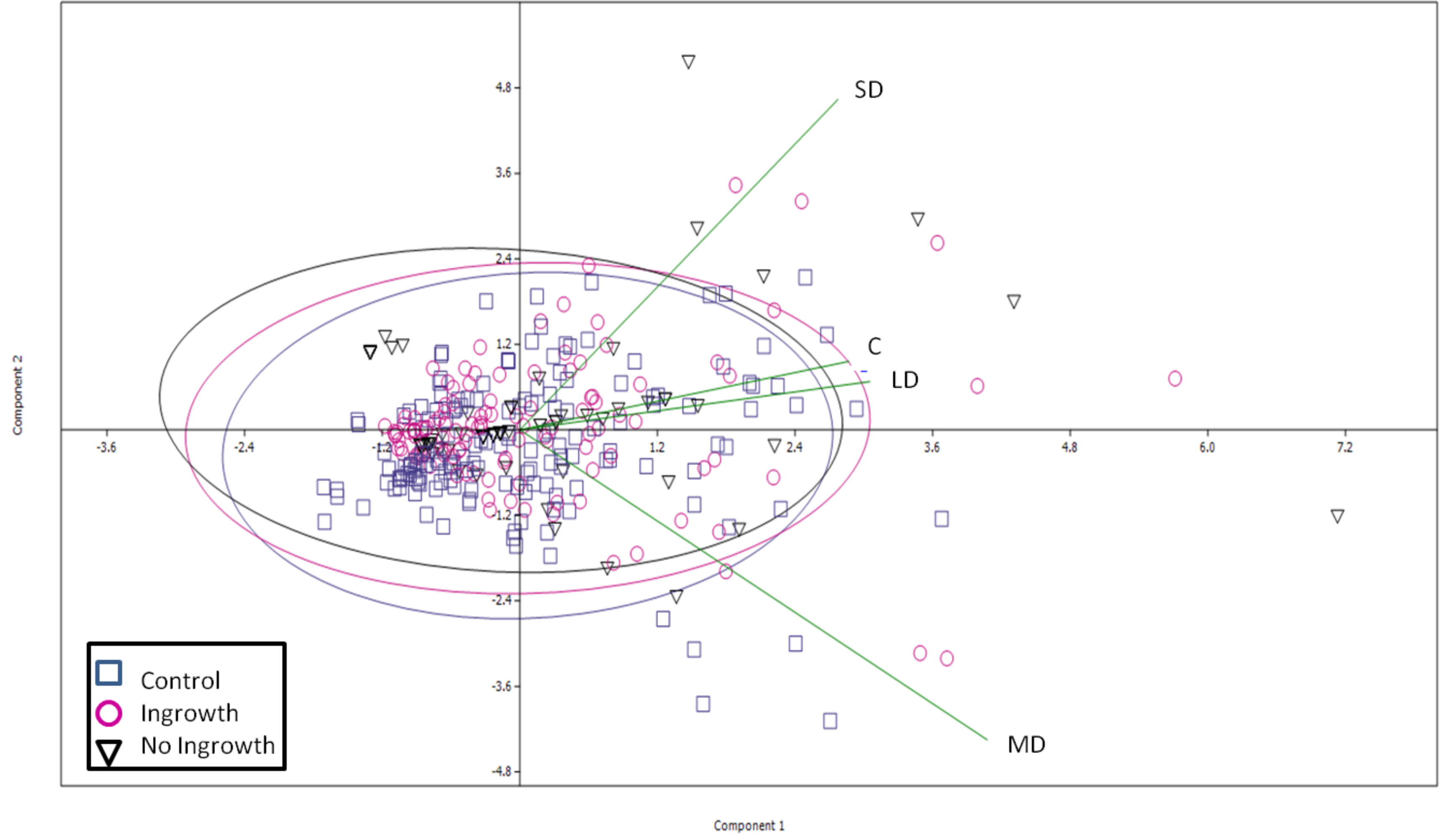


Figure 3.27: EM community for all harvests and all treatments. Principle component analysis (PCA) by exploration types (z-standardized) with component 1 and component 2. 95% confidence ellipses represent the overall EM communities.

4. Discussion

4 Discussion

The overarching goal of this thesis was to investigate root-associated fungal community structures of different lifestyles and with special regard on EM, functional diversity in relation to ecosystem functions and land-use intensity. Therefore one aim of the study was to investigate which environmental factors affected the richness, diversity and community structure of root-associated fungal community and how different lifestyles, here ectomycorrhizal and saprophytic fungi respond to them. The taxonomic diversity is strongly correlated with the functional diversity within ectomycorrhizal communities (Rineau and Courty, 2011). Variables influencing the fungal diversity might therefore also affect the functionality of the fungal community within a stable ecosystem. Whether or not forest management plays a role in this complex system was also investigated within this study.

4.1 Evaluation of pyrosequencing and richness calculation method

To investigate those aims, tree root-associated fungal community on forest plots of the Biodiversity Exploratories was analyzed by pyrosequencing. Moreover a small scale disturbance experiment was performed.

4.1.1 ITS2 region and 97% DNA identity for OTU clustering

Within this study 454 pyrosequencing was started from the ITS2 region. Ihrmark et al. (2012) found that the diversity and community composition were much better preserved in ITS2 than in the whole ITS region used. Another advantage was, that the ITS2 region is less variable in length compared with the ITS1 region and lacks the problem of an intron (5’ SSU) that is common in many Ascomycota (Lindahl et al., 2013). Blaalid et al. (2013) found a significantly higher BLAST similarity match of fungi for ITS2 than for ITS1. Additionally, ITS2 has a better representation within the databases (Nilsson et al., 2009).

For fungal community composition one might expect the same results with traditional Sanger-sequencing in comparison to 454 pyrosequencing, as the two methods yielded qualitatively similar

results (Tedersoo et al., 2010b). But they found significantly differences regarding the taxonomy of the fungal community on species level when comparing these two methods (Tedersoo et al., 2010b). Kauserud et al. (2012) found a relatively low overlap between a 454 and a clone library datasets. This fits to our results as the counted tips during morphotyping did not correlate with the number of OTU reads for four of the five tested EM species. Furthermore four of the morphotypes resulting in a species name during Sanger-sequencing were not detected as OTU. This phenomenon was already described by Tedersoo *et al.* 2010, who observed a roughly similar phylogenetic structure between the two methods but also that several taxa were not captured by either one of the two methods. Kauserud et al. (2012) observed a relationship of the GC/AT content of the OTU sequence and their proportional abundance in the 454 dataset versus the clone library datasets. This could be an explanation for the different abundances of species within the 454 and the morphotyping/Sanger sequencing. But for comparison, if working for all samples with the same method, 454 pyrosequencing is appropriate, as replicate samples from the same root system revealed very similar results (Kauserud et al., 2012).

With a 97% similarity cutoff a commonly used cutoff was chosen in this study as it represents a reasonable threshold for species estimation for ITS2 region (Blaalid et al., 2013). Several times more than one OTU were assigned to the same species, but this might rely on local differences of single species. Additionally not all fungi are represented within the databases and therefore sister taxa with a very similar sequence might be selected instead of the right species. This limitation of the ITS region as a marker is already known, based on the knowledge that different species might cluster together and that many species split into several OTUs (Blaalid et al., 2013). For example one single aspen tree (*Populus tremula*) was found to harbour 23 ITS genotypes from the EM fungus *Cenococcum geophilum* (Bahram et al., 2011). Therefore clustering at 97% was chosen as a commonly used compromise regarding this discussion (cf. Table 4.1).

The accuracy per base of 454 sequencing was tested by Huse et al. (2007) to 99.5% and with all the quality filtering steps therefore exceeding the accuracy of traditional methods. Therefore the 454 pyrosequencing is a good method for investigating the whole fungal root-associated community within this study. The results needs to be regarded with those limitations discussed above. Nevertheless morphotyping will be important for future studies, because only with this method those EM which formed a mycorrhiza can be selected. The other present EM species may serve as a pool of possible mycorrhizal partners. Danielsen et al. (2012) showed that species already present in one year may form a mycorrhiza in the next year. For example these cryptic species may become important if environmental conditions change. Within our Trenching experiment we found that a *Xerocomus* species, which was not detected before, became highly abundant as morphotype after a

drought period. This preference of host plants for *Xerocomus* species under drought was described before (Shi et al., 2002). Therefore *Xerocomus* with a long distance exploration type may be important for water supply as it occupies a several times bigger area than other exploration types (Weigt et al., 2011). This example illustrates that not all species present as hyphae or spore near the root, form mycorrhiza but they can be detected during pyrosequencing. The species diversity of EM fungi is linked to the functional diversity (Rineau and Courty, 2011) and therefore, if more potential EM species are available, indicated by a high diversity during pyrosequencing, even if they actually did not form a mycorrhiza, host plants have more opportunities for EM partners under changing environmental conditions. It can be concluded that a higher EM diversity might therefore be important also for plant species stability within an ecosystem.

4.1.2 Plant originated sequences

Around 66% of all sequences within this pyrosequencing study turned out to be plant originated. Between 0.12% and 99.9% of the sequences per plot were of plant and not of fungal origin. In the literature only small indications were found on this problem. For example (Lindahl et al., 2013) gave a short remark that the primers ITS1-ITS5 (White et al., 1990) could amplify the DNA of other eukaryotic lineages as well. Tedersoo *et al.* 2010 found 6.4% to be plant originated OTUs on root samples. When sequencing morphotypes (e.g. from the Trenching experiment) some sequences from the host tree were sequenced also by Sanger sequencing. This bias did not occur dependent on their origin (Exploratory), but spruce (*Picea abies*) or pine (*Pinus sylvestris*) were not detected. Over 50% of all plant sequences were assigned to beech (*Fagus sylvatica*). Other abundant tree genera were *Acer, Fraxinus, Betula* and shrubs of the genus *Rubus*. But also some herbal plant species were detected, therefore plant originated sequences were also present on conifer plots. Small fragments of their roots must have been remained between the tree roots. Nevertheless, our primers were chosen to select for fungal sequences and therefore this shows that the abundance of plant sequences per sample seems to be arbitrary. We assume that the relationship between fungal and plant originated DNA within the sample might influence this problem when leading to unspecific bonding during PCR. Maybe an increased annealing temperature may influence this in a positive way, as this might lead to fewer unspecific bindings during annealing.

4.1.3 Singletons

A common practice is to remove singletons before further calculations (Lindahl et al., 2013; Tedersoo et al., 2010b). Within our dataset less than 3% of all sequences were singletons. A procrustes test revealed that they had no significant influence on the community structure. Of course many of them may represent rare taxa (Kauserud et al., 2012), but it is also likely that they result from pyrosequencing errors (Hiiesalu et al., 2012; Huse et al., 2007), even if chimeras were excluded. This is supported by the finding of Tedersoo et al. (2010b) that most singletons were artifactual and contained more insertions compared with natural intra- and interspecific variation. Also within their study they found that none of the singletons matched to a species which was recovered by traditional identification methods. In the present study, some singletons matched to a species, but some were also completely unclassified. Additionally there could be methodological artefacts resulting from the 97% identity threshold during OTU determination (Quince et al., 2009; Reeder and Knight, 2009). The abundance of singletons makes extrapolation of richness and diversity indices more risky (Lindahl et al., 2013). Especially for species richness estimators the fact that singletons become an increasingly large proportion of the community could cause problems (Dickie, 2010). Therefore the singletons were removed from our dataset.

4.1.4 Number of OTUs

Overall 4,544 OTUs (without singletons) were detected in our dataset. With the reduction to 494 reads per plot 96% of them were covered, so this appeared to be an appropriate cutoff for normalization and richness/diversity calculation.

To estimate if this number of OTU is in the range of other studies the results of other fungal pyrosequencing studies were summarized in Table 4.1. Drawing a conclusion of the comparison between different pyrosequencing studies of soil or root fungal communities is disputable. The number of OTUs depends on many factors, like number of sequences per sample, identity cutoff during clustering and origin of the DNA. For example, different numbers of sequences were used to calculate the number of OTUs per plot or sample unit, even not normalized in some studies. Additionally different cutoffs for clustering OTUs were used, ranging from 95% over commonly used 97% to 98.5%, also frequently used. The next difference occurs when regarding the length of the sequences used for clustering. The minimal length ranged from 140 bp to 200 bp, whereof latest was also used in this study. Further on different parts or the whole ITS region were used. As already

discussed above ITS2 region was chosen for this study. The main differences occurred in the systems studied, ranging from roots of a tropical rainforest over soil and roots in temperate forests to an herb, forming EM. Studies dealing with soil and not with root fungal community like Wubet et al. (2012) are not useful for a comparison as soil inhabits much more fungi than roots (Danielsen et al., 2012).This demonstrates that our study is unique and therefore very important for understanding EM fungal dynamics in temperate forests.

Table 4.1: Other Pyrosequencing studies for comparison (norm.=normalization/normalized ; ws = without singltetons)

Literature	Study system	DNA region sequenced	Length of sequence [bp]	Cutoff	Number of sequences total	Number of sequences per sample unit	Number of fungal OTUs
This study	roots of temperate forests of different tree species in Germany	ITS 2	min. 200 bp	97%	before norm.: 325 797 /after norm.: 54 834	494	4544 / 4359
Blaalid et al. (2012).	herb; glacier foreland in Southern Norway	ITS 1	min 150 bp	98.5% / 97%	101 870	not norm.	1633 / 470
Buée et al. (2009)	soil of deciduous forests in France	ITS 1	min 100 bp	97%	166350	not norm.	590-1000 per sample
Danielsen et al. (2012)	soil / roots of a poplar plantation in France	ITS 1	150 bp	95%	381 845 / 304 208	not norm.	392-800 / 75-249
Hartmann et al. (2012)	soil of spruce and Douglas-fir sites in British Columbia	ITS 2	average length of 243 ±9 bp	97%	19 353	383 ± 107	1453
Jumpponen et al. (2010)	oak roots from City of Manhattan (Kansas USA)	ITS 1	200 bp	95%	33 959	150	1077
Kauserud et al. (2012)	herb; glacier foreland in Southern Norway	whole ITS	min 150bp	97%	10 430	not norm.	52
Tedersoo *et al.* (2010)	tropical rain forest in south-west Cameroon	ITS 1	min 140 bp,	97%	44 411	not norm.	243 fungal (312 total)
Toju et al. (2013)	roots from a temperate forest in Japan (mainly oak)	whole ITS	min 150bp	97%	134 996	not norm. (152 ± 48 reads per sample)	836
Wallander et al. (2010)	actively growing EM mycelia of spruce stands in Sweden	whole ITS	min 190 bp	98.5%	over 18 000	not norm. (300-1 100 reads per sample)	248
Wubet et al. (2012)	soil of temperate beech forests in Germany	whole ITS	200 bp- 450 bp	97%	29 169	856	2271 before norm. 1655 after norm.

4.1.5 Michaelis MentenFit und Shannon as richness and diversity estimators

There are many studies comparing richness estimators and all recommend different estimators for different taxa, sampling methods and measurements (Reese, 2012). As the rarefaction curves are still increasing in this study, it was indispensable to estimate species richness. Chao, commonly used for fungi, sometimes combined with abundance-based coverage estimators (ACE) (Buée et al., 2009; Jumpponen et al., 2010; Lang and Polle, 2011; Lim et al., 2010; Wubet et al., 2012) or Jackknife estimators (Aučina et al., 2010; Jones et al., 2008; Jumpponen et al., 2010; Toljander et al., 2006) are based on abundance and the extrapolation of a sampling curve (Dickie, 2010). Even if Chao 2 is recommended for computing fungal richness (Unterseher et al., 2008), for high abundances as expected by pyrosequencing this estimator is not recommended any more (Dickie, 2010; Jumpponen et al., 2010). Alternatively, rarefaction analysis can be used for accumulation calculations, which was the reason why Michaelis Menten Fit (MMF) was used in this study for estimating species richness. In general, it is important to point out that for community analyses based on molecular markers, it is important to remember that the abundance of sequences does not always reflect biomass or abundance in the samples (Lindahl et al., 2013). For example fungal species with long, filamentous cells like hyphae are likely to be underrepresented, whereas fungi with yeastlike growth and/or small cells may be overrepresented, because they differ in nucleus/DNA to biomass ratio (Lindahl et al., 2013). Therefore with one sampling/extraction method and within the same species an abundance based comparison should be possible (Amend et al., 2010). Because of this, within this study, the comparison of the richness and diversity of different EM species should be possible to some extent as they mostly have comparable growing structures, e.g. regarding the mycelia in comparison to yeast like fungi. In this study over 60% of the estimated OTUs (richness by MMF) were detected within the samples, which showed that the estimation by MMF is not out of range. The quantitative use of high-throughput sequencing data is much debated (Amend et al., 2010; Baldrian et al., 2013), but as one of the five tested EMs (*Russula integra*) showed a significant correlation between pyrosequencing and morphotyping data it is also not unrealistic to have some abundance effect.

Furthermore, counting root tips during Morphotyping is also not completely comparable with the total abundance of the fungi. Root tips are counted independtly from size, mantle thickness and abundance of hyphae. But all those factors influence the biomass of the fungi, and are probably better reflected within a molecular approach. Furthermore, not only the presence or biomass of a fungi, but also the activity is important for the host plant. Therefore for an ideal abundance calculation the measurement of the fungal biomass, for example with ergosterol, or the fungal enzyme activity would be a good approach. Other pyrosequencing studies also used Shannon

Diversity Index for fungal communities (e.g. Buée et al., 2009; Jumpponen et al., 2010; Urbanová et al., 2015; Wubet et al., 2012).

More important is that for the analysis to environmental variables like soil parameters for all plots the same method for observing the fungal community was used.

4.1.6 Lifestyle annotation

The majority of the root-associated fungal OTUs could be assigned to ectomycorrhizal lifestyle. Together with a few other mycorrhizal lifestyles, such as arbuscular mycorrhiza, ericoid mycorrhiza and orchid mycorrhiza they accounted for 42.9% of all OTUs and 55.5% of all sequences. In comparison to 64% of all OTUs with known lifestyle being annotated to EM, Danielsen et al. (2012) found 87% of the total abundance to account for EM. They found only 4% for saprophytic fungi in a poplar plantation, in comparison to 22% within this study. Within our study around 7% of all OTUs and 5% of all sequences with annotated lifestyle accounted for endophytes and 3% of all OTUs and 1% of all sequences for plant pathogens. In Danielsen et al. (2012) also 5% of all sequences were annotated to endophytes and 4% to plant pathogens. Studies on *Quercus* roots found around 71% to be of ectomycorrhizal origin, similar to this study, but only 8% of saprophytes. More potential pathogens with around 12% were detected (Jumpponen et al., 2010).

4.2 Characterization of the root-associated fungal community structure separated by Exploratory or dominant tree species

4.2.1 Shared OTUs

An important aim of the present study was the characterization of the root-associated fungal communities in temperate forests in Germany. The fungal species where classified according with their lifestyle (i.e., saprophytes, EM, AM, pathogen, endophytic). The three Exploratories shared around 10% of all OTUs. Interestingly, we found that the pattern of the number of OTUs shared between the Exploratories differed with the largest differences between Schorfheide and the two others (16-17%), while Alb and Hainich shared the highest numbers of OTUs (23%). The found patterns are explained by abiotic (e.g. soil texture, pH….) and biotic (e.g.,host plant species) features of the three sampling areas.

First, the soil texture and chemistry differs between the three Exploratories (Fischer et al., 2010). The dominant geological substrate in the Hainich exploratory is loess over Triassic limestone and within the Alb it is Jurassic shell limestone. In contrast to this the main geological substrate in the Schorfheide is glacial till and the soil has a more sandy structure (Fischer et al., 2010). This results in a lower pH value in the Schorfheide than in the other two Exploratories (Solly et al., 2014). As the pH

value influences the soil fungal community it might also influence the root fungal community (Smith and Read, 1996; Wubet et al., 2012). Moreover different climatic conditions, mainly regarding annual mean temperature, which is highest in Schorfheide, has a wide range in Hainich and is coldest with a smaller range in Alb (Material and Methods Table 2.1; Fischer et al. (2010)). Additionally the annual mean precipitation, which is lowest in Schorfheide and highest in the Alb (Material and Methods Table 2.1; Fischer et al. (2010)). This leads to dryer climatic conditions within the Schorfheide in comparison to the other two Exploratories.

The different tree species within the Exploratories might influence the differences in the root-associated fungal community at a large extent. Host preference of EM fungal communities is widely known (Dickie, 2007; Ishida et al., 2007; Lang et al., 2011; Tedersoo et al., 2012, 2008). This assumption is supported by our result that the root-associated fungal community differs between the different tree species. The different dominant tree species shared the doubled amount between two tree species respectively than between all.

Our results revealed also a strong difference regarding the shared life styles of the root-associated fungal taxa. Only 37% of all shared fungal OTUs between the different tree species are of ectomycorrhizal lifestyle, even if they represent over 41% of all fungi. Pine plots shared twice as many OTUs with ectomycorrhizal lifestyle with beech than with spruce. This might be due to the fact that spruce and pine does not grow within one Exploratory and was the reason why not summing them up to the group of "conifers". Beech shared a few EM more with pine than with spruce, which might result from EM generalists as for example some pine dominated plots within the Schorfheide have a higher percentage of beech trees than spruce dominated plots within the other two Exploratories. EM fungi shared a smaller percent of OTUs between all tree species than the saprophytic fungi. Interestingly, the number of saprophytic OTUs shared between the different tree species was very similar, so there is a clear separation between host specific EMs and non host specific saprophytes. For example in Ding et al. (2011) 74% and in Lang et al. (2011) 61% of the EM fungal species showed a host preference, whereas in Ishida et al. (2007) only 15% of the EM showed a strong host preference. The effect that Alb and Hainich shared much more fungal species than Alb or Hainich to Schorfheide is also detected within the ectomycorrhizal OTUs. For saprophytes again Alb and Hainich shared most OTUs, but unexpectedly Alb and Schorfheide shared a few more species than Hainich and Schorfheide.

4.2.2 Taxonomy

Overall, the main fungal group were the Basidiomycota, which accounted for more than twice as much of the sequences than the Ascomycota. Remarkably there were a few plots completely without Ascomycota. Within the Hainich and the Schorfheide there only were twice as much Basidiomycota than Ascomycota sequences. When regarding the mean number of OTUs there was only 1.7 times more Basidiomycota than Ascomycota in the Alb and nearly the same mean number of OTUs per plot within the Hainich and the Schorfheide. Similar relationships between Basidiomycota and Ascomycota were detected for the soil fungal community within the Exploratories (Wubet et al., 2012). The percentage of Ascomycota OTUs was the same with around 44% of all OTUs which could be assigned to any fungal phylum. Within the Alb region, Wubet et al. (2012) found nearly the same proportion of OTUs as the present study to originate from the Basidiomycota. Remarkably within the Alb 80% of all sequences with assignment to a fungal phylum were from the Basidiomycota. However, other authors, e.g. Jumpponen et al. (2010) detected 3% more Ascomycota than Basidiomycota on oak roots. In Jumpponens and in the present study accordingly only a few Glomeromycota respectively arbuscular mycorrhizas were detected. This result is in accordance with the fact that EM fungi dominate temperate forests (Allen et al., 1995; Courty et al., 2005). Nevertheless, the aim of the present study was to investigate the EM community and, therefore, the primer pair (ITS3 and ITS4) was selected. This pair was used in studies for EM fungal communities before (e.g. Blaalid et al., (2013) and Tedersoo et al. (2014)). The selection of the barcode primer combination might explain around 38% of the variation between fungal community analysis by high throughput methods (Tedersoo et al., 2015) and is therefore very important.

Regarding the differences in the fungal phyla between the different dominant tree species, Basidiomycota sequences were most abundant (86%) on oak plots resulting in 56% OTUs, mainly based on *Lactarius quietus* that has been described as an oak specialist (Courty et al., 2007; Suz et al., 2014). In comparison to this Jumpponen et al. (2010) detected 50% Ascomycota and only 47% Basidiomycota on oak roots. This result is supported as Suz et al. (2014) found 79% of EM on oak roots to be from the Basidiomycota. Within the forest soil of oak plots Buée et al. (2009) found 65% of the OTUs to be from the Basidiomycota. For spruce plots with over 100 Basidiomycota OTUs (58%) this groups was more abundant than on plots dominated by other tree species. For the soil of a spruce forest 28% of the OTUs were accounted to Basidiomycota (Buée et al., 2009). But within their study on spruce plots a high abundance of saprophytic lineages in forest soils were detected (Buée et al., 2009). On beech plots of the Biodiversity Exploratories within the soil around 54% Basidiomycota, exactly as in our study, and 36% Ascomycota were detected (Wubet et al., 2012). In our study 27% of

all OTUs were Ascomycota. For pine plots around 40% of all OTUs were from Basidiomycota and 45% from Ascomycota fungi. Trocha et al. (2012) found 80% of EM fungi on pine plots to be from Basidiomycotas. Nevertheless, in the present study all root-associated fungi were regarded, leading to a lower proportion of Basidiomycetes as in studies only focusing on EM.

The most abundant family were the Russulaceae (which contained 37% of sequences and 17.7% of OTUs). The Russulaceae with the russula-lactarius lineage are one of the most species-rich and abundant ectomycorrhizal family within temperate forests (Avis et al., 2003; Courty et al., 2008; Geml et al., 2010; Tedersoo et al., 2010a). For example, on oak roots 45% of all EM were found to be of the Russulaceae (Suz et al., 2014). Most of the OTUs were assigned to the genus *Lactarius* with more than 25% of the sequences and ~7% of all OTUs. In the Hainich the generalists *Cenococcum geophilum, Clavulina cristata*, *Russula delia* and *Inocybe maculata* were most abundant in a morphotyping/ Sanger sequencing study (Lang et al., 2011). Specialists for beech were *Lactarius subdulcis* and *Tomentella sublilacina*, which could explain the high abundance of *Lactarius* within our study. *Tomentella* species were most abundant in their mean number of sequences on beech plots, but did not differ remarkably between beech, oak and spruce dominated plots. *Tomentella* in contrast did in average only account for around 2 OTUs in all Exploratories. *Tomentella* is, together with *Lactarius* found in a high frequency from Hainich beech forests (Lang et al., 2011).

Additionally the genus *Russula* with for example *Russula ochroleuca, Russula formula* and *Russula integra* with in average 9.3% of the sequences and 7.3% of the OTUs was highly abundant over all Exploratories with highest abundance in Alb, followed by Schorfheide and least in Hainich, most probably caused by the dominance of *Lactarius* here. However *Russula* species like *Russula firmula* or *Russula integra* on spruce plots and *Russula ochroleuca* on both conifer dominated plots form the most abundant group here. *Russula* and especially *Russula ochroleuca* is a dominant EM on conifers (Cox et al., 2010). In the study of Pena et al. (2010) *Russula* was not highly abundant in the Swabian Alb, but this study was only conducted on beech plots.

Besides *Lactarius* and *Russula* the EM genera *Xerocomus, Sebacina, Tomentella, Inocybe* and *Hygrophorus Cenococcum* and *Tuber* were under the most abundant OTUs. Those genera are commonly found in studies on tree roots in temperate forests (Agerer, 2001; Lang et al., 2011; Lang and Polle, 2011; Pena et al., 2010; Tedersoo et al., 2010a, 2006; Toju et al., 2013). For example in an temperate forest in Japan also *Russula, Cortinarius* (which was not so frequent within our study) and *Lactarius* constituted more than a quarter of the total community (Toju et al., 2013). As already mentioned above within the Hainich and especially on beech dominated plots a high abundance of *Lactarius* was detected, which might result from *Lactarius subdulcis*, a fungus with high abundance within the Hainich beech plots (Lang et al., 2011). But surprisingly this species had a very low

abundance within the pyrosequencing data. This might have two reasons: first, especially for this fungus the primer might not match well, which might have caused an imprecise bonding. But as other *Lactarius* species were found with a high abundance this might not be the right explanation. An uncultured *Lactarius* was very frequent within the OTUs, so one sequence within the UNITE database with an incomplete name could cause this imprecise assignment to genus and not to species level.

Hygrophorus pustulatus, also one of the most dominant species, was mainly detected on spruce dominated plots. This species is known from spruce plots (Agerer et al., 1998; Peter et al., 2001). Overall our study detected the commonly found EM fungal species.

Mycena and *Trechispora* were the most abundant saprophytic genera. They were also detected as root-associated fungi in another study (Toju et al., 2013). Some of the *Mycena* species are host tree dependent, too (Tyier, 1991), which was also detected for some species within this study, as they only occur on sited dominated by one tree species. But this does not seem to influence the overall saprophytic community. There are only small differences in the mean number of saprophytic OTUs shared between the different tree species, but also differences between the community structures which will be discussed later. With *Lachnum, Phialocephala* and *Tetracladium* species commonly known endophytes were detected (Letourneau et al., 2010; Raviraja et al., 1996; Roldán et al., 1989; Sánchez Márquez et al., 2007). Overall, our study detected commonly known EM and soil fungal lineages, sometimes with other proportions of taxa like other studies did, which could be due to methodological, seasonal or origin effects.

4.2.3 Richness and Diversity of root-associated fungi

Another aim of this study was to investigate the differences of richness and diversity of root associate fungi between the different study regions (Exploratories) and dominant tree species. In general richness as well as the diversity showed a high variation within each Exploratory. The Alb had significantly more OTUs than the Schorfheide, whereas the richness within the Hainich plots was not different from either the Alb or the Schorfheide plots. In the Alb plots the diversity was higher in comparison to the other two study regions. In contrast to this, for the soil fungal community within the Biodiversity Exploratories (Wubet et al., 2012) found a similar richness in all three study regions. But in this study only the beech plots were considered. The fungal community of EM and other soil fungi can on the one hand vary spatially, but also vary temporally, even within one month (O'Hanlon, 2012). For our study samples were taken in May and for Wubet et al. (2012) in April in different years. Therefore it would be an important approach to investigate soil and root fungal community

within one harvest. Additionally a time series over one year could be a helpful approach in understanding the dynamics between soil and root fungal community and the influencing environmental factors.

The richness of the EM was significantly higher within the Alb, which might also explain the high Basidiomycota abundance, as within the Basidiomycota (37 lineages), in comparison to Ascomycota (27) or Zygomycota (2), most EM forming lineages were found (Tedersoo et al., 2010a). The significantly smallest diversity of EM fungi was found on Hainich plots, which could mainly be explained by the dominance of *Lactarius*. The EM diversity was highest within the Alb, differing significantly from Hainich and Schorfheide. Those results are surprising as a higher diversity within the Schorfheide was expected as there were plots with three different dominant tree species: beech, pine and oak. Alb and Hainich had only beech and spruce. But when considering not only the dominant but also the other tree species we found the most tree species within the Alb forest plots (6.1 ± 2.5), followed by Hainich (5.2 ± 2.3) and lowest in Schorfheide (4.4 ±2.1) (calculations based on the inventory of the vascular plant diversity within the Biodiversity Exploratories of 2009 for details see Supplementary Table S1). This reflects the richness of the root-associated fungal community, but not directly the diversity of all or EM fungi. It is assumed that the host plant genus-level diversity is a good predictor for EM diversity (Gao et al., 2013). This study was highly debated, because of a re-evaluation of the data by Tedersoo et al. (2014), pointing out a design with inconsistent species pool and poor data compilation for the meta-analysis this assumption is based on. So therefore this conclusion still needs to be verified (Gao et al., 2014; Tedersoo et al., 2014). Within our study positive correlations between the richness estimator MMF for all fungi and all mycorrhizas (including EM, AM, orchid and ericoid mycorrhiza) and the sum of tree species per plot were detected (All: rho = 0.200; p = 0.035, Myk: rho = 0.1975, p = 0.049), but no significant correlation between the diversity, represented by Shannon Index, of all fungi. From all other lifestyles (even EM) none showed a significant correlation neither for richness nor for diversity to the richness of tree species. Kernaghan et al. (2003b) demonstrated for mixed boreal forests that the diversity of EM correlates positively with the diversity of the trees. Similar results as in our study were detected by two studies within temperate beech forest (Lang et al., 2011; Lang and Polle, 2011): In one study a increasing number of EM forming tree species resulted in an increased EM diversity, whereas within another study this effect was counteracted by the presence of the non EM forming roots of ash (*Fraxinus excelsior*). Therefore this could only be a part of an explanation and needs further investigation.

The number of EM species in the Hainich can differ between study methods. Lang and Polle (2011) detected 86 EM species within the Hainich, whereas in our study over 730 EM OTUs were detected. This differences could be mainly explained by two factors: first, the methods differed as in Lang and

Polle (2011) morphotyping and Sanger sequencing was used and with the pyrosequencing here maybe species were separated which were not able to separate by morphotyping. Second, in this study additionally to beech plots also spruce plots were used which inhabits a different EM fungal community than beech roots. When eliminating the two spruce plots 306 EM OTUs were detected within the Hainich. But this is again more than in Lang and Polle (2011) or in Lang et al. (2011) where the number of EMs in beech plots was estimated to 74. A comparison of the two different methods used here (Sanger sequencing in Lang et al., (2011); Lang and Polle, (2011)) and pyrosequencing in our study) found 111 EM species with Sanger sequencing and 240 OTUs with pyrosequencing respectively in a tropical rainforest in Cameroon (Tedersoo et al., 2010b). As already discussed above the pyrosequencing method may detect species not forming a mycorrhiza, but are still there as an inoculum (Danielsen et al., 2012).

When comparing the richness and the diversity of all fungi between the different dominant tree species no significant differences between them were detected. For EM fungal richness, spruce dominated plots had significantly more EM than pine dominated ones. But all tree species did not differ in their EM diversity. For forest soils, Buée et al., (2009) found more fungal OTUs in the soil of oak, spruce and pine dominated than in beech plots. We also detected most OTUs in spruce plots, but here oak and pine plots had nearly the same richness as beech plots. As described before, comparisons with oak plots needs to be regarded with suspicion as statistical comparisons could become unbalanced, caused by the limiting number of five oak plots. As the curve progression of the rarefaction curves did not differ remarkably between the different tree species, the results of richness and diversity estimation should be well comparable for beech, spruce and pine. In oak plots we estimated between 26 and 63 EM OTUs, which is within the range of the 60 OTUs detected by Jumpponen et al. (2010) on oak roots, also with pyrosequencing and the usage of 500 sequences. For pine plots RFLP based studies, where 30 (Grogan et al., 2000), or 43 (Jonsson et al., 1999) EMs on pine roots were detected, range within the same amount of EM within this study, which was estimated to a wide range of 10-78 EM-OTUs. For spruce plots, where within this study 13-116 EM-OTUs were estimated, for example 16 EMs with RFLP (Kjøller et al., 2012) 18 EMs (Kalliokoski et al., 2010) or 34 EMs (Korkama et al., 2006), both with DGGE fingerprints were found in other studies. The richness and diversity of saprophytic fungi is significantly decreased in Schorfheide in comparison to the other two study regions. Beech plots had a higher richness and diversity than oak or pine plots.

The richness and diversity of saprophytic fungi was correlated with soil texture parameters (cf. Supplemenatry Table S5), which could shift those differences.As the fungi with unknown lifestyle show similar results in correlations and comparisons as all fungi, it could be assumed that they might

represent proportional the same lifestyles already annotated for all fungi. Their richness or diversity did not differ between plots dominated by different tree species. For other lifestyles like pathogens or endophytic fungi there were too few individuals for reliable statistics. Endophytes accounted for 3.3% of all sequences and 4,7% of all OTUs, plant pathogens for only 0.8% of all sequences and 1.9% of all OTUs.

4.2.4 Root-associated fungal community structure differed between study regions

The root-associated community of all fungi differed significantly between the three Exploratories. Also the soil fungal community differed between the Exploratories (Wubet et al., 2012). The difference is highest between Alb and Schorfheide. The fungal community within the Hainich is slightly more different to Schorfheide than to Alb. Those community differences could on the one hand be related to the different geological background with limestone in Alb and Schorfheide, but on the other hand also indicate some distance decay effect (Bahram et al., 2013), as the Hainich is geographically between Alb and Schorfheide, also the root-associated fungal community is "between" them. As a remark, the distance between Hainich and the other two regions is around 300 km, whereas Alb and Schorfheide are 600 km apart from each other. The communities of EM fungi were less different between the study regions than all fungi (shown by a lower R values of the ANOSIM), indicating a micro habitat, not influenced by environmental/regional factors as that fungal community influenced by surrounding soil. The saprophytic community was more different between the study regions than EM, all or unknown fungi.

4.3 Natural and anthropogenic environmental variables influencing root-associated community

4.3.1 Dominant tree species on the plots – influence on root-associated fungal community

As already mentioned above, besides generalists there are also ectomycorrhizal specialists, specified on one tree species or genus, like *Lactarius quietus* which is usually found to be associated with oak trees (Courty et al., 2007; Suz et al., 2014). Those host specific fungi seemed to be the reason for the clear separation of the root-associated fungal community by different tree species. The fungal community from the whole, the EM or the unknown fungal community were more distinct between

dominant tree species of the plot than between the Exploratories. This effect could not be detected for saprophytic fungal community so clearly. In contrast to the root-associated fungal community in the different study regions, when regarding fungal communities between different dominant tree species, the community of the saprophytes was less different between the tree species than the community of the EMs. This has shown once more, that tree species is a more important factor for shaping the community for EM than for saprophytic fungi.

Only pine and oak did not host a significantly different root-associated community, which might have been explained by two effects: first, on our sampling plots both tree species are only present in Schorfheide, second *Lactarius quietus* the oak specialist was found to be very dominant on oak. But one fungal species might not result in an overall community separation. Remarkably also for oak and pine dominated plots the EM community was more different than the saprophytic community. The other important microbial community within soil besides fungi is the bacterial community. Also within the Biodiversity Exploratories it appeared, that tree species was an important driver of soil bacterial community structure (Nacke et al., 2011). For example, Ding et al. (2011) showed that over 33% of the variance within EM fungal community was explained by host plant species and only 4.6% by soil origin. Changes of the EM community in tropical forests were reported to parallel changes of the tree community (Peay et al., 2010). Lang et al., (2011) reported that only 10% of all EM species were shared between *Fagus sylvatica, Tilia* spp. and *Carpinus betulus*, whereas over 60% of the EM species were only detected on one of the three hosts.

4.3.2 Soil properties – influence on root-associated fungal community

As already discussed above, the dominant tree species on the plot had a great influence on the community structure of root-associated fungal community, but not on the richness or diversity. Which other environmental variables or root properties might influence the richness, diversity or community structure of the root-associated fungi or different lifestyles is discussed below. Within our study the main attention should rely on root properties influencing the root-associated fungal community. Nevertheless the main soil properties for fungi like pH and N have to be considered.

The pH value is known as one of the basic soil variables influencing soil and root fungal community (Kernaghan et al., 2003b; Taylor and Finlay, 2003; Wang et al., 2015; Wubet et al., 2012). However, for the richness or diversity of root-associated fungi it was not an important variable within the overall model. But it explained the community structure better than other environmental variables. For EM the variable pH was removed from the model as one of the last variables, was not significant,

but more important than other environmental variables tested. Furthermore, there was a positive correlation between the richness and diversity of all, the richness of EM and the diversity of saprophytes to the pH value. Those findings are in agreement with Suz et al. (2014), thapH correlates positively with the EM fungal diversity on oak roots. Other reports indicate also, that AM colonization increases with pH on maple (*Acer saccharum*) trees (Coughlan et al., 2000). An increase in soil pH additionally was shown to increase fungal biomass in forest soils phospholipid fatty acid (PLFA) technique - (Bååth and Anderson, 2003). EM species may vary in their pH optimum (Smith and Read, 2008) and from culture studies it was reported that they react to pH in in different ways. EM taxa showed optimum growth rates at different pH spans from one to five units or increasing growth with increasing pH values (Hung and Trappe, 1983). In the present study the higher pH values in the Alb may cause the higher richness comparison to the Schorfheid. Soil fungal community composition on beech plots within the Biodiversity Exploratories was mainly explained by soil pH, sand content and litter cover (Wubet et al., 2012). In the present study, the pH value of the mineral soil was more important for the community structure of saprophytic fungi than for EM.

Soil C and N concentrations significantly affected EM diversity within the model, but that was not the case for saprophytes or fungi with unknown lifestyle. The total N of the soil correlated positively with the diversity of all and EM fungi. The amount of plant available nitrogen is not directly correlated with the total amount of nitrogen in the soil as a minimum of 95%-99% of the soil nitrogen is present in organic forms and is, therefore, not always directly available for the plant (Haynes, 2012; Stevenson, 1994). That may explain our result, showing that N within the soil and N within the roots were negatively correlated.

4.3.3 Forest Management – influence on root-associated fungal community

As the major plant symbiont EM fungi are among the most sensitive biota to harvesting induced disturbances. EM community was significantly altered a decade after harvesting in northern coniferous forests (Hartmann et al., 2012). The richness and diversity of EM fungi correlated positively with forest management index SMI. The amount of harvested biomass, one component of the ForMi, also showed a significant positive correlation with EM richness. The diversity of the saprophytic fungi negatively correlated with ForMI, which was also important in the model, which was built with all other variables. The same difference between saprophytic fungi and EMs was also true for the community structure. The structure of the community was better explained by the

management for EM than for the saprophytes. The management on those plots is a kind of sustainable management without for example clear-cut.

Further on, there could be an inderict effect, as with an increase in management more trees and with them their nutrients are removed from forests. The management indices differed between the plot stands that are dominated by various tree species from oak<beech<pine<<spruce (Schall and Ammer, 2013). But as the host tree species did not differ significantly (despite pine having less EM richness than spruce) in their EM richness this is only a partial explanation of the positive correlation between management and EM richness. Tree removal, especially if the whole tree and not only the stem is harvested, may lead to an alteration of resources available in the EM/plant habitat (Blanco et al., 2005; Johnson and Curtis, 2001), including for example nitrogen. This reduction in N could lead to an increase in EM richness (Kjøller et al., 2012; Lilleskov et al., 2002a). Both management indices and the amount of harvested biomass showed a significant negative correlation to root N concentration. Buée et al. (2005) found a significantly higher richness of EM in thinned than in not thinned forests. The silvicultural use of a forest might result in a soil structure disturbance and may induce a patchiness of nutrient supply. That is especially the case, where trees are cut and roots remain within the forest. This disturbance consequently leads to a higher diversity of microhabitats, which may explain a part of the forest management influenve on EM communities. Another component of the management index were the tree species, for the ForMI especially the number of non-native tree species (Kahl and Bauhus, 2014). When taking into account the host specificity of many EM fungi one can assume that a higher number of available hosts for EM could lead to an increase in richness and/or diversity of EM.

Studies in conifer forest showed, that even a decade after harvesting the diversity and structure of soil bacterial and fungal communities were still affected by the harvesting disturbances (Hartmann et al., 2012). In beech monospecific forests differences between strong thinned and non-thinned plots in the EM community structure were detected (Buée et al., 2005). Results pointing our in the same direction were found in a red pine (*Pinus densiflora*) forest, where the richness and diversity of EM increased in higher disturbed sites (Lee and Eom, 2013). In the present study, we showed on a large scale, with different tree species and with a continuous variation from unmanaged to intensively used forest the increase in EM richness with forest management intensity.

But this increase in EM richness with increasing management was only true for moderate management. For example in spruce forests with a high forest decline due to air pollution, causing a soil acidification, the EM richness was significantly decreased (Peter et al., 2008). Furthermore trees

in urban sites, which in contrast to a forest can be seen as highly disturbed, also had a lower richness of EMs (Karpati et al., 2011).

The belowground disturbance caused by moderate management was investigated in our experiment, in which disturbance of tree harvesting was simulated on the small scale. When a tree is harvested, the root system remains in the forest ground and decomposes. Our experiment simulated this formation of root litter by cutting the roots within the forest floor. The recolonization process of patches free from living roots was observed. In an litter decomposing experiment with pine litter, after two years decomposing, EM roots lost about 7% of their initial C mass (Langley et al., 2006). Within our experiment, fine root biomass from decomposing roots had a significantly mass loss of around 75% only within the last three month of the experiment. Caused by its woody structure, combined with mycorrhized root tips, fine root litter is not comparable to foliar litter. In boreal forests, the first year mass loss of root litter ranged between 19 and 40%, most variability have been caused by climatic conditions (Berg and McClaugherty, 2003). The initial C quality (e.g., cellulose concentration, lignin concentration) of fine roots was found to be correlated with fine-root decomposition rates in spruce and ash forests (Chen et al., 2002). In contrast, they found no correlation between initial N concentration/soil N availability and fine-root decomposition rates. The rate of N released from decomposing roots was positively correlated with the initial N concentration of the fine roots. It was suggested that in mature Douglas-fir forests decomposing fine roots could release about 20 kg N/ha annually (Chen et al., 2002). Therefore, two counteracting influences on EM richness and diversity were combined: with the disturbance, enrichment in EM could be expected, but with the enhanced N release from decomposing roots a decrease in EM richness and diversity could be assumed.

Within the first three month the Ingrowth and the No Ingrowth cores had the same decomposition rate. No new roots were observed in the Ingrowth cores after three months. (Lindahl et al., 2010) observed a decrease in EM abundance already after 14 days after root severing. Additionally no differences between the decomposing one and the EM fungal community within undisturbed controls were detected after three months. This indicates that all EM decompose with a similar proportion. *Cencoccum geophilum*, a melanized morphotype with short rhizomorphs, was one of the morphotypes which appeared vital for the longest time of six month. *C. geophilum* has been previously described as a very robust EM, as it was abundant on burnt sites for recolonization (Kipfer et al., 2011). Moreover, *C. geophilum* was unaffected by severe reduction of C flux toward roots by tree girdling (Pena et al., 2010) and is generally known as drought tolerant taxa (Pietro et al., 2007). Additionally a *Tomentella* species and *Lactarius subdulcis* were found in No Ingrowth cores even after 15 months, too. After six and twelve months the richness and diversity was lower in Ingrowth than in

control cores. This decrease in EM richness within disturbed sites was also observed in experiments that have investigated the EM recolonization of burnt sites (Kipfer et al., 2011). Nevertheless, also the root litter addition might have favoured a decrease in EM richness, excepting certain species that responded positively to litter addition (Cullings et al., 2003). Furthermore, as the rarefaction curve is still increasing and the Ingrowth cores had less vital tips than the Control cores the differences within the richness and diversity (especially during the first 12 months) could also be a sampling effect.

Six and twelve months after disturbance, the Ingrowth EM community differed significantly between Ingrowth and Control cores until. The pattern was maintained until 15 months after disturbance, when the richness and diversity of EM revealed no more differences between Ingrowth and Control sampling cores. However, the EM communities were still different, but became already more similar between Ingrowth and Control cores. After one and a half year after disturbance the EM community structure did not differ any more between Ingrowth soil cores and undisturbed Controls. We examined only a small patch of 0.08 m in diameter, whereas a whole root system of a tree may have a diameter of several meters, even exceeding the edges of the crown (summarized in Ammer and Wagner (2004); Lang et al. (2010)). Moreover, Lang et al. (2010) reported that the roots of an individual beech tree could span several meters and that even directly next to a tree, roots from other tree individuals were detected. Given all these considerations, estimation of root system decomposition time span and recolonization prosesses on a larger scae are still difficult to assess. For example on brunt pine sides, comparable with a large scale disturbance, it took 15-18 years to re-establish the climax state of undisturbed sites (Kipfer et al., 2011). A faster re-establishment was observed after a thinning treatment in an *Quercus robur* forest, where the biodiversity of EMs already re-established after 10 months (Mosca et al., 2007). Nearly the same species, which commonly colonize undisturbed roots, were important for the recolonization process. For example *Xerocomus chrysenteron*, with Long distance exploration type mycelia, was one of the two taxa highly abundant in Ingrowth cores. The other one was an un-sequenced morphotype with contact exploration type. Exploration type is regarded as an important trait in colonization process. Interestingly, in our study the Short distance exploration type appeared to be favourable for recolonization. Contact and Short distance exploration types were frequently detected in mineral soils where they have access to soluble forms of N like amino acids, ammonium and nitrate (Hobbie and Agerer, 2010). Pena et al. (2013b) for example found different EM species to be different effective in ^{15}N uptake released from root litter. *Tomentella* sp., with a Short distance exploration type, was most effective in N uptake.

There is the theory, that EM fungi on dying roots autolyse their mycelia and attack the roots to maintain themselves searching for a new host (Baldrian, 2009; Cullings and Courty, 2009). EM fungi

typically revela the cellulase enzyme activity at lower levels than those measured by their saprophytic relatives (Baldrian, 2009). The decomposition of dying roots may shorten mineralization pathways, because EMs living on decomposing roots may recycle their own resources (Kerley and Read, 1998; Langley et al., 2006; Lindahl et al., 2002) and, have therefore, primary access to their own N, supporting the new ingrowing roots with those resources. The hypothesis that EM fungi are not strict mutualistic but they range along a biotrophy-saprotrophy continuum might be an explanation for the lysis of the dying host root (Cullings and Courty, 2009; Koide et al., 2008). Until now, no clear pattern for N release from decomposing roots has been described (Berg and McClaugherty, 2003). For example in beech root litter the rapid release of soluble N from the litter was reported to be balanced by the incorporation of exogenous N (Zeller et al., 2000). EM root litter from pine immobilized additional 15% N from soil (Langley et al., 2006). Overall the N concentration of root litter varied during decay and between different tree species, with conifer roots decaying much more slowly than roots of deciduous trees (Berg and McClaugherty, 2003; Silver and Miya, 2001). As we had mainly beech roots within our cores, we expect that decomposition of the roots might have discharged N sources, favouring short distance exploration type within the Ingrowth cores over the first period of ingrowth (12 months).

In our experiment we observed a typical secondary succession as many, especially generalistic species were present over the entire development period and could therefore be present over the whole recolonization process (Twieg et al., 2007). They shifted in dominance over time and therefore represent different life history strategies or competition of resources. With our experimental setup, we did not discriminate for any type of EM fungi, neither for fungi depending on carbon supply by the host tree nor for fungi who might not need this supply as germinating from spores and then entering the host roots (Fleming, 1984). Therefore, the whole fungal community had the same chance to enter recolonizing roots. For example *Lactarius subdulcis*, the most abundant species in undisturbed controls was also highly abundant in Ingrowth cores. Generally, the pattern that a few EM species dominate the EM community with many rare species has been detected previously several times (Byrd et al., 2000; Cullings et al., 2003; Pena et al., 2010).

Altogether this demonstrated that forest management creates temporal niches with a changed EM community. Nevertheless, the EM fungal community has a high resilience to disturbances on small scale. Harvesting a tree might have a much longer impact on EM fungal community than our small scale experiment. We did not find an increase in richness or diversity within the Ingrowth cores, but the communities between Ingrowth and Controls differed for 15 month. Increasing forest management may result in more niches within the forest belowground habitats, therefore additionally favouring the increased EM richness, which was shown in the large scale study. Different

EM fungi are known to react differentially on canopy gap opening, one aspect of forest management we did not regard within our small scale study (Grebenc et al., 2009). The resilience of the EM community might need further investigations on the large scale.

4.3.4 Root nitrogen concentration affects EM richness

Our results support the assumption that richness of all root-associated and EM fungi depended mainly on root nitrogen. Plant-available N forms include nitrate, ammonium and small soluble organic compounds like amino acids via EM (Jackson et al., 2008; Persson and Näsholm, 2001). EM fungi have several opportunities to use N from soil: from ammonium and nitrate from mineralization processes (Finlay et al., 1992) from soil organic matter (Näsholm et al., 1998) and from small organic particles released from litter (Perez-Moreno and Read, 2000). In comparison to plant roots, the nutrient absorbing surface of EM increases with the formation of an external mycelia and improves N uptake (Bending and Read, 1995). The advantages of these hyphae are that they can absorb less mobile forms of nutrients, grow further than the tree roots and reach, due to their fine structure, soil microsites which otherwise would be inaccessible for roots (Finlay and Read, 1986; Gobert and Plassard, 2008; Tuomi et al., 2001). In our study we found that the more root N the less root-associated fungal species were detected. However, the saprophytic fungi did not show this dependency in the overall model, but their diversity was also negatively correlated with root N concentration. The large scale dependency on root N concentration of EM was also found by (Cox et al., 2010) in conifer stands in Europe. But not only the diversity and richness was influenced, also the community composition of EM fungi changed on the large scale (Cox et al., 2010) and on the local scale (Wallenda and Kottke, 1998). Local scales in the mentioned study refered to less than > 500 m^2, as within plot gradients might be too small to affect a whole EM community (Avis et al., 2003; Cox et al., 2010). Other influences like competition may be more important (Koide et al., 2005). Pena et al. (2013a) detected an increased N uptake of light exposed plants in contrast to not mycorrhized beech seedlings within a pot experiment, but not for shaded plants. This indicates that the functionality of EM species strongly depends on environmental conditions.

In the present study, we observed a higher dependency of all and EM fungal communities on root N than the saprophytic fungi. Many other studies already detected the effect of increased N availability and the loss of EM richness and diversity (Avis et al., 2003; Lilleskov et al., 2002a; Taylor et al., 2000). Nitrogen is a limiting nutrient within boreal and temperate forests (LeBauer and Treseder, 2008; Vitousek and Howarth, 1991) and therefore, the plants may profit from their ectomycorrhizal

symbiosis partner through a better N supply (Jackson et al., 2008; Smith and Read, 2008). The more species are hosted by a plant the more functional resources are available (Phillips et al., 2014; Talbot et al., 2013). With these functional resources, like enzymes for N mobilization, the plant might have a better supply in N poor soils. As the EM symbiosis needs a high carbohydrate supply by the plant, under shortage of carbohydrates cryptic EM species disappear faster than dominant ones (Pena et al., 2010) and therefore possible functional resources by a higher EM diversity might get lost for the plant. The effects of concentrations of rare elements on EM community composition revealed no clear pattern. For example, Fe was positively important for EM diversity, whereas Ca (positive correlation) and K (negative correlation) showed significant influence on EM richness. Previously studies have shown that the iron uptake differed between EM species (Rineau et al., 2008; Szaniszlo et al., 1981). Distinct EM species were found to differentially accumulate chemical elements (Seven and Polle, 2014). Nevertheless, rare elements like Mg, Na and S were shown in our study to be important for the diversity saprophytic fungal community.

Root N concentration was mainly independent from Exploratory, whereas the tree species played a major role. Spruce plots showed the lowest root N concentration whereas the roots from oak plots, together with some beech and pine ones, exhibited the highest root N concentration. Additionally for the community structure of all root-associated fungi there was a clear separation to higher CN values for conifers. This is in accordance to a study that found, that N availability is higher in deciduous than in conifer stands, caused mainly by the low N content of the litter needle compared to leave litter (Jerabkova et al., 2006), indicating a lower quality for nutrient supply under coniferous stands (Côté et al., 2000).

Human activities, mainly fertilization in agriculture increased N deposition (Holland et al., 1999). Our results support the assumption that this deposition, can cause the reduction of EM fungal richness. Forest fungi are known to have a rapid response to N deposition changes (Baron et al., 2014) and may therefore be used as indicators of a general biodiversity loss. Thus, a higher N deposition might not only reduce the diversity of plants (Phoenix et al., 2006), but also of their mycorrhizal symbiosis partners.

4.3.5 Carbohydrates: influences on EM and saprophytic diversity and community structure

Our results showed that EM diversity dependet significantly on root glucose concentration and that EM community structure was better explaines by glucose than by fructose.

By measuring glucose and fructose we measured those carbohydrates which are most important for plant-fungus interaction (Nehls et al., 2007). Glucose and fructose occur in both partners of this symbiosis, as for example sucrose would be host-specific (Shi et al., 2002). For glucose and fructose it is known, that they support EM growth (Nehls and Hampp, 2000; Schaeffer et al., 1995). Differentiating the fungal partner of the mycorrhizal interaction preferably absorbs glucose, but also some fructose (Nehls et al., 2007). Fungal specific carbohydrates like arabitol, mannitol and trehalose were not determined in the present study. We measured the carbohydrates that are most important for symbiosis. Root glucose showed a stronger correlation to EM richness than root fructose concentration. The EM community composition fitted better to root glucose than to fructose concentration, whereas for saprophytes no difference between the two carbohydrates on the fit of the community structure was detected. Nevertheless, the richness of saprophytic fungi correlated positively with the root fructose concentration, but not with root glucose concentration. The community structure of both lifestyles fitted similar to root fructose concentration. This supports the result that the EM diversity, but not the richness, depended on the glucose concentration of the roots. Druebert et al. (2009) showed that plant carbon productivity was the reason and not the result of a higher EM diversity. Sucrose is hydrolysed in the plant cell to glucose and fructose (Hampp and Schaeffer, 1999) before being transported to the fungal partner (Nehls et al., 2007). Most EM lack the invertase and therefore sucrose needs to be hydrolized by plant-derived invertase (reviewed in Nehls (2008)). The total amounts of carbohydrates found were in the same range for root carbohydrate concentrations as found by Druebert et al. (2009). The high amount of glucose in comparison to fructose in roots from all tree species indicated that plants support their fungal partners, maybe additionally by converting fructose to glucose. For example the basidiomycete sugar transporter AmMst1, described from spruce roots symbiosis, strongly favours glucose (Nehls and Hampp, 2000) and therefore preferentially uses glucose from the glucose - fructose mixture (Nehls et al., 2007). The AmMst1 revealed KM values of 0.46 mM for glucose and 4.2 mM for fructose, that indicates a strong preference for glucose (Wiese et al., 2000). At the plant fungus interface, both the hexose importer genes from plants and from fungi are up regulated to mainly avoid fungal parasitism and not to lose many carbohydrates within a mycorrhizal symbiosis (Nehls, 2008; Nehls et al., 2007). Hyphae from *Amanita muscaria*, a known EM fungus from *Populus* sp., also strongly favoured glucose over fructose uptake, even in the presence of excess fructose (20 mM vs. 1 mM; Nehls et al., (2001)).

The glucose preference was also reported for *Cenococcum geophilum* (Stülten et al., 1995). Our study showed that root associated fungal community depends on root carbohydrate concentration, with a trend to glucose for EM and a trend for fructose to saprophytes. Pena et al., (2010) reported also a significant correlation of EM diversity to the glucose concentration in roots, but in contradiction with our results, the correlation was valid also for the fructose concentration in roots.

Fungal carbohydrate preferences may impact our result, which showed that within the overall model the richness of saprophytic fungi mainly depended on the total carbon concentration of the roots, including the carbohydrates and, as mentioned above, on forest management. Their diversity positively depended on the amount of mainly fructose within the roots and not on glucose, whereas the richness dependet on both carbohydrates. The saprophytic community may profit from general root exudates via rhizodeposition (Jones et al., 2009). The model for fructose and the community structure of all different lifestyles tested (all, EM, saprophytic and unknown fungi) fitted with a very similar GCV score indicating no different influences of fructose to their communities.

Here, the amount of glucose within the fine roots was negatively correlated with the percentage of nitrogen in the roots, but it was positively correlated with the C to N ratio. This fact is supported by the theory, that EM diversity and the energy invested by plants to form mycorrhiza is negatively associated with the amount of available nutrients. More fertile stands show a higher biomass of external hyphae of EM and a low diversity of EM fungal communities (Kalliokoski et al., 2010). Our results show, that the tree stands with a diameter at breast height of 15-30 cm, a middle age class forest, showed the highest glucose concentration and the highest EM richness. Young stands are known to harbour a lower EM diversity than older stands between 26-100 years (Twieg et al., 2007). Additionally it is known that stand age influences EM community structure (Smith et al., 2002). Saprophytic fungi were independent from stand structure.

The negative correlation between N concentration of the fine roots and the amount of glucose supports the hypothesis, that the host plant allocates carbon to its roots to stimulate N uptake. This increase would not be necessary, if N would be readily available for the plant. The so called "*plant-economic theory"* predicts that trees invest less carbohydrates in EM when nutrients like N are easily available (Read, 1991). It was supported by labelling experiments of the soil with ammonium ($^{15}NH_4^+$). Those experiment showed that the ectomycorrhizal roots, which were the strongest sinks for carbohydrates, were also the largest sinks for N (Jones et al., 2009). In contrast to this, Valtanen et al. (2014) showed with labelling of ^{15}N and ^{13}C of beech seedlings, that C and N fluxes were unrelated under long term conditions. But within this study, N was not a limiting factor as they

exceeded the saturation of N uptake of mycorrhizal roots. Under N saturation, plants are not thought to be reliant on favouring N supply. Within a field study on pine, the accumulation of N was temporarily positively correlated with C signatures of EM root tips for one week after labeling, but disappeared after one month (Högberg et al., 2008). Our results are also in accordance to Kobe et al. (2010) who reported an increase in total nonstructural carbohydrates in response to low nitrogen conditions. If carbonflux from the plant to the mycorrhizal fungi is partitioned under drought stress nitrogen in the fungal vacuoles increases (Shi et al., 2002). The fact that the extent of plant N limitation is essential for the strength of plant carbon investment was reported in a meta-analysis from Corrêa et al. (2012). Nehls (2008) discussed a general link between the plant controlled carbon drain towards the fungal partner and the dependence on the fungus-derived mineral nutrition. Jonsson et al. (2000) found in a Norway spruce forest that N availability changes the EM community composition, but on roots the EM were not reduced in richness or diversity. Nevertheless, they found a reduction in EM sporocarps. Pena and Polle (2014) showed that only under stressconditions the plants may benefit from functional diversity within EM assemblages. It is suggested that EM communities can regulate the N supply by diminishing plant-available N, which might increase the carbon flux for their own nutrition (Pena et al., 2013a). Those studies indicate that there are many other functions besides N supply, which EM fungi offer to their hosts. For example protection against heavy metals (Schützendübel and Polle, 2002) or parasites (Chakravarty and Unestam, 1987) and support during drought (Lehto and Zwiazek, 2011). Thus, the relation between N and EM found in the present study is only a part of the various variables influencing this symbiosis.

5. Conclusion

5 Conclusion

The overarching goal of this thesis was to investigate root-associated fungal community structures and functional diversity in relation to ecosystem functions and land-use intensity. With our large scale, high throughput pyrosequencing study on the root-associated fungal community we focused the aim of a characterization of the root-associated fungal community. This community was split to different fungal lifestyles. EM and saprophytic fungi revealed differences between study regions, dominant tree species and different relations on abiotic and biotic environmental variables.

5.1 Functional differences between ectomycorrhizal and saprophytic fungi are related to different environmental drivers

One goal of this study was to determine the relationships of environmental variables to different fungal lifestyles. It was hypothesized, that abiotic and biotic environmental variables influence the richness, diversity and community of EM and saprophytes in different ways, based on their fundamental differences in their lifestyles.

For all results regarding tree species it was difficult to compare spruce to pine and oak as they did not grow within one exploratory. Nevertheless, the large scale of the study allowed us to conclude that the dominant tree species on the plot had more influence on community distribution for EM fungi than for the saprophytic fungal community. Furthermore, the richness of all root-associated and EM fungi depended mainly on root N, whereas the saprophytic fungi were more dependent on the overall root C, especially the orangic C concentration, and some rare elements. Forest management had a negative effect on the diversity of saprophytic fungi and a positive on the diversity and richness of EM fungi. Additionally, we support results which were reported from laboratory experiments, that carbohydrates, mainly glucose, favour EM diversity in temperate forests. Saprophytes might mainly profit from fructose, but also to some extend from glucose, via root exudates.

The present study demonstrated on the large scale the ecological relevance of different environmental variables on different fungal lifestyles on roots. When additionally being supported by their host plant carbohydrates EMs may outcompete saprophytes from nutrient rich substrates (Lindahl et al., 2007). This is not in discrepancy regarding the biotrophy-saprotrophy continuum hypothesis, but indicates the clearly different roles of the two distinct fungal groups in ecosystem function. Even if there is some cellulolytic activity shown for some ectomycorrhizal fungi, their

decomposition rate is too slow to cover the fungal carbohydrate demand (Entry et al., 1991; Haselwandter et al., 1990; Nehls et al., 2007; Trojanowski et al., 1984). Nevertheless, in laboratory microcosms, EM fungi have been found to compete successfully with saprophytes for space and nutrients (discussed in Lindahl et al. (2010)). Thus, even if EMs might have the ability to extraxellular enzyme activities like saprophytes, their functionality within an ecosystem is different. This is supported by a study in a coastal pine forest, that showed that EM and saprophytic fungi have independent roles in the cycling of N-, C- and P-rich molecules (Talbot et al., 2013). Lindahl and Tunlid (2014) review that the saprophytic capacity of EM fungi is to a lesser extent used for C than for N mobilization and that a number of EMs lost most of enzymes acting on cell wall material, which were present in their saprophytic ancestors.

5.2 Dynamic interaction between forest management, root carbohydrate supply and EM diversity

It was hypothesized that there is a link between forest management, root nitrogen supply, carbohydrate concentration in roots and EM richness, diversity or community structure. We found that a combination of origin, which was represented by a combination of dominant tree species and soil properties, root carbohydrate concentration and forest management were the main drivers of the richness, diversity and community structure of ectomycorrhizal fungi. In comparison to that saprophytic fungi were not affected so clearly by differences between tree species. We found that the root glucose concentration is an important variable for EM diversity and community structure. This interacts with the root nitrogen concentration in a negative way, supporting the "*plant economy theory*", that under low N availability plants invest more carbohydrates in their mycorrhizal partner.

Forest management was negatively correlated with root N concentration. Altogether this indicates a dynamic interaction between forest management causing less N in the roots and a higher carbohydrate concentration within the roots and therefore maybe to a higher supply of mycorrhizal partner ending up in a higher EM diversity. More nutrients might be available for a single tree if a neighbouring tree was harvested, but overall with removing biomass from the forest, in managed forests there is a net nutrient output by harvesting (Achat et al., 2015). Scenarios predicting nitrogen enhancement caused by intensive agriculture may negatively influence EM diversity in the long run (Sala et al., 2000). This increasing deposition of N to forests could be counteracted with the removal of nutrients via thinning (Teste et al., 2012). Therefore moderate forest management could be an important point for the protection of EM diversity and therefore also the functionality of the

temperate forest ecosystem (Rineau and Courty, 2011). Nevertheless some dead wood within forests is essential for other organisms, like saprophytic fungi, insects, birds or bats. The amount of decaying wood is higher in un- or low managed forests (Verkerk et al., 2011), which could cause the reduction in the diversity of saprophytic fungi with increasing forest management.

5.3 Simulated disturbance locally affects EM community

We hypothesize that a stimulated disturbance may locally affect EM community. To simulate this disturbance caused by forest management an experiment was investigated, observing the decomposition and recolonization process of root litter in patches free of living roots. On a small scale we demonstrated a high resilience of the EM fungal community. The recolonizing community first differed from undisturbed controls for 15 months. Short distance exploration type was identified to preferably recolonize root free patches during the first year, possibly caused by the release of soluble N from the decomposing roots. Therefore, the influence of forest management on EM fungal community may be due to two drivers: In the short term disturbance via tree harvesting may change EM fungal community. But as we showed a high resilience of the EM community this might only be a short term effect in relation to tree age. On the long term, removal of nutrients due to biomass removal and higher carbohydrate production by higher light availability could be more severe and the consequences on EM fungi remain to be investigated.

5.4 Outlook

First approaches were done to include EM in forest ecosystem C and N cycling models (Deckmyn et al., 2014). This inclusion could enhance our understanding of environmental processes within forest ecosystems. It would be an important approach to investigate soil and root fungal community and their influencing environmental variables within one harvest. Additionally a time series over one or several years could be a helpful approach in understanding the dynamics between soil and root fungal community, carbohydrate supply and the influencing environmental variables. Those seasonal measurements would enhance our understanding especially regarding responses of root-associated fungal communities to climate change. Therefore the resilience of the EM community might need further investigations on the large scale. As one major goal of forest management is to enhance the robustness of forests to environmental and climate change, studying the most important symbiosis partners of trees is indispensable.

6. References

6 References

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7 *Supplementary*

Supplementary: Detailed carbohydrate analysis

For carbohydrate analysis 25 mg freeze dried root material (exact weight was determined) was used for analysis. Carbohydrates were extracted in 1.5 ml DMSO/HCl (dimethylsulfoxide: 25% HCl = 80:20 (v:v)) at 60°C for 30 min after intensive mixing. Samples were cooled on ice and centrifuged for 5 min at 4°C and 5000 rpm (Centrifuge 5417R, Eppendorf, Hamburg). The supernatant was used for determination of carbohydrates.

From the supernatant 200 ml was mixed with 1250 µl of 0.2 M Natriumcitrat-Dihydrat (pH 10.6) buffer. The sample was mixed and centrifuged for 5 min at 4°C and 5000 rpm. From this supernatant 200 µl was used for mixing with 200 µl 50 µM Natriumcitrat-Dihydrat (pH 4.6) buffer. 100 µl from this mixture in each cuvette (Sarstedt, Ref:67.742) were mixed with 400µl bidestilled water and 250 µl of the NADH-ATP-solution: 4mM NADP (NADP-Na_2 from Merck Ref:1245410001), 10mM ATP (Roche, Lot: 93414721), 9mM $MgSO_4$ (Merck Ref: 1058861000), 0.75M Triethanolamin (Merck Ref: 1083790250) at pH 7.6. Three replicates were measured per extract/sample. Before adding the first enzyme from each sample a value for comparing the extinctions afterwards were measured using a Photometer (Beckman Photometer Typ UV-DU 640) at 340 nm (→E1). At this wavelength absorbance of NADPH is measured. Then 10 µl of the enzyme hexokinase (Hexkoinase/Glucose -6-Photphate Dehydrogenase from Roche, Ref: 10737275001; 30 mg/10 ml) was added, which catalyses the conversion of glucose to gluconat-6-phosphate (incubation for 5 min at RT, under darkness). Extinction was measured again at 340 nm (→E2). The amount of NADPH generated in this step was equivalent to the amount of converted glucose. Hexokinase also catalyzes the conversion of fructose to fructose-6-phosphate. In the second step, to determine the content of fructose, 5 µl of the enzyme phosphoglucose isomerase (PGI from Roche Ref: 10128139001; 10mg/ml) was added which

converted fructose-6-phosphate to glucose-6-phosphate, which was further converted to gluconat-6-phosphate (incubation for 15 min at RT, under darkness). Extinction was measured again at 340 nm (→E3). The generated NADPH was measured which was here equivalent to the amount of fructose in the supernatant. In the last step sucrose was determined by adding 10 µl of the enzyme β-fructosidase/invertase (Sigma Lot: 060M1589 using a concentration of 10 mg/ml) which hydrolyses the sucrose to glucose and fructose (incubation for 20 min at 55°C, cuvettes closed with parafilm). Glucose and fructose were converted to gluconat-6-phosphate and the absorption of generated NADPH was measured (→E4).

NADPH was measured as carbohydrate equivalents. For correlations with osmolarity the concentrations of sugars were transformed into mol as follows: sugar concentration [mg g-1 DW] / molecular weight 180.16 [mol g-1].

$$c[mg/ml] = \frac{testvolume\ [ml] * molecular\ weight\ of\ the\ carbohydrate[g/mol]}{(\varepsilon\ NADPH * coat\ thicknes\ [\ cm] * Samplevolume\ [ml]} * \Delta E$$

c [gm/ml] = concentration of the carbohydrate

molecular weight of the carbohydrate = 180.16 [g/mol]

ε NADPH= extinction coefficient of NADPH = 6300 1 / (6300 l/(mmol*cm))

ΔE = difference of Extinctions between measurements

for glucose: E2-E1.

for fructose: E3-E2

for sucrose: (E4-E3)/2

Supplementary Table S1: Overview about used datasets from other working groups

Dataset (Bexis Acession Number)	Data Owner	Metadata Bexis	Publication
6240_Vascular plant diversity in forest EPs 2009_1.6.12	Steffen Boch, Stephanie Socher, Jörg Müller, Dani Patri and Markus Fischer	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=6240	(Boch et al., 2013)
10580_EP_all_exploratories_2.5.6	Jens Nieschulze and Ernst-Detlef Schulze (data given by the local implementation teams and Dani Prati (grasslands), Ingo Schöning (soils), Dominik Hessenmöller (forest))	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=10580	
14446_MinSoil_2011_Mineral_Soil_CN_1.5.1	Ingo Schöning, Emily Solly, Theresa Klötzing and Susan Trumbore	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=14446	(Solly et al., 2013)
14447_MinSoil_2011_Mineral_Soil_pH_1.9.6	Ingo Schöning, Emily Solly, Theresa Klötzing and Susan Trumbore	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=14447	(Solly et al., 2013)
14448_MinSoil_2011_Roots_Biomass_1.1.8	Ingo Schöning, Emily Solly, Theresa Klötzing and Susan Trumbore	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=14448	(Solly et al., 2013)
14566_MinSoil_2011_Organic_Horizons_CN_1.2.7	Ingo Schöning, Emily Solly, Theresa Klötzing and Marion Schrumpf	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=14566	
14686_MinSoil_2011_Mineral_Soil_Texture_1.9.1	Ingo Schöning, Emily Solly, Theresa Klötzing and Susan Trumbore	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=14686	
14866_MinSoil_2011_Mineral_Soil_delta13C_1.4.3	Ingo Schöning and Susan Trumbore	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=14866	(Solly et al., 2013)
15866_MinSoil_2011_Roots_14C_1.1.3	Ingo Schöning, Emily Solly, Theresa Klötzing and Marion Schrumpf	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=15866	(Solly et al., 2013)
15867_MinSoil_2011_Root_decomposition_1.1.1	Ingo Schöning, Emily Solly, Theresa Klötzing and Marion Schrumpf	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=14566	(Solly et al., 2014)
16466_ForMI - Forest Management Intensity Index_1.3.2 von Kahl und Bauhus	Tiemo Kahl and Juergen Bauhus	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=16466	(Kahl and Bauhus, 2014)
17706_Forest EP - new forest type classification_1.2.1	Peter Schall and Christian Ammer	https://www.bexis.uni-jena.de/Data/ShowXml.aspx?DatasetId=17706	(Schall and Ammer, 2013)

Supplementary Table S2: Taxonomical classification and lifestyle annotation of the OTUs resulted from Pyroseqeuncing. OTUs with the same name were grouped in this table (see “according OTU_IDs”. This Supplementary has an own Reference section.

Please see Supplementary Table S2 on attached CD.

Supplementary Table S3: Number of fungal sequences per plot, remaining after quality filtering, trimming, denoising, chimera check and singleton removal.

Alb		Hainich		Schorfheide	
EP_Plotid	Sum seq.	EP_Plotid	Sum Seq	EP_Plotid	Sum Seq
AEW01	6936	**HEW01**	200	**SEW01**	1681
AEW02	4612	**HEW02**	18667	**SEW02**	2354
AEW03	3193	**HEW03**	4914	**SEW03**	4978
AEW04	50	**HEW04**	655	**SEW04**	3437
AEW05	1628	**HEW05**	889	**SEW05**	2800
AEW06	873	**HEW06**	620	**SEW06**	2429
AEW07	685	**HEW07**	4891	**SEW07**	4595
AEW08	2537	**HEW08**	1764	**SEW08**	4121
AEW09	2091	**HEW09**	16539	**SEW09**	286
AEW10	5184	**HEW10**	2307	**SEW10**	3321
AEW11	4659	**HEW11**	214	**SEW11**	3115
AEW12	3240	**HEW12**	2240	**SEW12**	749
AEW13	1205	**HEW13**	1392	**SEW13**	5611
AEW14	2844	**HEW14**	895	**SEW14**	3496
AEW15	299	**HEW15**	548	**SEW15**	7496
AEW16	416	**HEW16**	269	**SEW16**	3342
AEW17	122	**HEW17**	1401	**SEW17**	289
AEW18	92	**HEW18**	314	**SEW18**	1240
AEW19	575	**HEW19**	21	**SEW19**	3949
AEW20	2958	**HEW20**	193	**SEW20**	681
AEW21	69	**HEW21**	773	**SEW21**	1326
AEW22	178	**HEW22**	57	**SEW22**	247
AEW23	501	**HEW23**	1763	**SEW23**	531
AEW24	4	**HEW24**	656	**SEW24**	3511
AEW25	27	**HEW25**	641	**SEW25**	4097
AEW26	184	**HEW26**	1264	**SEW26**	11153
AEW27	494	**HEW27**	210	**SEW27**	54
AEW28	525	**HEW28**	105	**SEW28**	1167
AEW29	734	**HEW29**	5308	**SEW29**	690
AEW30	841	**HEW30**	6280	**SEW30**	2756
AEW31	1955	**HEW31**	88	**SEW31**	4234
AEW32	4973	**HEW32**	1443	**SEW32**	2293
AEW33	2681	**HEW33**	2949	**SEW33**	817
AEW34	2971	**HEW34**	3152	**SEW34**	691
AEW35	143	**HEW35**	268	**SEW35**	1470
AEW36	21	**HEW36**	848	**SEW36**	1182
AEW37	185	**HEW37**	7785	**SEW37**	3243
AEW38	103	**HEW38**	3854	**SEW38**	2380
AEW39	872	**HEW39**	2798	**SEW39**	1638
AEW40	4123	**HEW40**	1116	**SEW40**	1495
AEW41	951	**HEW41**	1295	**SEW41**	3210
AEW42	93	**HEW42**	3269	**SEW42**	8414
AEW43	523	**HEW43**	7	**SEW43**	3713
AEW44	89	**HEW44**	7	**SEW44**	907
AEW45	19	**HEW45**	217	**SEW45**	3327
AEW46	18	**HEW46**	61	**SEW46**	4176
AEW47	1951	**HEW47**	1249	**SEW47**	4915
AEW48	149	**HEW48**	146	**SEW48**	9160
AEW49	537	**HEW49**	1051	**SEW49**	1482
AEW50	1623	**HEW50**	1109	**SEW50**	1110

Supplementary table S 4.1.: Table with the number of sequences, Percentages of sequences and the number of resulting OTUs for the according taxonomy, calculated for each taxonomic level (a) Phylum, b) Class, c) Order, d) Family, e) Genus, f) Species). For this all OTUs/Sequences which were detected were used.

Supplementary table S4.2.: Mean number of sequences and OTUs per plot (based on standardized abundance matrix with 494 sequences per plot) according to different taxonomic levels: a) Phylum, b) Class, c) Order, d) Family, e) Genus, f) Species.

S 4.2.1.: splitted by Exploratory

S 4.2.2.: splitted by dominant tree species per plot

Please see Supplementary Table S4 on attached CD.

Supplementary Table S5: Spearman rank correlation of all variables used for calculations (rho and p-values).For p=0 → p<0.0001

	All MMF		All observed		All Shannon		EM MMF		EM observed		EM Shannon		Myk total MMF		Myk total observed		Myk total Shannon	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
All MMF	1	0	0.9653	0	0.7955	0	0.7562	0	0.7426	0	0.5728	0	0.7821	0	0.7571	0	0.5890	0
All observed	0.9653	0	1	0	0.9127	0	0.7588	0	0.8103	0	0.7105	0	0.7760	0	0.8241	0	0.7264	0
All Shannon	0.7955	0	0.9127	0	1	0	0.6181	0	0.7559	0	0.8331	0	0.6389	0	0.7776	0	0.8502	0
EM MMF	0.7562	0	0.7588	0	0.6181	0	1	0	0.9398	0	0.6843	0	0.9609	0	0.9156	0	0.6797	0
EM observed	0.7426	0	0.8103	0	0.7559	0	0.9398	0	1	0	0.8530	0	0.9183	0	0.9759	0	0.8460	0
EM Shannon	0.5728	0	0.7105	0	0.8331	0	0.6843	0	0.8530	0	1	0	0.6916	0	0.8647	0	0.9926	0
Myk total MMF	0.7821	0	0.7760	0	0.6389	0	0.9609	0	0.9183	0	0.6916	0	1	0	0.9407	0	0.7008	0
Myk total observed	0.7571	0	0.8241	0	0.7776	0	0.9156	0	0.9759	0	0.8647	0	0.9407	0	1	0	0.8735	0
Myk total Shannon	0.5890	0	0.7264	0	0.8502	0	0.6797	0	0.8460	0	0.9926	0	0.7008	0	0.8735	0	1	0
Sapro MMF	0.5308	0	0.4629	0	0.2971	0.0030	0.2806	0.0085	0.2364	0.0275	0.1063	0.3273	0.2609	0.0147	0.2097	0.0513	0.0843	0.4377
Sapro observed	0.5440	0	0.4900	0	0.3363	0.0007	0.3011	0.0046	0.2571	0.0162	0.1234	0.2549	0.2791	0.0089	0.2446	0.0224	0.1113	0.3049
Sapro Shannon	0.4487	0	0.4331	0	0.3314	0.0009	0.2393	0.0256	0.2184	0.0421	0.1140	0.2930	0.2119	0.0488	0.2258	0.0355	0.1143	0.2916
unknown MMF	0.7616	0	0.7005	0	0.4974	0	0.4328	0	0.4432	0	0.3083	0.0042	0.4383	0	0.4197	0.0001	0.3111	0.0038
unknown observed	0.7508	0	0.7064	0	0.5227	0	0.4175	0.0001	0.4440	0	0.3228	0.0026	0.4160	0.0001	0.4158	0.0001	0.3257	0.0024
unknown Shannon	0.6479	0	0.6344	0	0.5033	0	0.3622	0.0007	0.4057	0.0001	0.3168	0.0031	0.3599	0.0007	0.3837	0.0003	0.3178	0.0030
Fine Roots Biomass	0.0226	0.8135	-0.0283	0.7678	-0.1251	0.1909	0.0411	0.6850	0.0200	0.8434	-0.0987	0.3286	0.0008	0.9937	-0.0252	0.8032	-0.1140	0.2588
Coarse Roots Biomass	-0.0552	0.5935	-0.1083	0.2935	-0.1843	0.0723	-0.3015	0.0045	-0.3417	0.0012	-0.3666	0.0005	-0.2829	0.0079	-0.3125	0.0032	-0.3461	0.0010
Organic Horizon total C	0.0330	0.7304	0.0063	0.9475	-0.0241	0.8019	0.0445	0.6595	0.0185	0.8550	0.0177	0.8607	0.0768	0.4470	0.0529	0.6010	0.0367	0.7168
Organic Horizon total N	-0.1510	0.1136	-0.1605	0.0924	-0.1554	0.1034	-0.1073	0.2873	-0.1196	0.2358	-0.1684	0.0941	-0.1732	0.0849	-0.1871	0.0624	-0.1986	0.0476
Organic Horizon CN ratio	0.1320	0.1671	0.1264	0.1862	0.1045	0.2751	0.1099	0.2760	0.1022	0.3115	0.1256	0.2128	0.1748	0.0819	0.1631	0.1050	0.1547	0.1244
Mineral soil Total C	0.2997	0.0015	0.3043	0.0012	0.2535	0.0073	0.3302	0.0008	0.3114	0.0016	0.2401	0.0163	0.2822	0.0046	0.2905	0.0034	0.2171	0.0300
Mineral soil Inorganic C	0.2592	0.0060	0.2485	0.0085	0.1985	0.0367	0.2275	0.0229	0.2125	0.0338	0.1401	0.1646	0.1922	0.0554	0.1950	0.0519	0.1300	0.1974
Mineral soil Organic C	0.2990	0.0014	0.3038	0.0012	0.2532	0.0073	0.3292	0.0008	0.3104	0.0017	0.2394	0.0164	0.2812	0.0046	0.2895	0.0035	0.2165	0.0305
Mineral soil Total N	0.2912	0.0019	0.2854	0.0024	0.2186	0.0212	0.3111	0.0016	0.2837	0.0042	0.1821	0.0697	0.2547	0.0106	0.2521	0.0114	0.1592	0.1136
Mineral Soil CN ratio	-0.2216	0.0194	-0.1749	0.0663	-0.0645	0.5011	-0.1176	0.2439	-0.0599	0.5536	0.0775	0.4433	-0.0577	0.5684	-0.0166	0.8697	0.0940	0.3523
Mineral soil pH 1	0.3092	0.0010	0.3016	0.0013	0.2387	0.0116	0.2177	0.0295	0.1979	0.0484	0.0996	0.3240	0.1740	0.0833	0.1735	0.0842	0.0978	0.3330
Mineral Soil Texture Clay	0.2818	0.0027	0.2767	0.0033	0.2224	0.0190	0.2917	0.0032	0.2636	0.0081	0.1549	0.1238	0.2265	0.0235	0.2205	0.0275	0.1310	0.1939

	All MMF		All observed		All Shannon		EM MMF		EM observed		EM Shannon		Myk total MMF		Myk total observed		Myk total Shannon	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Mineral Soil Texture Fine Silt	0.2939	0.0017	0.2481	0.0086	0.1319	0.1677	0.2197	0.0281	0.1671	0.0966	0.0490	0.6281	0.1513	0.1330	0.1343	0.1827	0.0328	0.7460
Mineral Soil Texture Medium Silt	0.2289	0.0157	0.1648	0.0839	0.0418	0.6633	0.1067	0.2908	0.0421	0.6776	-0.0839	0.4068	0.0716	0.4791	0.0235	0.8165	-0.0921	0.3621
Mineral Soil Texture Coarse Silt	0.1398	0.1434	0.0617	0.5198	-0.0762	0.4270	0.0457	0.6519	-0.0363	0.7202	-0.1801	0.0729	-0.0036	0.9715	-0.0678	0.5024	-0.1955	0.0512
Mineral Soil Texture Fine Sand	-0.3608	0.0001	-0.3429	0.0002	-0.2597	0.0059	-0.3229	0.0011	-0.3040	0.0021	-0.1649	0.1010	-0.2873	0.0038	-0.2784	0.0050	-0.1577	0.1172
Mineral Soil Texture Medium Sand	-0.1865	0.0501	-0.1099	0.2507	0.0220	0.8185	-0.0524	0.6047	0.0328	0.7461	0.1715	0.0880	-0.0040	0.9684	0.0527	0.6023	0.1777	0.0770
Mineral Soil Texture Coarse Sand	-0.1986	0.0366	-0.1425	0.1356	-0.0324	0.7358	-0.1291	0.2006	-0.0661	0.5137	0.0589	0.5604	-0.0817	0.4193	-0.0430	0.6713	0.0709	0.4835
Root C concentration	-0.3296	0.0005	-0.2950	0.0018	-0.2240	0.0186	-0.1620	0.1091	-0.1370	0.1762	-0.0533	0.5997	-0.1757	0.0819	-0.1483	0.1428	-0.0656	0.5191
Root N concentration	-0.3718	0.0001	-0.4148	0	-0.4383	0	-0.2713	0.0068	-0.3161	0.0014	-0.3117	0.0018	-0.2967	0.0030	-0.3587	0.0003	-0.3371	0.0006
Root CN ratio	0.2473	0.0094	0.3051	0.0012	0.3651	0.0001	0.2261	0.0246	0.2865	0.0040	0.3263	0.0010	0.2477	0.0136	0.3281	0.0009	0.3513	0.0004
SMId	0.1004	0.2946	0.1485	0.1200	0.1652	0.0832	0.1804	0.0725	0.1692	0.0923	0.1127	0.2643	0.1804	0.0725	0.1711	0.0887	0.0965	0.3397
SMIr	0.0782	0.4144	0.1234	0.1969	0.1862	0.0504	0.1985	0.0477	0.2354	0.0184	0.2530	0.0111	0.2164	0.0306	0.2611	0.0087	0.2521	0.0114
SMI	0.1733	0.0690	0.2260	0.0171	0.2606	0.0057	0.2911	0.0033	0.3029	0.0022	0.2671	0.0072	0.2852	0.0040	0.3125	0.0015	0.2550	0.0105
ForMI	0.1092	0.2584	0.1448	0.1329	0.1950	0.0422	0.1485	0.1444	0.1686	0.0971	0.1668	0.1008	0.1758	0.0833	0.1952	0.0541	0.1757	0.0836
Iharv	0.1189	0.2182	0.1268	0.1889	0.0873	0.3667	0.2093	0.0386	0.1874	0.0646	0.0413	0.6866	0.2031	0.0449	0.1675	0.0993	0.0231	0.8213
Inonat	0.1315	0.1730	0.1952	0.0420	0.2920	0.0021	0.1843	0.0692	0.2420	0.0164	0.3288	0.0009	0.2252	0.0258	0.2864	0.0043	0.3386	0.0006
Idwcut	0.0422	0.6632	0.0696	0.4721	0.1124	0.2446	0.0362	0.7231	0.0473	0.6439	0.0530	0.6044	0.0447	0.6623	0.0542	0.5960	0.0610	0.5508
Number of tree species per plot	0.2005	0.0349	0.2043	0.0315	0.1838	0.0534	0.1676	0.0956	0.1626	0.1061	0.1699	0.0911	0.1975	0.0489	0.2053	0.0404	0.1928	0.0546
Root Al concentration	0.3878	0	0.3470	0.0003	0.2493	0.0100	0.2882	0.0045	0.2458	0.0158	0.1067	0.3001	0.2787	0.0061	0.2475	0.0150	0.1043	0.3121
Root Ca concentration	0.3238	0.0008	0.2978	0.0019	0.2236	0.0212	0.2709	0.0078	0.2457	0.0158	0.1514	0.1408	0.2332	0.0224	0.2261	0.0268	0.1339	0.1935
Root Fe concentration	0.3084	0.0014	0.2926	0.0023	0.2486	0.0102	0.2364	0.0206	0.2462	0.0156	0.1952	0.0567	0.2937	0.0038	0.2869	0.0046	0.2108	0.0393
Root K concentration	0.0635	0.5171	0.0033	0.9729	-0.0936	0.3397	-0.1960	0.0557	-0.2289	0.0249	-0.2696	0.0081	-0.1999	0.0511	-0.2391	0.0190	-0.2747	0.0068
Root Mg concentration	0.3686	0.0001	0.3344	0.0005	0.2298	0.0178	0.2079	0.0423	0.1967	0.0548	0.0763	0.4596	0.1744	0.0892	0.1773	0.0840	0.0624	0.5457
Root Mn concentration	0.1638	0.0933	0.1829	0.0606	0.1811	0.0632	0.2219	0.0300	0.2241	0.0282	0.1896	0.0644	0.2641	0.0095	0.2595	0.0107	0.1952	0.0566
Root Na concentration	0.3001	0.0018	0.2979	0.0019	0.2563	0.0080	0.0751	0.4663	0.0409	0.6924	-0.0254	0.8058	0.0531	0.6071	0.0434	0.6749	-0.0178	0.8632
Root P concentration	-0.2342	0.0159	-0.2056	0.0345	-0.1365	0.1628	-0.1232	0.2312	-0.0951	0.3564	0.0081	0.9377	-0.1188	0.2487	-0.0945	0.3598	0.0077	0.9403
Root S concentration	-0.1176	0.2295	-0.1350	0.1676	-0.1332	0.1733	-0.2684	0.0084	-0.2914	0.0040	-0.2479	0.0151	-0.2079	0.0423	-0.2673	0.0085	-0.2452	0.0160
Root glucose concentration	0.0819	0.3990	0.1500	0.1211	0.2435	0.0111	0.2416	0.0173	0.3162	0.0016	0.3944	0.0001	0.2657	0.0087	0.3422	0.0006	0.3836	0.0001
Root fructose concentration	0.1553	0.1086	0.1529	0.1142	0.1555	0.1081	0.1300	0.2042	0.1722	0.0917	0.1927	0.0586	0.1301	0.2041	0.1737	0.0888	0.1899	0.0625

	Sapro MMF		Sapro observed		Sapro Shannon		unknown MMF		unknown observed		unknown Shannon	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
All MMF	0.5308	0	0.5440	0	0.4487	0	0.7616	0	0.7508	0	0.6479	0
All observed	0.4629	0	0.4900	0	0.4331	0	0.7005	0	0.7064	0	0.6344	0
All Shannon	0.2971	0.0030	0.3363	0.0007	0.3314	0.0009	0.4974	0	0.5227	0	0.5033	0
EM MMF	0.2806	0.0085	0.3011	0.0046	0.2393	0.0256	0.4328	0	0.4175	0.0001	0.3622	0.0007
EM observed	0.2364	0.0275	0.2571	0.0162	0.2184	0.0421	0.4432	0	0.4440	0	0.4057	0.0001
EM Shannon	0.1063	0.3273	0.1234	0.2549	0.1140	0.2930	0.3083	0.0042	0.3228	0.0026	0.3168	0.0031
Myk total MMF	0.2609	0.0147	0.2791	0.0089	0.2119	0.0488	0.4383	0	0.4160	0.0001	0.3599	0.0007
Myk total observed	0.2097	0.0513	0.2446	0.0224	0.2258	0.0355	0.4197	0.0001	0.4158	0.0001	0.3837	0.0003
Myk total Shannon	0.0843	0.4377	0.1113	0.3049	0.1143	0.2916	0.3111	0.0038	0.3257	0.0024	0.3178	0.0030
Sapro MMF	1	0	0.9379	0	0.7461	0	0.4005	0.0001	0.3942	0.0002	0.3363	0.0016
Sapro observed	0.9379	0	1	0	0.9144	0	0.4382	0	0.4360	0	0.3865	0.0003
Sapro Shannon	0.7461	0	0.9144	0	1	0	0.3533	0.0009	0.3566	0.0008	0.3271	0.0022
unknown MMF	0.4005	0.0001	0.4382	0	0.3533	0.0009	1	0	0.9589	0	0.8132	0
unknown observed	0.3942	0.0002	0.4360	0	0.3566	0.0008	0.9589	0	1	0	0.9345	0
unknown Shannon	0.3363	0.0016	0.3865	0.0003	0.3271	0.0022	0.8132	0	0.9345	0	1	0
Fine Roots Biomass	0.0458	0.6512	0.0203	0.8409	0.0450	0.6567	-0.0073	0.9425	0.0044	0.9651	0.0094	0.9260
Coarse Roots Biomass	0.1566	0.1475	0.1171	0.2801	0.1295	0.2318	-0.1759	0.1012	-0.1424	0.1858	-0.1459	0.1751
Organic Horizon total C	-0.1477	0.1426	-0.1259	0.2118	-0.1400	0.1648	0.0410	0.6851	-0.0156	0.8777	-0.0825	0.4143
Organic Horizon total N	-0.0380	0.7072	-0.0153	0.8800	0.0302	0.7656	-0.0921	0.3613	-0.0607	0.5484	0.0058	0.9540
Organic Horizon CN ratio	-0.0333	0.7426	-0.0514	0.6113	-0.1049	0.2989	0.0897	0.3743	0.0395	0.6963	-0.0493	0.6263
Mineral soil Total C	0.1196	0.2358	0.1253	0.2143	0.1427	0.1566	0.2478	0.0131	0.2520	0.0114	0.2480	0.0128
Mineral soil Inorganic C	0.0940	0.3522	0.1025	0.3100	0.1288	0.2014	0.2710	0.0064	0.2684	0.0069	0.2419	0.0153
Mineral soil Organic C	0.1191	0.2379	0.1246	0.2167	0.1423	0.1578	0.2545	0.0108	0.2577	0.0096	0.2528	0.0112
Mineral soil Total N	0.1666	0.0976	0.1867	0.0629	0.2170	0.0301	0.2586	0.0094	0.2677	0.0071	0.2665	0.0074
Mineral Soil CN ratio	-0.3040	0.0021	-0.3518	0.0003	-0.3982	0	-0.1921	0.0555	-0.2127	0.0336	-0.2124	0.0339
Mineral soil pH 1	0.1675	0.0958	0.2006	0.0453	0.2512	0.0117	0.3235	0.0010	0.3359	0.0006	0.3141	0.0015
Mineral Soil Texture Clay	0.2112	0.0349	0.2304	0.0211	0.2602	0.0089	0.3028	0.0022	0.3114	0.0016	0.3066	0.0019
Mineral Soil Texture Fine Silt	0.3315	0.0008	0.3572	0.0003	0.3689	0.0002	0.2839	0.0042	0.2790	0.0049	0.2746	0.0057

	Sapro MMF		Sapro observed		Sapro Shannon		unknown MMF		unknown observed		unknown Shannon	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Mineral Soil Texture Medium Silt	0.3139	0.0015	0.3683	0.0002	0.3716	0.0001	0.1642	0.1025	0.1927	0.0548	0.2280	0.0225
Mineral Soil Texture Coarse Silt	0.2717	0.0062	0.3194	0.0012	0.3400	0.0005	0.1342	0.1831	0.1434	0.1546	0.1614	0.1087
Mineral Soil Texture Fine Sand	-0.2547	0.0105	-0.2894	0.0035	-0.2944	0.0029	-0.3445	0.0004	-0.3587	0.0002	-0.3710	0.0001
Mineral Soil Texture Medium Sand	-0.2873	0.0038	-0.3253	0.0010	-0.3487	0.0004	-0.1392	0.1673	-0.1670	0.0968	-0.1873	0.0621
Mineral Soil Texture Coarse Sand	-0.2072	0.0386	-0.2328	0.0198	-0.2624	0.0084	-0.1561	0.1209	-0.1679	0.0950	-0.1691	0.0925
Root C concentration	-0.2161	0.0308	-0.2485	0.0127	-0.2397	0.0163	-0.2897	0.0037	-0.2587	0.0097	-0.1991	0.0482
Root N concentration	-0.0561	0.5796	-0.1604	0.1108	-0.2203	0.0276	-0.3780	0.0001	-0.3450	0.0005	-0.2972	0.0028
Root CN ratio	-0.0534	0.5978	0.0575	0.5698	0.1381	0.1705	0.2605	0.0094	0.2400	0.0167	0.2155	0.0322
SMId	0.1143	0.2574	0.0759	0.4528	0.0300	0.7667	-0.0099	0.9222	0.0409	0.6860	0.1296	0.1986
SMIr	-0.2198	0.0280	-0.2356	0.0183	-0.2762	0.0054	0.1661	0.0987	0.1346	0.1817	0.1211	0.2300
SMI	-0.0082	0.9355	-0.0501	0.6208	-0.1166	0.2482	0.1463	0.1465	0.1698	0.0912	0.2257	0.0240
ForMI	-0.1902	0.0607	-0.2309	0.0222	-0.2950	0.0032	0.1308	0.1992	0.1359	0.1821	0.1627	0.1094
Iharv	0.0212	0.8356	0.0010	0.9922	-0.0216	0.8328	0.0911	0.3723	0.0866	0.3967	0.1269	0.2132
Inonat	-0.1383	0.1744	-0.1757	0.0836	-0.2275	0.0243	0.1457	0.1523	0.1257	0.2173	0.1067	0.2959
Idwcut	-0.1467	0.1495	-0.1612	0.1127	-0.2048	0.0430	0.0376	0.7132	0.0653	0.5229	0.1119	0.2727
Number of tree species per plot	0.0285	0.7780	0.0109	0.9139	-0.0203	0.8409	0.3415	0.0005	0.3394	0.0006	0.3165	0.0013
Root Al concentration	0.2121	0.0370	0.2583	0.0106	0.2881	0.0042	0.3328	0.0010	0.3183	0.0017	0.2705	0.0080
Root Ca concentration	0.1437	0.1603	0.1495	0.1439	0.1790	0.0794	0.3233	0.0015	0.3210	0.0015	0.2871	0.0048
Root Fe concentration	0.0519	0.6135	0.0888	0.3873	0.0984	0.3375	0.2451	0.0169	0.1904	0.0646	0.1106	0.2858
Root K concentration	0.2226	0.0284	0.1977	0.0522	0.2065	0.0424	0.0656	0.5273	0.0523	0.6150	0.0023	0.9821
Root Mg concentration	0.2639	0.0090	0.2949	0.0034	0.3288	0.0010	0.3726	0.0002	0.3495	0.0005	0.2934	0.0039
Root Mn concentration	0.0197	0.8479	0.0340	0.7410	0.0498	0.6278	0.0775	0.4548	0.0693	0.5048	0.0796	0.4429
Root Na concentration	0.1989	0.0508	0.3005	0.0028	0.3994	0.0001	0.1239	0.2313	0.1011	0.3294	0.0599	0.5643
Root P concentration	-0.1233	0.2288	-0.2011	0.0482	-0.2344	0.0208	-0.2479	0.0156	-0.2375	0.0205	-0.1962	0.0568
Root S concentration	-0.1331	0.1938	-0.1807	0.0765	-0.1663	0.1035	-0.1799	0.0810	-0.1525	0.1402	-0.1318	0.2029
Root glucose concentration	0.0331	0.7453	0.0419	0.6802	0.0174	0.8646	0.0922	0.3684	0.0427	0.6778	0.0566	0.5816
Root fructose concentration	0.2087	0.0381	0.2687	0.0072	0.2485	0.0131	0.1570	0.1247	0.1583	0.1214	0.1783	0.0805

	Fine Roots Biomass		Coarse Roots Biomass		Organic Horizon total C		Organic Horizon total N		Organic Horizon CN ratio		Mineral soil Total C		Mineral soil Inorganic C	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
All MMF	0.0226	0.8135	-0.0552	0.5935	0.0330	0.7304	-0.1510	0.1136	0.1320	0.1671	0.2997	0.0015	0.2592	0.0060
All observed	-0.0283	0.7678	-0.1083	0.2935	0.0063	0.9475	-0.1605	0.0924	0.1264	0.1862	0.3043	0.0012	0.2485	0.0085
All Shannon	-0.1251	0.1909	-0.1843	0.0723	-0.0241	0.8019	-0.1554	0.1034	0.1045	0.2751	0.2535	0.0073	0.1985	0.0367
EM MMF	0.0411	0.6850	-0.3015	0.0045	0.0445	0.6595	-0.1073	0.2873	0.1099	0.2760	0.3302	0.0008	0.2275	0.0229
EM observed	0.0200	0.8434	-0.3417	0.0012	0.0185	0.8550	-0.1196	0.2358	0.1022	0.3115	0.3114	0.0016	0.2125	0.0338
EM Shannon	-0.0987	0.3286	-0.3666	0.0005	0.0177	0.8607	-0.1684	0.0941	0.1256	0.2128	0.2401	0.0163	0.1401	0.1646
Myk total MMF	0.0008	0.9937	-0.2829	0.0079	0.0768	0.4470	-0.1732	0.0849	0.1748	0.0819	0.2822	0.0046	0.1922	0.0554
Myk total observed	-0.0252	0.8032	-0.3125	0.0032	0.0529	0.6010	-0.1871	0.0624	0.1631	0.1050	0.2905	0.0034	0.1950	0.0519
Myk total Shannon	-0.1140	0.2588	-0.3461	0.0010	0.0367	0.7168	-0.1986	0.0476	0.1547	0.1244	0.2171	0.0300	0.1300	0.1974
Sapro MMF	0.0458	0.6512	0.1566	0.1475	-0.1477	0.1426	-0.0380	0.7072	-0.0333	0.7426	0.1196	0.2358	0.0940	0.3522
Sapro observed	0.0203	0.8409	0.1171	0.2801	-0.1259	0.2118	-0.0153	0.8800	-0.0514	0.6113	0.1253	0.2143	0.1025	0.3100
Sapro Shannon	0.0450	0.6567	0.1295	0.2318	-0.1400	0.1648	0.0302	0.7656	-0.1049	0.2989	0.1427	0.1566	0.1288	0.2014
unknown MMF	-0.0073	0.9425	-0.1759	0.1012	0.0410	0.6851	-0.0921	0.3613	0.0897	0.3743	0.2478	0.0131	0.2710	0.0064
unknown observed	0.0044	0.9651	-0.1424	0.1858	-0.0156	0.8777	-0.0607	0.5484	0.0395	0.6963	0.2520	0.0114	0.2684	0.0069
unknown Shannon	0.0094	0.9260	-0.1459	0.1751	-0.0825	0.4143	0.0058	0.9540	-0.0493	0.6263	0.2480	0.0128	0.2419	0.0153
Fine Roots Biomass	1	0	0.3010	0.0006	-0.0372	0.6511	-0.0406	0.6216	0.1132	0.1678	0.0702	0.3931	0.1880	0.0212
Coarse Roots Biomass	0.3010	0.0006	1	0	0.0982	0.2719	-0.0706	0.4303	0.1220	0.1718	-0.1159	0.1943	0.0180	0.8407
Organic Horizon total C	-0.0372	0.6511	0.0982	0.2719	1	0	0.0036	0.9653	0.2982	0.0002	-0.1174	0.1525	-0.1403	0.0869
Organic Horizon total N	-0.0406	0.6216	-0.0706	0.4303	0.0036	0.9653	1	0	-0.9034	0	0.0843	0.3050	0.0197	0.8105
Organic Horizon CN ratio	0.1132	0.1678	0.1220	0.1718	0.2982	0.0002	-0.9034	0	1	0	-0.1065	0.1948	-0.0380	0.6444
Mineral soil Total C	0.0702	0.3931	-0.1159	0.1943	-0.1174	0.1525	0.0843	0.3050	-0.1065	0.1948	1	0	0.8565	0
Mineral soil Inorganic C	0.1880	0.0212	0.0180	0.8407	-0.1403	0.0869	0.0197	0.8105	-0.0380	0.6444	0.8565	0	1	0
Mineral soil Organic C	0.0646	0.4322	-0.1223	0.1707	-0.1220	0.1369	0.0882	0.2833	-0.1114	0.1748	0.9990	0	0.8505	0
Mineral soil Total N	0.1009	0.2195	-0.1124	0.2082	-0.1617	0.0481	0.1280	0.1186	-0.1608	0.0494	0.9802	0	0.8610	0
Mineral Soil CN ratio	-0.2224	0.0062	-0.1481	0.0965	0.2360	0.0036	-0.1828	0.0251	0.2261	0.0054	-0.6532	0	-0.6780	0
Mineral soil pH 1	0.2590	0.0014	0.0300	0.7376	-0.2177	0.0074	0.0070	0.9325	-0.0453	0.5818	0.7969	0	0.8965	0
Mineral Soil Texture Clay	0.0929	0.2584	-0.1343	0.1321	-0.2091	0.0102	0.2607	0.0013	-0.2948	0.0003	0.8798	0	0.7929	0
Mineral Soil Texture Fine Silt	0.1913	0.0190	0.2179	0.0139	-0.2685	0.0009	0.0955	0.2452	-0.1613	0.0486	0.6114	0	0.6677	0
Mineral Soil Texture Medium Silt	0.2553	0.0016	0.3256	0.0002	-0.1852	0.0233	0.1252	0.1269	-0.1566	0.0557	0.3979	0	0.4721	0

	Fine Roots Biomass		Coarse Roots Biomass		Organic Horizon total C		Organic Horizon total N		Organic Horizon CN ratio		Mineral soil Total C		Mineral soil Inorganic C	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Mineral Soil Texture Coarse Silt	0.3188	0.0001	0.3228	0.0002	-0.2206	0.0067	0.1794	0.0280	-0.1932	0.0178	0.3383	0	0.3948	0
Mineral Soil Texture Fine Sand	-0.0608	0.4595	0.0387	0.6656	0.1812	0.0265	-0.1590	0.0519	0.1913	0.0190	-0.7583	0	-0.7034	0
Mineral Soil Texture Medium Sand	-0.2839	0.0004	-0.3661	0	0.2090	0.0103	-0.1587	0.0524	0.1782	0.0291	-0.4378	0	-0.5035	0
Mineral Soil Texture Coarse Sand	-0.2428	0.0028	-0.2664	0.0025	0.1858	0.0228	-0.1239	0.1308	0.1452	0.0763	-0.5071	0	-0.5471	0
Root C concentration	-0.1625	0.0478	-0.0355	0.6930	0.1776	0.0304	0.0957	0.2451	-0.0555	0.5013	-0.4269	0	-0.4212	0
Root N concentration	-0.1119	0.1742	0.1325	0.1392	-0.0226	0.7843	0.1889	0.0212	-0.1751	0.0328	-0.2222	0.0065	-0.2171	0.0078
Root CN ratio	0.0504	0.5414	-0.1375	0.1246	0.1020	0.2157	-0.1738	0.0342	0.1725	0.0355	0.0531	0.5197	0.0605	0.4639
SMId	0.1060	0.1965	-0.0702	0.4327	-0.0587	0.4754	-0.0104	0.8996	-0.0273	0.7399	0.1546	0.0588	0.1630	0.0462
SMIr	-0.1575	0.0543	-0.1851	0.0372	0.1192	0.1464	-0.2127	0.0090	0.1780	0.0293	0.0489	0.5527	-0.0149	0.8567
SMI	-0.0409	0.6195	-0.1208	0.1762	-0.0411	0.6174	-0.0875	0.2868	0.0241	0.7699	0.2792	0.0005	0.2176	0.0075
ForMI	-0.1365	0.0981	-0.1899	0.0339	0.0692	0.4033	-0.0903	0.2749	0.0346	0.6761	0.2103	0.0103	0.1434	0.0822
Iharv	0.1316	0.1109	-0.1504	0.0942	-0.1261	0.1266	0.1158	0.1611	-0.1782	0.0303	0.4051	0	0.3772	0
Inonat	-0.3815	0	-0.2973	0.0008	0.1602	0.0517	-0.1685	0.0407	0.1479	0.0728	0.0932	0.2598	-0.0290	0.7263
Idwcut	-0.0525	0.5259	-0.1616	0.0718	-0.0332	0.6884	-0.0368	0.6567	-0.0380	0.6463	0.0628	0.4483	0.0725	0.3810
Number of tree species per plot	-0.0870	0.2897	0.0228	0.7990	-0.0282	0.7323	-0.0211	0.7981	-0.0383	0.6418	0.3265	0	0.2727	0.0007
Root Al concentration	0.1189	0.1558	-0.0569	0.5355	-0.0996	0.2348	0.1224	0.1436	-0.1142	0.1727	0.7728	0	0.7090	0
Root Ca concentration	0.1797	0.0312	-0.1103	0.2283	-0.2518	0.0024	0.0246	0.7699	-0.0868	0.3006	0.8641	0	0.8717	0
Root Fe concentration	-0.0346	0.6809	-0.0967	0.2916	0.0085	0.9193	-0.0719	0.3911	0.1019	0.2237	0.3968	0	0.3671	0
Root K concentration	0.2780	0.0007	0.4084	0	-0.2143	0.0100	0.0402	0.6318	-0.0841	0.3161	0.2205	0.0079	0.3788	0
Root Mg concentration	0.2768	0.0008	0.1628	0.0744	-0.1971	0.0180	0.0514	0.5404	-0.0961	0.2515	0.6927	0	0.7508	0
Root Mn concentration	0.0101	0.9045	0.0097	0.9157	-0.1751	0.0359	0.1500	0.0728	-0.1753	0.0357	-0.0499	0.5525	-0.0812	0.3333
Root Na concentration	0.1289	0.1235	0.1704	0.0616	-0.0808	0.3353	-0.0449	0.5926	0.0091	0.9138	0.3089	0.0002	0.3177	0.0001
Root P concentration	-0.3182	0.0001	-0.0359	0.6956	-0.0074	0.9294	-0.0482	0.5655	-0.0226	0.7878	-0.2754	0.0008	-0.2947	0.0003
Root S concentration	0.1745	0.0365	0.2984	0.0009	-0.0469	0.5764	-0.0305	0.7166	0.0292	0.7282	-0.0641	0.4451	0.0285	0.7341
Root glucose concentration	-0.2631	0.0013	-0.2349	0.0086	-0.0268	0.7474	-0.0727	0.3810	0.0156	0.8508	0.0199	0.8108	-0.0637	0.4434
Root fructose concentration	-0.2036	0.0134	-0.0190	0.8341	0.0566	0.4962	0.0727	0.3815	-0.0673	0.4180	-0.1918	0.0200	-0.2300	0.0051

	Mineral soil Organic C		Mineral soil Total N		Mineral Soil CN ratio		Mineral soil pH 1		Mineral Soil Texture Clay		Mineral Soil Texture Fine Silt		Mineral Soil Texture Medium Silt	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
All MMF	0.2990	0.0014	0.2912	0.0019	-0.2216	0.0194	0.3092	0.0010	0.2818	0.0027	0.2939	0.0017	0.2289	0.0157
All observed	0.3038	0.0012	0.2854	0.0024	-0.1749	0.0663	0.3016	0.0013	0.2767	0.0033	0.2481	0.0086	0.1648	0.0839
All Shannon	0.2532	0.0073	0.2186	0.0212	-0.0645	0.5011	0.2387	0.0116	0.2224	0.0190	0.1319	0.1677	0.0418	0.6633
EM MMF	0.3292	0.0008	0.3111	0.0016	-0.1176	0.2439	0.2177	0.0295	0.2917	0.0032	0.2197	0.0281	0.1067	0.2908
EM observed	0.3104	0.0017	0.2837	0.0042	-0.0599	0.5536	0.1979	0.0484	0.2636	0.0081	0.1671	0.0966	0.0421	0.6776
EM Shannon	0.2394	0.0164	0.1821	0.0697	0.0775	0.4433	0.0996	0.3240	0.1549	0.1238	0.0490	0.6281	-0.0839	0.4068
Myk total MMF	0.2812	0.0046	0.2547	0.0106	-0.0577	0.5684	0.1740	0.0833	0.2265	0.0235	0.1513	0.1330	0.0716	0.4791
Myk total observed	0.2895	0.0035	0.2521	0.0114	-0.0166	0.8697	0.1735	0.0842	0.2205	0.0275	0.1343	0.1827	0.0235	0.8165
Myk total Shannon	0.2165	0.0305	0.1592	0.1136	0.0940	0.3523	0.0978	0.3330	0.1310	0.1939	0.0328	0.7460	-0.0921	0.3621
Sapro MMF	0.1191	0.2379	0.1666	0.0976	-0.3040	0.0021	0.1675	0.0958	0.2112	0.0349	0.3315	0.0008	0.3139	0.0015
Sapro observed	0.1246	0.2167	0.1867	0.0629	-0.3518	0.0003	0.2006	0.0453	0.2304	0.0211	0.3572	0.0003	0.3683	0.0002
Sapro Shannon	0.1423	0.1578	0.2170	0.0301	-0.3982	0	0.2512	0.0117	0.2602	0.0089	0.3689	0.0002	0.3716	0.0001
unknown MMF	0.2545	0.0108	0.2586	0.0094	-0.1921	0.0555	0.3235	0.0010	0.3028	0.0022	0.2839	0.0042	0.1642	0.1025
unknown observed	0.2577	0.0096	0.2677	0.0071	-0.2127	0.0336	0.3359	0.0006	0.3114	0.0016	0.2790	0.0049	0.1927	0.0548
unknown Shannon	0.2528	0.0112	0.2665	0.0074	-0.2124	0.0339	0.3141	0.0015	0.3066	0.0019	0.2746	0.0057	0.2280	0.0225
Fine Roots Biomass	0.0646	0.4322	0.1009	0.2195	-0.2224	0.0062	0.2590	0.0014	0.0929	0.2584	0.1913	0.0190	0.2553	0.0016
Coarse Roots Biomass	-0.1223	0.1707	-0.1124	0.2082	-0.1481	0.0965	0.0300	0.7376	-0.1343	0.1321	0.2179	0.0139	0.3256	0.0002
Organic Horizon total C	-0.1220	0.1369	-0.1617	0.0481	0.2360	0.0036	-0.2177	0.0074	-0.2091	0.0102	-0.2685	0.0009	-0.1852	0.0233
Organic Horizon total N	0.0882	0.2833	0.1280	0.1186	-0.1828	0.0251	0.0070	0.9325	0.2607	0.0013	0.0955	0.2452	0.1252	0.1269
Organic Horizon CN ratio	-0.1114	0.1748	-0.1608	0.0494	0.2261	0.0054	-0.0453	0.5818	-0.2948	0.0003	-0.1613	0.0486	-0.1566	0.0557
Mineral soil Total C	0.9990	0	0.9802	0	-0.6532	0	0.7969	0	0.8798	0	0.6114	0	0.3979	0
Mineral soil Inorganic C	0.8505	0	0.8610	0	-0.6780	0	0.8965	0	0.7929	0	0.6677	0	0.4721	0
Mineral soil Organic C	1	0	0.9805	0	-0.6519	0	0.7913	0	0.8863	0	0.6166	0	0.3913	0
Mineral soil Total N	0.9805	0	1	0	-0.7476	0	0.8336	0	0.9114	0	0.6591	0	0.4535	0
Mineral Soil CN ratio	-0.6519	0	-0.7476	0	1	0	-0.7201	0	-0.7045	0	-0.7309	0	-0.6448	0
Mineral soil pH 1	0.7913	0	0.8336	0	-0.7201	0	1	0	0.7948	0	0.6911	0	0.5478	0
Mineral Soil Texture Clay	0.8863	0	0.9114	0	-0.7045	0	0.7948	0	1	0	0.6692	0	0.4014	0
Mineral Soil Texture Fine Silt	0.6166	0	0.6591	0	-0.7309	0	0.6911	0	0.6692	0	1	0	0.7171	0

	Mineral soil Organic C		Mineral soil Total N		Mineral Soil CN ratio		Mineral soil pH 1		Mineral Soil Texture Clay		Mineral Soil Texture Fine Silt		Mineral Soil Texture Medium Silt	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Mineral Soil Texture Medium Silt	0.3913	0	0.4535	0	-0.6448	0	0.5478	0	0.4014	0	0.7171	0	1	0
Mineral Soil Texture Coarse Silt	0.3349	0	0.4047	0	-0.6376	0	0.4939	0	0.3913	0	0.6565	0	0.9130	0
Mineral Soil Texture Fine Sand	-0.7609	0	-0.7782	0	0.6482	0	-0.7242	0	-0.8001	0	-0.7329	0	-0.5860	0
Mineral Soil Texture Medium Sand	-0.4362	0	-0.5044	0	0.7021	0	-0.5566	0	-0.5240	0	-0.7325	0	-0.8436	0
Mineral Soil Texture Coarse Sand	-0.5067	0	-0.5598	0	0.6671	0	-0.5750	0	-0.5715	0	-0.6872	0	-0.7624	0
Root C concentration	-0.4271	0	-0.4775	0	0.5107	0	-0.5232	0	-0.4313	0	-0.4725	0	-0.4177	0
Root N concentration	-0.2222	0.0064	-0.1949	0.0172	0.0053	0.9486	-0.2994	0.0002	-0.2275	0.0053	-0.1411	0.0861	-0.1794	0.0286
Root CN ratio	0.0521	0.5280	0.0050	0.9522	0.1927	0.0185	0.1037	0.2082	0.0538	0.5148	-0.0407	0.6221	0.0312	0.7057
SMId	0.1527	0.0620	0.1425	0.0820	-0.0394	0.6324	0.2063	0.0113	0.1267	0.1223	0.0827	0.3142	0.0681	0.4074
SMIr	0.0457	0.5785	-0.0401	0.6259	0.3500	0	-0.1039	0.2059	-0.1024	0.2124	-0.2170	0.0076	-0.2848	0.0004
SMI	0.2772	0.0006	0.2064	0.0113	0.1112	0.1756	0.1819	0.0259	0.1817	0.0261	0.0826	0.3148	0.0255	0.7563
ForMI	0.2113	0.0099	0.1277	0.1218	0.2257	0.0058	0.0662	0.4241	0.1088	0.1880	-0.0213	0.7969	-0.0914	0.2690
Iharv	0.4068	0	0.4098	0	-0.2089	0.0108	0.3938	0	0.3876	0	0.3010	0.0002	0.2025	0.0136
Inonat	0.0956	0.2477	-0.0168	0.8395	0.4297	0	-0.1376	0.0954	-0.0253	0.7599	-0.2412	0.0031	-0.3811	0
Idwcut	0.0622	0.4528	0.0286	0.7303	0.1118	0.1762	0.0437	0.5983	0.0584	0.4804	-0.0011	0.9898	-0.0192	0.8166
Number of tree species per plot	0.3307	0	0.2795	0.0005	-0.0962	0.2418	0.2288	0.0049	0.3170	0.0001	0.2193	0.0070	0.0509	0.5365
Root Al concentration	0.7741	0	0.8182	0	-0.7387	0	0.7540	0	0.7772	0	0.6078	0	0.4772	0
Root Ca concentration	0.8598	0	0.8837	0	-0.6567	0	0.9206	0	0.8355	0	0.6449	0	0.4538	0
Root Fe concentration	0.3979	0	0.4253	0	-0.3543	0	0.4186	0	0.3730	0	0.2894	0.0004	0.1541	0.0652
Root K concentration	0.2147	0.0098	0.2847	0.0005	-0.6314	0	0.4325	0	0.2533	0.0022	0.5797	0	0.4766	0
Root Mg concentration	0.6874	0	0.7416	0	-0.8078	0	0.8035	0	0.6688	0	0.7542	0	0.6055	0
Root Mn concentration	-0.0443	0.5982	0.0060	0.9427	-0.1815	0.0294	-0.0272	0.7460	0.0134	0.8737	0.1285	0.1247	0.2450	0.0031
Root Na concentration	0.3094	0.0002	0.3389	0	-0.5503	0	0.4027	0	0.2839	0.0006	0.3547	0	0.3450	0
Root P concentration	-0.2761	0.0008	-0.2869	0.0005	0.2499	0.0025	-0.3814	0	-0.3366	0	-0.2710	0.0010	-0.4352	0
Root S concentration	-0.0694	0.4088	-0.0391	0.6416	-0.2068	0.0129	0.0007	0.9934	-0.1509	0.0710	-0.0178	0.8325	-0.0386	0.6457
Root glucose concentration	0.0258	0.7560	-0.0154	0.8530	0.1551	0.0607	-0.1427	0.0847	0.0055	0.9477	-0.0418	0.6155	-0.0689	0.4068
Root fructose concentration	-0.1892	0.0217	-0.2275	0.0056	0.2587	0.0016	-0.2306	0.0050	-0.1172	0.1574	-0.0284	0.7324	0.0198	0.8118

	SMId		SMIr		SMI		ForMI		Iharv		Inonat		Idwcut		Number of tree species per plot	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
All MMF	0.1004	0.2946	0.0782	0.4144	0.1733	0.0690	0.1092	0.2584	0.1189	0.2182	0.1315	0.1730	0.0422	0.6632	0.2005	0.0349
All observed	0.1485	0.1200	0.1234	0.1969	0.2260	0.0171	0.1448	0.1329	0.1268	0.1889	0.1952	0.0420	0.0696	0.4721	0.2043	0.0315
All Shannon	0.1652	0.0832	0.1862	0.0504	0.2606	0.0057	0.1950	0.0422	0.0873	0.3667	0.2920	0.0021	0.1124	0.2446	0.1838	0.0534
EM MMF	0.1804	0.0725	0.1985	0.0477	0.2911	0.0033	0.1485	0.1444	0.2093	0.0386	0.1843	0.0692	0.0362	0.7231	0.1676	0.0956
EM observed	0.1692	0.0923	0.2354	0.0184	0.3029	0.0022	0.1686	0.0971	0.1874	0.0646	0.2420	0.0164	0.0473	0.6439	0.1626	0.1061
EM Shannon	0.1127	0.2643	0.2530	0.0111	0.2671	0.0072	0.1668	0.1008	0.0413	0.6866	0.3288	0.0009	0.0530	0.6044	0.1699	0.0911
Myk total MMF	0.1804	0.0725	0.2164	0.0306	0.2852	0.0040	0.1758	0.0833	0.2031	0.0449	0.2252	0.0258	0.0447	0.6623	0.1975	0.0489
Myk total observed	0.1711	0.0887	0.2611	0.0087	0.3125	0.0015	0.1952	0.0541	0.1675	0.0993	0.2864	0.0043	0.0542	0.5960	0.2053	0.0404
Myk total Shannon	0.0965	0.3397	0.2521	0.0114	0.2550	0.0105	0.1757	0.0836	0.0231	0.8213	0.3386	0.0006	0.0610	0.5508	0.1928	0.0546
Sapro MMF	0.1143	0.2574	-0.2198	0.0280	-0.0082	0.9355	-0.1902	0.0607	0.0212	0.8356	-0.1383	0.1744	-0.1467	0.1495	0.0285	0.7780
Sapro observed	0.0759	0.4528	-0.2356	0.0183	-0.0501	0.6208	-0.2309	0.0222	0.0010	0.9922	-0.1757	0.0836	-0.1612	0.1127	0.0109	0.9139
Sapro Shannon	0.0300	0.7667	-0.2762	0.0054	-0.1166	0.2482	-0.2950	0.0032	-0.0216	0.8328	-0.2275	0.0243	-0.2048	0.0430	-0.0203	0.8409
unknown MMF	-0.0099	0.9222	0.1661	0.0987	0.1463	0.1465	0.1308	0.1992	0.0911	0.3723	0.1457	0.1523	0.0376	0.7132	0.3415	0.0005
unknown observed	0.0409	0.6860	0.1346	0.1817	0.1698	0.0912	0.1359	0.1821	0.0866	0.3967	0.1257	0.2173	0.0653	0.5229	0.3394	0.0006
unknown Shannon	0.1296	0.1986	0.1211	0.2300	0.2257	0.0240	0.1627	0.1094	0.1269	0.2132	0.1067	0.2959	0.1119	0.2727	0.3165	0.0013
Fine Roots Biomass	0.1060	0.1965	-0.1575	0.0543	-0.0409	0.6195	-0.1365	0.0981	0.1316	0.1109	-0.3815	0	-0.0525	0.5259	-0.0870	0.2897
Coarse Roots Biomass	-0.0702	0.4327	-0.1851	0.0372	-0.1208	0.1762	-0.1899	0.0339	-0.1504	0.0942	-0.2973	0.0008	-0.1616	0.0718	0.0228	0.7990
Organic Horizon total C	-0.0587	0.4754	0.1192	0.1464	-0.0411	0.6174	0.0692	0.4033	-0.1261	0.1266	0.1602	0.0517	-0.0332	0.6884	-0.0282	0.7323
Organic Horizon total N	-0.0104	0.8996	-0.2127	0.0090	-0.0875	0.2868	-0.0903	0.2749	0.1158	0.1611	-0.1685	0.0407	-0.0368	0.6567	-0.0211	0.7981
Organic Horizon CN ratio	-0.0273	0.7399	0.1780	0.0293	0.0241	0.7699	0.0346	0.6761	-0.1782	0.0303	0.1479	0.0728	-0.0380	0.6463	-0.0383	0.6418
Mineral soil Total C	0.1546	0.0588	0.0489	0.5527	0.2792	0.0005	0.2103	0.0103	0.4051	0	0.0932	0.2598	0.0628	0.4483	0.3265	0
Mineral soil Inorganic C	0.1630	0.0462	-0.0149	0.8567	0.2176	0.0075	0.1434	0.0822	0.3772	0	-0.0290	0.7263	0.0725	0.3810	0.2727	0.0007
Mineral soil Organic C	0.1527	0.0620	0.0457	0.5785	0.2772	0.0006	0.2113	0.0099	0.4068	0	0.0956	0.2477	0.0622	0.4528	0.3307	0
Mineral soil Total N	0.1425	0.0820	-0.0401	0.6259	0.2064	0.0113	0.1277	0.1218	0.4098	0	-0.0168	0.8395	0.0286	0.7303	0.2795	0.0005
Mineral Soil CN ratio	-0.0394	0.6324	0.3500	0	0.1112	0.1756	0.2257	0.0058	-0.2089	0.0108	0.4297	0	0.1118	0.1762	-0.0962	0.2418
Mineral soil pH 1	0.2063	0.0113	-0.1039	0.2059	0.1819	0.0259	0.0662	0.4241	0.3938	0	-0.1376	0.0954	0.0437	0.5983	0.2288	0.0049
Mineral Soil Texture Clay	0.1267	0.1223	-0.1024	0.2124	0.1817	0.0261	0.1088	0.1880	0.3876	0	-0.0253	0.7599	0.0584	0.4804	0.3170	0.0001
Mineral Soil Texture Fine Silt	0.0827	0.3142	-0.2170	0.0076	0.0826	0.3148	-0.0213	0.7969	0.3010	0.0002	-0.2412	0.0031	-0.0011	0.9898	0.2193	0.0070
Mineral Soil Texture Medium Silt	0.0681	0.4074	-0.2848	0.0004	0.0255	0.7563	-0.0914	0.2690	0.2025	0.0136	-0.3811	0	-0.0192	0.8166	0.0509	0.5365

	SMId		SMIr		SMI		ForMI		Iharv		Inonat		Idwcut		Number of tree species per plot	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Mineral Soil Texture Coarse Silt	0.0131	0.8739	-0.3690	0	-0.0810	0.3246	-0.2075	0.0114	0.1030	0.2128	-0.4469	0	-0.0833	0.3144	-0.0136	0.8692
Mineral Soil Texture Fine Sand	-0.0725	0.3781	0.0334	0.6847	-0.2145	0.0084	-0.1327	0.1079	-0.3374	0	-0.0160	0.8466	-0.0654	0.4295	-0.2561	0.0016
Mineral Soil Texture Medium Sand	0.0618	0.4524	0.3816	0	0.1165	0.1556	0.1871	0.0228	-0.1019	0.2176	0.4167	0	0.0468	0.5725	-0.0321	0.6969
Mineral Soil Texture Coarse Sand	0.0460	0.5765	0.3770	0	0.1079	0.1890	0.1404	0.0887	-0.1274	0.1230	0.3301	0	0.0112	0.8922	-0.0775	0.3457
Root C concentration	-0.2235	0.0061	-0.0329	0.6902	-0.2190	0.0073	-0.1485	0.0726	-0.2445	0.0028	0.0305	0.7134	-0.0668	0.4215	-0.2872	0.0004
Root N concentration	-0.2949	0.0003	-0.2433	0.0028	-0.3917	0	-0.3263	0.0001	-0.2391	0.0035	-0.2289	0.0053	-0.1976	0.0164	-0.1981	0.0154
Root CN ratio	0.1740	0.0338	0.2368	0.0036	0.2955	0.0003	0.2696	0.0010	0.1114	0.1793	0.2570	0.0017	0.1694	0.0403	0.0860	0.2969
SMId	1	0	0.1832	0.0248	0.7086	0	0.4421	0	0.6193	0	0.0558	0.5004	0.4297	0	0.0884	0.2821
SMIr	0.1832	0.0248	1	0	0.6993	0	0.7666	0	0.2992	0.0002	0.6954	0	0.4157	0	0.3760	0
SMI	0.7086	0	0.6993	0	1	0	0.7945	0	0.5837	0	0.5370	0	0.5002	0	0.4347	0
ForMI	0.4421	0	0.7666	0	0.7945	0	1	0	0.5963	0	0.6715	0	0.7412	0	0.4039	0
Iharv	0.6193	0	0.2992	0.0002	0.5837	0	0.5963	0	1	0	0.0702	0.3963	0.4826	0	0.1097	0.1844
Inonat	0.0558	0.5004	0.6954	0	0.5370	0	0.6715	0	0.0702	0.3963	1	0	0.2458	0.0026	0.4488	0
Idwcut	0.4297	0	0.4157	0	0.5002	0	0.7412	0	0.4826	0	0.2458	0.0026	1	0	0.0603	0.4665
Number of tree species per plot	0.0884	0.2821	0.3760	0	0.4347	0	0.4039	0	0.1097	0.1844	0.4488	0	0.0603	0.4665	1	0
Root Al concentration	0.2195	0.0082	-0.0384	0.6479	0.1775	0.0333	0.1179	0.1623	0.3779	0	-0.0733	0.3862	0.0460	0.5865	0.2552	0.0020
Root Ca concentration	0.1314	0.1163	-0.0348	0.6785	0.1866	0.0251	0.0966	0.2526	0.3833	0	-0.0517	0.5415	0.0591	0.4848	0.1946	0.0194
Root Fe concentration	0.2241	0.0069	0.1492	0.0743	0.2207	0.0079	0.2217	0.0080	0.2696	0.0012	0.1145	0.1747	0.0836	0.3226	0.2131	0.0103
Root K concentration	0.0550	0.5128	-0.2952	0.0003	-0.1368	0.1021	-0.1994	0.0174	0.1428	0.0901	-0.4762	0	-0.0255	0.7628	-0.0367	0.6624
Root Mg concentration	0.1828	0.0283	-0.0287	0.7328	0.1579	0.0587	0.0632	0.4551	0.3945	0	-0.1941	0.0206	0.0493	0.5602	0.2188	0.0084
Root Mn concentration	0.0013	0.9875	-0.0181	0.8293	-0.0352	0.6757	-0.1173	0.1644	-0.0071	0.9331	-0.1704	0.0427	-0.0614	0.4677	-0.0626	0.4559
Root Na concentration	0.1345	0.1079	-0.0907	0.2795	0.0121	0.8854	-0.0050	0.9528	0.1776	0.0344	-0.1700	0.0431	0.0181	0.8306	0.1005	0.2309
Root P concentration	-0.0638	0.4471	0.2468	0.0029	0.0607	0.4697	0.0758	0.3701	-0.1182	0.1611	0.1889	0.0243	0.0463	0.5840	-0.0420	0.6168
Root S concentration	0.0380	0.6511	-0.0658	0.4331	-0.1408	0.0923	-0.1190	0.1582	-0.0197	0.8161	-0.2159	0.0099	-0.0818	0.3331	-0.0607	0.4696
Root glucose concentration	-0.1217	0.1420	0.2955	0.0003	0.1574	0.0568	0.1647	0.0477	0.0178	0.8319	0.2722	0.0009	0.1451	0.0816	0.0658	0.4282
Root fructose concentration	-0.1726	0.0366	0.0548	0.5097	-0.0026	0.9752	0.0255	0.7606	-0.1675	0.0441	0.1880	0.0236	0.0099	0.9059	-0.0531	0.5230

	Root Al concentration		Root Ca concentration		Root Fe concentration		Root K concentration		Root Mg concentration		Root Mn concentration		Root Na concentration		Root P concentration	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
All MMF	0.3878	0	0.3238	0.0008	0.3084	0.0014	0.0635	0.5171	0.3686	0.0001	0.1638	0.0933	0.3001	0.0018	-0.2342	0.0159
All observed	0.3470	0.0003	0.2978	0.0019	0.2926	0.0023	0.0033	0.9729	0.3344	0.0005	0.1829	0.0606	0.2979	0.0019	-0.2056	0.0345
All Shannon	0.2493	0.0100	0.2236	0.0212	0.2486	0.0102	-0.0936	0.3397	0.2298	0.0178	0.1811	0.0632	0.2563	0.0080	-0.1365	0.1628
EM MMF	0.2882	0.0045	0.2709	0.0078	0.2364	0.0206	-0.1960	0.0557	0.2079	0.0423	0.2219	0.0300	0.0751	0.4663	-0.1232	0.2312
EM observed	0.2458	0.0158	0.2457	0.0158	0.2462	0.0156	-0.2289	0.0249	0.1967	0.0548	0.2241	0.0282	0.0409	0.6924	-0.0951	0.3564
EM Shannon	0.1067	0.3001	0.1514	0.1408	0.1952	0.0567	-0.2696	0.0081	0.0763	0.4596	0.1896	0.0644	-0.0254	0.8058	0.0081	0.9377
Myk total MMF	0.2787	0.0061	0.2332	0.0224	0.2937	0.0038	-0.1999	0.0511	0.1744	0.0892	0.2641	0.0095	0.0531	0.6071	-0.1188	0.2487
Myk total observed	0.2475	0.0150	0.2261	0.0268	0.2869	0.0046	-0.2391	0.0190	0.1773	0.0840	0.2595	0.0107	0.0434	0.6749	-0.0945	0.3598
Myk total Shannon	0.1043	0.3121	0.1339	0.1935	0.2108	0.0393	-0.2747	0.0068	0.0624	0.5457	0.1952	0.0566	-0.0178	0.8632	0.0077	0.9403
Sapro MMF	0.2121	0.0370	0.1437	0.1603	0.0519	0.6135	0.2226	0.0284	0.2639	0.0090	0.0197	0.8479	0.1989	0.0508	-0.1233	0.2288
Sapro observed	0.2583	0.0106	0.1495	0.1439	0.0888	0.3873	0.1977	0.0522	0.2949	0.0034	0.0340	0.7410	0.3005	0.0028	-0.2011	0.0482
Sapro Shannon	0.2881	0.0042	0.1790	0.0794	0.0984	0.3375	0.2065	0.0424	0.3288	0.0010	0.0498	0.6278	0.3994	0.0001	-0.2344	0.0208
unknown MMF	0.3328	0.0010	0.3233	0.0015	0.2451	0.0169	0.0656	0.5273	0.3726	0.0002	0.0775	0.4548	0.1239	0.2313	-0.2479	0.0156
unknown observed	0.3183	0.0017	0.3210	0.0015	0.1904	0.0646	0.0523	0.6150	0.3495	0.0005	0.0693	0.5048	0.1011	0.3294	-0.2375	0.0205
unknown Shannon	0.2705	0.0080	0.2871	0.0048	0.1106	0.2858	0.0023	0.9821	0.2934	0.0039	0.0796	0.4429	0.0599	0.5643	-0.1962	0.0568
Fine Roots Biomass	0.1189	0.1558	0.1797	0.0312	-0.0346	0.6809	0.2780	0.0007	0.2768	0.0008	0.0101	0.9045	0.1289	0.1235	-0.3182	0.0001
Coarse Roots Biomass	-0.0569	0.5355	-0.1103	0.2283	-0.0967	0.2916	0.4084	0	0.1628	0.0744	0.0097	0.9157	0.1704	0.0616	-0.0359	0.6956
Organic Horizon total C	-0.0996	0.2348	-0.2518	0.0024	0.0085	0.9193	-0.2143	0.0100	-0.1971	0.0180	-0.1751	0.0359	-0.0808	0.3353	-0.0074	0.9294
Organic Horizon total N	0.1224	0.1436	0.0246	0.7699	-0.0719	0.3911	0.0402	0.6318	0.0514	0.5404	0.1500	0.0728	-0.0449	0.5926	-0.0482	0.5655
Organic Horizon CN ratio	-0.1142	0.1727	-0.0868	0.3006	0.1019	0.2237	-0.0841	0.3161	-0.0961	0.2515	-0.1753	0.0357	0.0091	0.9138	-0.0226	0.7878
Mineral soil Total C	0.7728	0	0.8641	0	0.3968	0	0.2205	0.0079	0.6927	0	-0.0499	0.5525	0.3089	0.0002	-0.2754	0.0008
Mineral soil Inorganic C	0.7090	0	0.8717	0	0.3671	0	0.3788	0	0.7508	0	-0.0812	0.3333	0.3177	0.0001	-0.2947	0.0003
Mineral soil Organic C	0.7741	0	0.8598	0	0.3979	0	0.2147	0.0098	0.6874	0	-0.0443	0.5982	0.3094	0.0002	-0.2761	0.0008
Mineral soil Total N	0.8182	0	0.8837	0	0.4253	0	0.2847	0.0005	0.7416	0	0.0060	0.9427	0.3389	0	-0.2869	0.0005
Mineral Soil CN ratio	-0.7387	0	-0.6567	0	-0.3543	0	-0.6314	0	-0.8078	0	-0.1815	0.0294	-0.5503	0	0.2499	0.0025
Mineral soil pH 1	0.7540	0	0.9206	0	0.4186	0	0.4325	0	0.8035	0	-0.0272	0.7460	0.4027	0	-0.3814	0
Mineral Soil Texture Clay	0.7772	0	0.8355	0	0.3730	0	0.2533	0.0022	0.6688	0	0.0134	0.8737	0.2839	0.0006	-0.3366	0
Mineral Soil Texture Fine Silt	0.6078	0	0.6449	0	0.2894	0.0004	0.5797	0	0.7542	0	0.1285	0.1247	0.3547	0	-0.2710	0.0010

	Root Al concentration		Root Ca concentration		Root Fe concentration		Root K concentration		Root Mg concentration		Root Mn concentration		Root Na concentration		Root P concentration	
	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
Mineral Soil Texture Medium Silt	0.4772	0	0.4538	0	0.1541	0.0652	0.4766	0	0.6055	0	0.2450	0.0031	0.3450	0	-0.4352	0
Mineral Soil Texture Coarse Silt	0.4434	0	0.3991	0	0.1240	0.1387	0.4971	0	0.5357	0	0.2675	0.0012	0.3171	0.0001	-0.4736	0
Mineral Soil Texture Fine Sand	-0.6918	0	-0.7385	0	-0.3284	0.0001	-0.2720	0.0010	-0.6540	0	-0.1107	0.1866	-0.3007	0.0002	0.2837	0.0006
Mineral Soil Texture Medium Sand	-0.4935	0	-0.4798	0	-0.1286	0.1244	-0.5591	0	-0.6018	0	-0.1814	0.0296	-0.3409	0	0.4603	0
Mineral Soil Texture Coarse Sand	-0.4885	0	-0.5383	0	-0.0804	0.3383	-0.4595	0	-0.5602	0	-0.0629	0.4540	-0.3159	0.0001	0.4909	0
Root C concentration	-0.7831	0	-0.4621	0	-0.8058	0	-0.4542	0	-0.6722	0	-0.3071	0.0002	-0.4541	0	0.2078	0.0126
Root N concentration	-0.2513	0.0024	-0.2817	0.0007	-0.2241	0.0070	0.1960	0.0187	-0.1199	0.1522	0.1211	0.1480	-0.0217	0.7964	0.4924	0
Root CN ratio	-0.0436	0.6035	0.1142	0.1727	-0.0755	0.3683	-0.3741	0	-0.1298	0.1208	-0.2438	0.0033	-0.1550	0.0636	-0.4261	0
SMId	0.2195	0.0082	0.1314	0.1163	0.2241	0.0069	0.0550	0.5128	0.1828	0.0283	0.0013	0.9875	0.1345	0.1079	-0.0638	0.4471
SMIr	-0.0384	0.6479	-0.0348	0.6785	0.1492	0.0743	-0.2952	0.0003	-0.0287	0.7328	-0.0181	0.8293	-0.0907	0.2795	0.2468	0.0029
SMI	0.1775	0.0333	0.1866	0.0251	0.2207	0.0079	-0.1368	0.1021	0.1579	0.0587	-0.0352	0.6757	0.0121	0.8854	0.0607	0.4697
ForMI	0.1179	0.1623	0.0966	0.2526	0.2217	0.0080	-0.1994	0.0174	0.0632	0.4551	-0.1173	0.1644	-0.0050	0.9528	0.0758	0.3701
Iharv	0.3779	0	0.3833	0	0.2696	0.0012	0.1428	0.0901	0.3945	0	-0.0071	0.9331	0.1776	0.0344	-0.1182	0.1611
Inonat	-0.0733	0.3862	-0.0517	0.5415	0.1145	0.1747	-0.4762	0	-0.1941	0.0206	-0.1704	0.0427	-0.1700	0.0431	0.1889	0.0243
Idwcut	0.0460	0.5865	0.0591	0.4848	0.0836	0.3226	-0.0255	0.7628	0.0493	0.5602	-0.0614	0.4677	0.0181	0.8306	0.0463	0.5840
Num. of tree species per plot	0.2552	0.0020	0.1946	0.0194	0.2131	0.0103	-0.0367	0.6624	0.2188	0.0084	-0.0626	0.4559	0.1005	0.2309	-0.0420	0.6168
Root Al concentration	1	0	0.7444	0	0.7626	0	0.4158	0	0.8101	0	0.2597	0.0017	0.5115	0	-0.3108	0.0002
Root Ca concentration	0.7444	0	1	0	0.3723	0	0.3490	0	0.7516	0	-0.0258	0.7585	0.2904	0.0004	-0.3537	0
Root Fe concentration	0.7626	0	0.3723	0	1	0	0.2870	0.0005	0.5397	0	0.3366	0	0.4201	0	-0.0156	0.8525
Root K concentration	0.4158	0	0.3490	0	0.2870	0.0005	1	0	0.7088	0	0.1962	0.0185	0.4804	0	0.0280	0.7391
Root Mg concentration	0.8101	0	0.7516	0	0.5397	0	0.7088	0	1	0	0.2182	0.0087	0.5977	0	-0.1971	0.0180
Root Mn concentration	0.2597	0.0017	-0.0258	0.7585	0.3366	0	0.1962	0.0185	0.2182	0.0087	1	0	0.1920	0.0213	0.0538	0.5217
Root Na concentration	0.5115	0	0.2904	0.0004	0.4201	0	0.4804	0	0.5977	0	0.1920	0.0213	1	0	-0.0461	0.5826
Root P concentration	-0.3108	0.0002	-0.3537	0	-0.0156	0.8525	0.0280	0.7391	-0.1971	0.0180	0.0538	0.5217	-0.0461	0.5826	1	0
Root S concentration	0.0501	0.5505	-0.0876	0.2960	0.1385	0.0977	0.5251	0	0.2888	0.0005	0.2086	0.0122	0.4406	0	0.3926	0
Root glucose concentration	-0.1158	0.1668	-0.0191	0.8204	-0.0845	0.3135	-0.2891	0.0005	-0.1224	0.1436	0.0888	0.2894	-0.1248	0.1361	0.0899	0.2836
Root fructose concentration	-0.1724	0.0388	-0.1931	0.0204	-0.1135	0.1756	-0.2576	0.0018	-0.2395	0.0038	0.0209	0.8036	-0.1575	0.0594	-0.0571	0.4965

	Root S concentration		Root glucose concentration		Root fructose concentration	
	rho	p	rho	p	rho	p
All MMF	-0.1176	0.2295	0.0819	0.3990	0.1553	0.1086
All observed	-0.1350	0.1676	0.1500	0.1211	0.1529	0.1142
All Shannon	-0.1332	0.1733	0.2435	0.0111	0.1555	0.1081
EM MMF	-0.2684	0.0084	0.2416	0.0173	0.1300	0.2042
EM observed	-0.2914	0.0040	0.3162	0.0016	0.1722	0.0917
EM Shannon	-0.2479	0.0151	0.3944	0.0001	0.1927	0.0586
Myk total MMF	-0.2079	0.0423	0.2657	0.0087	0.1301	0.2041
Myk total observed	-0.2673	0.0085	0.3422	0.0006	0.1737	0.0888
Myk total Shannon	-0.2452	0.0160	0.3836	0.0001	0.1899	0.0625
Sapro MMF	-0.1331	0.1938	0.0331	0.7453	0.2087	0.0381
Sapro observed	-0.1807	0.0765	0.0419	0.6802	0.2687	0.0072
Sapro Shannon	-0.1663	0.1035	0.0174	0.8646	0.2485	0.0131
unknown MMF	-0.1799	0.0810	0.0922	0.3684	0.1570	0.1247
unknown observed	-0.1525	0.1402	0.0427	0.6778	0.1583	0.1214
unknown Shannon	-0.1318	0.2029	0.0566	0.5816	0.1783	0.0805
Fine Roots Biomass	0.1745	0.0365	-0.2631	0.0013	-0.2036	0.0134
Coarse Roots Biomass	0.2984	0.0009	-0.2349	0.0086	-0.0190	0.8341
Organic Horizon total C	-0.0469	0.5764	-0.0268	0.7474	0.0566	0.4962
Organic Horizon total N	-0.0305	0.7166	-0.0727	0.3810	0.0727	0.3815
Organic Horizon CN ratio	0.0292	0.7282	0.0156	0.8508	-0.0673	0.4180
Mineral soil Total C	-0.0641	0.4451	0.0199	0.8108	-0.1918	0.0200
Mineral soil Inorganic C	0.0285	0.7341	-0.0637	0.4434	-0.2300	0.0051
Mineral soil Organic C	-0.0694	0.4088	0.0258	0.7560	-0.1892	0.0217
Mineral soil Total N	-0.0391	0.6416	-0.0154	0.8530	-0.2275	0.0056
Mineral Soil CN ratio	-0.2068	0.0129	0.1551	0.0607	0.2587	0.0016
Mineral soil pH 1	0.0007	0.9934	-0.1427	0.0847	-0.2306	0.0050
Mineral Soil Texture Clay	-0.1509	0.0710	0.0055	0.9477	-0.1172	0.1574

	Root S concentration		Root glucose concentration		Root fructose concentration	
	rho	p	rho	p	rho	p
Mineral Soil Texture Fine Silt	-0.0178	0.8325	-0.0418	0.6155	-0.0284	0.7324
Mineral Soil Texture Medium Silt	-0.0386	0.6457	-0.0689	0.4068	0.0198	0.8118
Mineral Soil Texture Coarse Silt	-0.0092	0.9131	-0.1576	0.0566	0.0078	0.9253
Mineral Soil Texture Fine Sand	0.1921	0.0211	-0.0945	0.2548	-0.0514	0.5360
Mineral Soil Texture Medium Sand	-0.0142	0.8663	0.0881	0.2888	-0.0072	0.9315
Mineral Soil Texture Coarse Sand	0.0306	0.7161	0.0910	0.2731	0.0084	0.9195
Root C concentration	-0.0894	0.2864	0.1509	0.0681	0.1109	0.1812
Root N concentration	0.5457	0	-0.1970	0.0169	-0.2070	0.0119
Root CN ratio	-0.6106	0	0.2709	0.0009	0.2927	0.0003
SMId	0.0380	0.6511	-0.1217	0.1420	-0.1726	0.0366
SMIr	-0.0658	0.4331	0.2955	0.0003	0.0548	0.5097
SMI	-0.1408	0.0923	0.1574	0.0568	-0.0026	0.9752
ForMI	-0.1190	0.1582	0.1647	0.0477	0.0255	0.7606
Iharv	-0.0197	0.8161	0.0178	0.8319	-0.1675	0.0441
Inonat	-0.2159	0.0099	0.2722	0.0009	0.1880	0.0236
Idwcut	-0.0818	0.3331	0.1451	0.0816	0.0099	0.9059
Number of tree species per plot	-0.0607	0.4696	0.0658	0.4282	-0.0531	0.5230
Root Al concentration	0.0501	0.5505	-0.1158	0.1668	-0.1724	0.0388
Root Ca concentration	-0.0876	0.2960	-0.0191	0.8204	-0.1931	0.0204
Root Fe concentration	0.1385	0.0977	-0.0845	0.3135	-0.1135	0.1756
Root K concentration	0.5251	0	-0.2891	0.0005	-0.2576	0.0018
Root Mg concentration	0.2888	0.0005	-0.1224	0.1436	-0.2395	0.0038
Root Mn concentration	0.2086	0.0122	0.0888	0.2894	0.0209	0.8036
Root Na concentration	0.4406	0	-0.1248	0.1361	-0.1575	0.0594
Root P concentration	0.3926	0	0.0899	0.2836	-0.0571	0.4965
Root S concentration	1	0	-0.3087	0.0002	-0.4241	0
Root glucose concentration	-0.3087	0.0002	1	0	0.4204	0
Root fructose concentration	-0.4241	0	0.4204	0	1	0

Supplementary Table S7: Results of ITS Sequencing from representative tips of each morphotypes defined for the Trenches-Experiment. ITS sequence information was deposite in NCBI databank. The sequences were blasted in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and UNITE (https://unite.ut.ee/analysis.php) database respectively. The results were compared and the best consensus for species name or a higher taxonomic range was chosen. For some morphotypes another blast-hit is listed for explanation why the respective name was chosen. If for two or more morphotypes the same species name was chosen pictures (Supplementar Figure S4) were compared and if this was in accordance, too, they were grouped together. Species for which sequence information was not available were called by their internal morphotye (MTH) number. If two relative species shared the best hit, that one with the highest score was chosen, or, if the species from NCBI or UNITE appeared within the other database at a lower ranking the other species was chosen. (ACC-nr = Accession number)

Morpho-type number	Group	Species	NCBI gen Bank ACC -nr	Fragment length	NCBI				UNITE			
					Best Blast match	ACC-nr	Score	Sequence identity	Best Blast match	ACC-nr	Score	Sequence identity
MTH_01	1	*Lactarius subdulcis*	KT020767	584	*Lactarius subdulcis*	KF432969.1	1062	99 %	*Lactarius subdulcis*	UDB000380 SH248407.06FU	1062	99.32 %
MTH_02	2	*Inocybe* sp.	KT020768	602	*Inocybe* cf. *splendens*	FN550912.1	1086	99 %	*Inocybe*	UDB011621 SH022086.06FU	1092	99.34 %
MTH_03	3	*Clavulina* sp.	KT020797	668	*Clavulina* cf. *cinerea*	EU862222.1	1218	99 %	*Clavulina cinerea*	UDB011250 SH001336.06FU	1031	94.63 %
MTH_04		*Inocybe maculata*	KT020769	588	*Inocybe maculata*	AM882957.2	828	99 %	*Inocybe cookei*	UDB018191 SH018136.06FU	797	91.57 %
		2. hit							*Inocybe maculata*	UDB011889 SH018136.06FU	797	91.57 %
MTH_05		*Tomentella cinerascens*	KT020770	614	Thelephoraceae sp. B249	FN669274.1|	1000	98 %	*Tomentella cinerascens*	UDB016498 SH264074.06FU	1114	99.67 %
		2. hit			*Tomentella cinerascens*	AF272915.1|AF272915	998	99 %				
MTH_06	5	*Amanita* sp.	KT020771	573	*Amanita vaginata*	AJ889925.1	1014	99 %	*Amanita mortenii*	UDB002335 SH134953.06FU	1027	99.48 %
MTH_07		*Inocybe* sp. 2	KT020772	639	*Inocybe cookei*	AM882956.2	1105	99 %	*Inocybe perlata*	UDB017942 SH027341.06FU	353	83.82 %
		6. hit							*Inocybe cookei*	UDB018191 SH018136.06FU	348	78.16 %
MTH_08		*Sebacina* sp. 2	KT020773	616	Sebacinaceae sp. F42	AJ534908.1	911	94 %	*Sebacina*	UDB014255 SH030769.06FU	874	93.46 %
MTH_09	5	*Amanita* sp.		560	*Amanita vaginata*	AJ889925.1	1020	99 %	*Amanita mortenii*	UDB002335 SH134953.06FU	1009	99.46 %

Morpho-type number	Group	Species	NCBI gen Bank ACC -nr	Fragment length	NCBI				UNITE			
					Best Blast match	ACC-nr	Score	Sequence identity	Best Blast match	ACC-nr	Score	Sequence identity
MTH_10		*Cenococcum geophilum*	already known									
MTH_11		*Tomentella* sp. 1	KT020774	619	*Tomentella* sp. O41	AJ534912.1	977	95 %	*Tomentella*	UDB010514 SH021579.06FU	1110	99.35 %
MTH_12		Uncultured ectomycorrhiza (Pezizacea) 1	KT020775	730	Pezizales sp. MS8	KF850619.1	1182	99 %	*Genea verrucosa*	UDB001186 SH000457.06FU	147	82.82 %
MTH_13		Uncultured ectomycorrhiza (Pezizacea)c1	KT020776	467	Pezizaceae sp. GB359	JN102406.1	389	94 %	*Peziza succosa*	UDB015873 SH057304.06FU	228	91.62 %
MTH_14	9	*Russula ochroleuca*	KT020777	677	*Russula ochroleuca*	HM189930.1	1247	100 %	*Russula ochroleuca*	UDB016009 SH301201.06FU	1243	100 %
MTH_15		*Tricholoma sciodes*	KT020778	619	*Tricholoma sciodes*	AF377226.1	1105	99 %	*Tricholoma sciodes*	UDB000548 SH090510.06FU	1116	99.19 %
MTH_16	5	*Amanita* sp.		572	*Amanita vaginata*	AJ889925.1	1014	99 %	*Amanita mortenii*	UDB002335 SH134953.06FU	1033	99.65 %
MTH_17	10	*Xerocomus pruinatus*	KT020779	676	*Xerocomus pruinatus*	HM190109.1	1236	99 %	*Xerocomus pruinatus*	UDB000477 SH144059.06FU	1236	99.56 %
MTH_18	13	*Clavulina* sp. 1	KT020780	609	*Clavulina* cf. *cinerea*	EU862222.1	1092	99 %	*Clavulina cristata*	UDB018841 SH001338.06FU	915	94.33 %
		2.hit							*Clavulina cinerea*	UDB011250 SH001336.06FU	915	93.66%
MTH_19		*Genea hispidula*	KT020781	631	*Genea hispidula*	JX679370.1	1146	99 %	*Genea hispidula*	UDB001408 SH032169.06FU	1146	99.52 %
MTH_20	4	*Clavulina cristata* 2	KT020798	703	*Clavulina cristata*	EU862223.1	1223	99 %	*Clavulina cristata*	UDB001121 SH001336.06FU	1206	99.4 %
MTH_21	10	*Xerocomus pruinatus*	KT020782	705	*Xerocomus pruinatus*	HM190109.1	1293	99 %	*Xerocomus pruinatus*	UDB000477 SH144059.06FU	1293	99.57 %
MTH_22		*Tomentella* sp. (cf. *coerulea*)		602	Uncultured *Tomentella* clone ECM_alnus_Tom esp 1	JQ890249.1	*1009*	97%	*Tomentella*	UDB018457	888	94%

Morpho-type number	Group	Species	NCBI gen Bank ACC -nr	Fragment length	NCBI				UNITE			
					Best Blast match	ACC-nr	Score	Sequence identity	Best Blast match	ACC-nr	Score	Sequence identity
									Tomentella coerulea	UDB018457	884	
MTH_23												
MTH_24		Thelephoraceae	KT020799	671	Thelephoraceae sp. EMF47	JF273547.1	983	94 %	*Tomentella*	UDB018504 SH021685.06FU	952	93.74 %
MTH_25		*Russula acrifolia*	KT020783	612	*Russula acrifolia*	DQ421998.1	1066	98 %	*Russula acrifolia*	UDB002471 SH013037.06FU	1066	97.72 %
MTH_26												
MTH_27		Thelephoraceae 1	KT020800	640	Thelephoraceae sp. C.t.-3	AF184742.1|AF184742	955	94 %	*Tomentella*	UDB018677 SH021691.06FU	970	94.09 %
MTH_28	6	*Tarzetta* sp.	KT020801	592	*Tarzetta catinus*	DQ200833.1	1037	99 %	*Tarzetta*	UDB000992 SH027874.06FU	885	97.15 %
		3. hit							*Tarzetta catinus*	UDB008235 SH328298.06FU	475	83.01 %
MTH_29												
MTH_30		Uncultured ectomycorrhiza 2	KT020802	469	Fungal sp. 6 RB-2011	JQ272426.1	787	97 %	*Skyttea nitschkei*	UDB016230 SH014157.06FU	396	86.85 %
MTH_31	6	*Tarzetta* sp.	KT020801	596	*Tarzetta catinus*	DQ200833.1	1033	99 %	*Tarzetta*	UDB000992 SH027874.06FU	881	96.97 %
		3. hit							*Tarzetta catinus*	UDB008235 SH328298.06FU	472	82.87 %
MTH_32												
MTH_33		*Sebacina* sp. 3	KT020784	592	*Sebacina sp.*	HG796953.1|	848	93 %	*Sebacina*	UDB014255 SH030769.06FU	845	93.21 %
MTH_34		*Tomentella* sp. 2	KT020823	607	Thelephoraceae sp. EMF47	JF273547.1	881	93 %	uncultured *Tomentella*	AM159589 SH021569.06FU	1098	99.17 %
MTH_35	18	*Inocybe* sp. 1	KT020785	660	*Inocybe* sp. P38	AJ534923.1	1190	99 %	*Inocybe*	UDB011621 SH022086.06FU	531	99.39 %
MTH_36		*Melanogaster broomeianus*	KT020803	744	*Melanogaster broomeianus*	EU784370.1	1321	99 %	*Melanogaster*	UDB016279 SH035822.06FU	1243	98.72 %
MTH_37												
MTH_38		*Russula*	KT020804	877	*Russula olivacea*	AF418634.1	1611	99 %	*Russula*	UDB002548	1472	96.73 %

Morpho-type number	Group	Species	NCBI gen Bank ACC -nr	Fragment length	NCBI				UNITE			
					Best Blast match	ACC-nr	Score	Sequence identity	Best Blast match	ACC-nr	Score	Sequence identity
		vinosobrunnea			_*near relative to R. vinosobrunnea_				_vinosobrunnea_	SH005480.06FU		
		2. hit							_Russula olivacea_	UDB000322 SH254924.06FU	1367	95.1 %
MTH_39		Uncultured ectomycorrhiza (Leotiomycetes)	KT020786	544	Vouchered mycorrhizae (Fungi) clone	EF026068.1	952	99 %	_Trichopezizella relicina_	UDB003048 SH013876.06FU	616	88.89 %
MTH_40		Helotiales sp. 1	KT020805	590	_Rhizoscyphus ericae_	JQ711893.1	819	92 %	_Skyttea nitschkei_	UDB016230 SH014157.06FU	503	86.03 %
		2. hit			Helotiales sp. 1 CG-2012	HE814143.1	813					
MTH_41												
MTH_42		_Tomentella castanea_	KT020787	634	_Fagus sylvatica_	KC952702.1	1158	99%	_Tomentella castanea_	UDB000120 SH004530.06FU	1158	99.11 %
comment: all other hits belonged to _Tomentella_ → _Fagus must be a database bug_		2. hit			_Tomentella_ cf. _sublilacina_	AJ889982.1	1158	99 %				
		3. hit			_Fagus sylvatica_	KC952708.1	1144	99 %				
		4. hit			_Tomentella castanea_	KC952674.1	1092	99 %				
		5. hit			_Tomentella sp. 2_	HM189969.1	1081	97 %				
MTH_43	6	_Tarzetta_ sp.	KT020801	423	_Tarzetta catinus_	DQ200833.1	704	98 %	_Tarzetta_	UDB000992 SH027874.06FU	652	96.68 %
		3. hit							_Tarzetta catinus_	UDB008235 SH328298.06FU	388	84.24 %
MTH_44												
MTH_45		_Tomentella coerulea_	KT020806	676	Vouchered mycorrhizae (Thelephoraceae	GQ979996.1	1099	96 %	_Tomentella coerulea_	UDB018451 SH009165.06FU	1157	98.38 %

Morpho-type number	Group	Species	NCBI gen Bank ACC -nr	Fragment length	NCBI				UNITE			
					Best Blast match	ACC-nr	Score	Sequence identity	Best Blast match	ACC-nr	Score	Sequence identity
					)							
					Tomentella sp. 3	JQ711817.1	1096					
MTH_46		*Sebacina* sp. 1	KT020807	634	*Sebacina* sp. F6	AF465191.1	1166	99 %	*Sebacina*	UDB000773 SH313340.06FU	983	99.52 %
MTH_47		*Laccaria amethystina* 1	KT020808	670	*Laccaria amethystina*	HM189774.1	1234	100 %	*Laccaria amethystina*	UDB000006 SH010958.06FU	1227	99.85 %
MTH_48		*Tomentella* sp. 3	KT020809	648	Thelephoraceae sp. EC117 A52	AY751562.1	1066	97 %	*Tomentella*	UDB018555 SH044218.06FU	1081	96.76 %
		3. hit			*Tomentella* sp.	AB211278.1	750	88 %				
MTH_49	9	*Russula ochroleuca*	KT020777	675	*Russula ochroleuca*	HM189930.1	1247	100 %	*Russula ochroleuca*	UDB016009 SH301201.06FU	1243	100 %
MTH_50		*Inocybe* sp. 3	KT020788	663	*Inocybe* sp. P38	AJ534923.1	1201	99 %	*Inocybe*	UDB011621 SH022086.06FU	1195	99.52 %
MTH_51	11	*Xerocomus chrysenteron*	KT020810	748	*Xerocomus chrysenteron*	HQ207691.1	354	99 %	*Xerocomus chrysenteron*	UDB000441 SH325085.06FU	1301	99.17 %
MTH_52		Helotiales sp. 2	KT020811	537	Helotiales 1 RB-2011	JQ272327.1	856	95 %	*Trichopezizella relicina*	UDB003048 SH013876.06FU	737	93.25 %
MTH_53		*Tomentella ramosissima*	KT020812	677	*Tomentella ramosissima*	U83480.1|TRU83480	1214	99 %	*Tomentella lapida*	UDB001659 SH257993.06FU	1171	99.69 %
MTH_54		*Inocybe hirtella*	KT020789	620	*Inocybe hirtella*	AM882934.2	1109	99 %	*Inocybe*	UDB018787 SH011130.06FU	1050	96.82 %
		2. hit							*Inocybe hirtella*	UDB000642 SH268730.06FU	1038	98.63 %
MTH_55	14	*Clavulina cristata* 1	KT020790	655	*Clavulina cristata*	EU862223.1	1205	99 %	*Clavulina cristata*	EU862223 SH001336.06FU	1205	99.54 %
MTH_56	15	*Clavulina cristata* 1	KT020791	664	*Clavulina cristata*	EU862223.1	1155	99 %	*Clavulina cristata*	EU862223 SH001336.06FU	1208	99.69 %
MTH_57												
MTH_58												
MTH_59												
MTH_60												
MTH_61												

Morpho-type number	Group	Species	NCBI gen Bank ACC -nr	Fragment length	NCBI				UNITE			
					Best Blast match	ACC-nr	Score	Sequence identity	Best Blast match	ACC-nr	Score	Sequence identity
MTH_62		*Laccaria amethystina* 2	KT020820	701	*Laccaria amethystina*	HM189774.1	1295	100 %	*Laccaria amethystina*	UDB000006 SH010958.06FU	1279	99.86 %
MTH_63		*Byssocorticium atrovirens*	KT020792	618	*Byssocorticium atrovirens*	AJ889936.1	1123	99 %	*Byssocorticium atrovirens*	UDB000075 SH031867.06FU	1123	99.51 %
MTH_64												
MTH_65		uncultured ectomycorrhiza (Helotiales sp.)	KT020821	582	Helotiales sp. GMU_LL_04_G7	KC180683.1	974	95 %	*Trichopezizella relicina*	UDB003048 SH013876.06FU	712	99.81 %
MTH_66												
MTH_67		Helotiales sp. 3	KT020813		Helotiales sp. GMU_LL_04_G7	KC180683.1	974	100 %	Trichopezizella relicina	UDB003048 SH013876.06FU	712	91.60 %
MTH_68	8	*Lactarius pallidus*	KT020794	704	*Lactarius helvus*	JF908304.1	1234	98 %	*Lactarius pallidus*	UDB000366 SH238121.06FU	1339	99.58 %
		2. hit			*Lactarius pallidus*	JF908268.1	1221	99 %				
MTH_69	8	*Lactarius pallidus*	KT020794	725	*Lactarius pallidus*	AY606951.1	1304	99 %	*Lactarius pallidus*	UDB000366 SH238121.06FU	1332	99.85%
MTH_70		*Russula romellii*	KT020795	693	*Russula romellii*	KF002752.1	1110	98 %	*Russula romellii*	UDB018798 SH008484.06FU	1218	99.7 %
MTH_71		*Hygrophorus discoxanthus*		647	*Hygrophorus eburneus*	AY463485.1	1101	99 %	*Hygrophorus discoxanthus*	UDB000021 SH274681.06FU	1133	99.84 %
		2. hit (high difference within the score to 1. Hit in UNITE)							*Hygrophorus eburneus*	UDB000555 SH013801.06FU	981	97.73 %
MTH_72		Uncultured ectomycorrhiza 1 (*Piloderma*)	KT020796	631	*Piloderma sp. 9*	JQ711824.1	830	94 %	*Piloderma*	UDB001726 SH280643.06FU	821	89.83 %
MTH_73		*Inocybe albomarginata*	KT020814	726	*Inocybe glabripes*	AM882971.2|	1264	99 %	*Inocybe albomarginata*	UDB017929 SH244662.06FU	1277	99.86 %
		2. hit							*Inocybe*	UDB000099	1264	

Morpho-type number	Group	Species	NCBI gen Bank ACC -nr	Fragment length	NCBI				UNITE			
					Best Blast match	ACC-nr	Score	Sequence identity	Best Blast match	ACC-nr	Score	Sequence identity
									glabripes	SH244662.06FU		
MTH_74	1	*Lactarius subdulcis*	KT020767	707	*Lactarius subdulcis*	HM189807.1	1301	99 %	*Lactarius subdulcis*	UDB000048 SH248407.06FU	1301	99.86 %
MTH_75	12	*Lactarius vellereus*	KT020822	456	*Lactarius vellereus*	AY606958.1	791	99 %	*Lactarius vellereus*	FR852039 SH002089.06FU	791	98.87 %
MTH_76		*Lactarius azonites*	KT020815	710	*Lactarius ruginosus*	JQ446150.1	1293	99 %	*Lactarius azonites*	UDB000828 SH034425.06FU	1243	98.31 %
		2. hit							*Lactarius ruginosus*	UDB000394 SH034425.06FU	1230	98.34 %
MTH_77	17	*Lactarius vellereus*	KT020824		*Lactarius vellereus*	AY606958.1	1330	99 %	*Lactarius vellereus*	UDB000396 SH002089.06FU	1325	99.73%
		Picture: no *Lactarius!*--> MTH_77										
MTH_78		Tomentella sp. 4	KT020816	656	Vouchered mycorrhizae (Tomentella)	EU570331.1	1181	99 %	Thelephoraceae	UDB010511 SH006633.06FU	1206	100 %
					Fungal sp. *Tomentella* myco-symbiont	AB605659.1	1134		*Tomentella*	UDB018677 SH021691.06FU	1092	
MTH_79	11	*Xerocomus chrysenteron*	KT020793	706	*Xerocomus chrysenteron*	HQ207693.1	1273	99 %	*Xerocomus chrysenteron*	UDB001403 SH325085.06FU	1273	99.17 %
MTH_80	11	*Xerocomus chrysenteron*	KT020793	735	*Xerocomus chrysenteron*	HQ207691.1	1341	99 %	*Xerocomus chrysenteron*	UDB000441 SH325085.06FU	1280	99.43%
MTH_81	7	*Tomentella subtestacea*	*KT020817*	679	*Tomentella subtestacea*	KF500232.1	1098	97%	*Tomentella cinerascens*	UDB018459 SH009106.06FU	1043	96.25 %
MTH_82		*Xerocomus porosporus*	KT020818	752	*Xerocomus porosporus*	HM190086.1	1210	98 %	*Xerocomus porosporus*	DB000475 SH144062.06FU	1275	98.09 %

Supplementary-Table S8: Relative Abdunace of each Morphotype/fungal Taxon per Harvest and Treatment. C= Control In =Ingrwoth No In = No Ingrowth. Colour intenstity indicated abundances.

	Installtion	3 Month			6 Month			12 Month			15 Month			18 Month		
	C	C	In	No In	C	In	No In	C	In	No In	C	In	No In	C	In	No In
Amanita sp.	11.42	1.88	0.28		8.49	0.45		9.05	0.92		0.57			0.38	1.60	
Byssocorticium atrovirens		0.07			0.14						0.72	0.16				
Cenococcum geophilum	5.58	3.69	3.08	1.66	5.40	0.39	2.47	1.28	0.28		2.10	0.26				
Clavulina cristata 1a		0.52	0.33		0.17	0.37		2.68	0.18		0.42	0.04				
Clavulina cristata.1b		0.73	0.14	0.01	2.10											
Clavulina cristata 2a	4.73	0.01			0.87						1.67	0.11				
Clavulina sp.	0.06				1.65			0.08								
Clavulina sp.1	1.69	1.08	0.05	0.11	0.06		0.25	0.18	0.71		0.37	0.06		1.25	0.36	
Genea hispidula	2.15	0.41	0.65	0.41	0.16	3.11	0.14	1.38	0.15		2.35	0.96				
Helotiales sp. 1		0.17	1.16	0.17			0.99	3.52		0.54	0.03	0.72				
Helotiales sp. 2		0.22			0.16						0.10					
Helotiales sp. 3			1.55		0.04	0.16		0.46			1.28	0.90		2.74	1.13	
Hygrophorus discoxanthus					0.06						0.97	0.19				
Inocybe albomarginata								0.43			0.56	0.49				
Inocybe hirtella		0.14	0.20		0.02	1.32		4.77	4.31	0.20	1.12	0.05				
Inocybe maculata	1.81	0.01	0.13	2.21	0.43	0.29		0.18								
Inocybe sp.	8.99	0.36	0.04	0.04	0.17			0.56	0.43		0.22					
Inocybe sp. 1		0.58	0.50	0.19							0.31					
Inocybe sp. 2	1.32	0.85	0.40	0.07	0.91			3.19	0.87		1.49	0.30				
Inocybe sp. 3		0.71	0.66	0.01	0.85			0.48	0.10							
Laccaria amethystina		0.47			0.25						0.27	0.42				
Lactarius azonites or *Lactarius ruginosus*											0.28	2.90				
Lactarius pallidus					4.52	0.93		0.41			8.29	0.01				
Lactarius subdulcis	21.75	10.42	2.14	7.14	17.11	4.56	0.37	12.34	1.58	0.26	12.49	7.34	0.33	11.74	14.53	
Lactarius vellereus											0.15	0.08				

	Installtion	3 Month			6 Month			12 Month			15 Month			18 Month		
	C	C	In	No In	C	In	No In	C	In	No In	C	In	No In	C	In	No In
Melanogaster broomeianus		1.23	0.76		0.83						0.01					
Russula acrifolia	0.07	0.08			0.54			2.01	0.08		0.07	0.01				
Russula ochroleuca	2.53	0.29	0.01					0.56	0.05		0.26			0.25	0.38	
Russula romellii					0.14	0.06					0.98	3.90				
Russula vinosobrunnea		0.01														
Sebacina sp. 1		0.20	0.10	0.10										1.81	3.41	
Sebacina sp. 2	2.80	0.02	0.02					1.43			0.15					
Sebacina sp. 3	2.50	0.17	0.02	0.35	1.90			0.20	0.18		0.73	0.47				
Tarzetta sp.	0.64	0.23	0.27	0.12	0.35	0.82		1.17			1.32	0.40				
Thelephoraceae	0.01				0.04			0.08								
Thelephoraceae 1	2.28		0.20		0.25			1.02			1.01	2.01				
Tomentella castanea		0.35	0.05	0.04	0.41	0.04						0.77				
Tomentella cinerascens	1.26				0.25			3.77	1.35	0.05						
Tomentella coerulea								0.08	0.03		1.07	0.44				
Tomentella ramosissima		2.68	0.10	0.02								0.14		0.75	1.46	
Tomentella sp. possibly *coerulea*	1.06	0.05	0.39	0.85	1.32			1.66	2.14		1.19	0.13				
Tomentella sp. 1	4.78	0.66	0.36	0.03				0.18			2.78	0.73				
Tomentella sp. 2		0.12	0.95		0.80									0.25	0.31	0.07
Tomentella sp. 3		0.06										0.06				
Tomentella sp. 4											2.77	1.82				
Tomentella subtestacea														0.04		
Tricholoma sciodes	5.99	4.21	0.68	0.44	0.25						3.71	0.31				
uncultured ectomycorrhiza Helotiales sp.		0.10		0.19	0.66	0.04					0.12	0.19				
Uncultured ectomycorrhiza Leotiomycetes		3.15	1.96	2.72				9.79	3.49		0.11					
Uncultured ectomycorrhiza Pezizacea 1	1.99	0.07			0.06	0.29					0.27					
Uncultured ectomycorrhiza Pezizacea c1	2.59							1.33	0.05		0.07	0.32		1.05	1.83	
Uncultured ectomycorrhiza 1					0.45			1.20								
Uncultured ectomycorrhiza 2	3.63	1.99	0.03	0.61	1.44			1.33	0.18		0.78	1.00		0.59	0.70	

	Installtion	3 Month			6 Month			12 Month			15 Month			18 Month		
	C	C	In	No In	C	In	No In	C	In	No In	C	In	No In	C	In	No In
Xerocomus chrysenteron		4.20	0.09		0.49	3.18					1.52	7.10		0.04		
Xerocomu porosporus														23.35	11.34	0.36
Xerocomus pruinatus	1.24	0.69	0.30		4.39	0.93	0.10	2.58	0.05	0.05	0.19	0.12				
MTH_23	0.12															
MTH_26	0.49							1.10								
MTH_29	6.39		4.39	0.49	0.27	0.06		0.38	1.07					4.52	1.79	
MTH_32	0.14				0.33	0.02								1.34	0.38	
MTH_37		4.41	3.27	3.82	8.43	3.22		2.45	0.05		0.49					
MTH_41		0.06	0.03					0.03								
MTH_44		0.05			2.58	0.19		0.13								
MTH_57		0.11			0.62	0.58		0.10			0.66	0.20				
MTH_58		0.46		0.06	1.07	0.10		2.65			0.46	0.42				
MTH_59		1.36			0.27	1.20			0.69		0.93	1.22				
MTH_60		1.47												4.48	3.81	
MTH_61		0.68	0.10	0.68	0.31			2.86			1.41	0.47				
MTH_64		0.33			0.17	0.04						0.20		1.66	0.32	
MTH_66		0.32			0.16	1.01		0.89				0.94				
MTH_77											1.61	0.90				

Supplementary Table S9: exploration types for the Morphotypes. First it was checked in in the Literature (Agerer 2001; Courty *et al.* 2008; http://deemy.de/) if the exploration type is known. Otherwise own pictures and descriptions were used for assigning an exploration type. Those two were combined to the final exploration type which was used for further calculations. C= Contact type, SD = Short Distance, MD = Medium Distance and LD =Long Distance

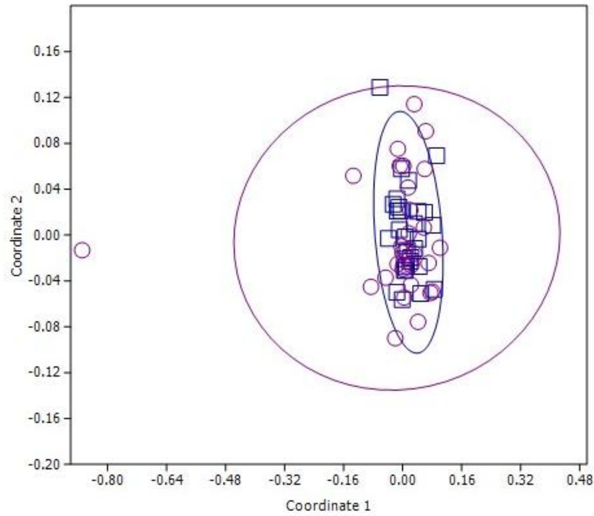
Name	exploration types from Literature	exploration types my classification	Explo Final
Amanita sp.	MD	MD	**MD**
Byssocorticium atrovirens	SD	MD	**SD**
Cenococcum geophilum	SD	SD	**SD**
Clavulina cristata 1 a	MD	MD	**MD**
Clavulina cristata 1 b	MD	MD	**MD**
Clavulina cristata 2a	MD	MD	**MD**
Clavulina sp.	MD	MD	**MD**
Clavulina sp. 1	MD	SD	**MD**
Genea hispidula	SD	SD	**SD**
Helotiales sp. 1	unknown	C	**C**
Helotiales sp. 2	unknown	SD	**SD**
Helotiales sp. 3	unknown	SD	**SD**
Hygrophorus discoxanthus	MD	SD	**MD**
Inocybe albomarginata	SD	SD	**SD**
Inocybe hirtella	SD	SD	**SD**
Inocybe maculata	SD	SD	**SD**
Inocybe sp.	SD	SD	**SD**
Inocybe sp. 1	SD	SD	**SD**
Inocybe sp. 2	SD	SD	**SD**
Inocybe sp. 3	SD	SD	**SD**
Laccaria amethystina	MD	MD	**MD**
Lactarius azonites or *Lactarius ruginosus*	C	C	**C**
Lactarius pallidus	C	C	**C**
Lactarius subdulcis	C	C	**C**
Lactarius vellereus	C	C	**C**
Melanogaster broomeianus	LD	LD	**LD**
MTH_77	C	MD	**C**
MTH23	unknown	NA	**NA**
MTH26	unknown	C	**C**
MTH29	unknown	MD	**MD**
MTH32	unknown	MD	**MD**
MTH37	unknown	MD	**MD**
MTH41	unknown	SD	**SD**
MTH44	unknown	SD	**SD**
MTH57	unknown	SD	**SD**
MTH58	unknown	C	**C**
MTH59	unknown	MD	**MD**
MTH60	unknown	MD	**MD**
MTH61	unknown	SD	**SD**

Name	**exploration types from Literature**	**exploration types my classification**	**Explo Final**
MTH64	unknown	SD	**SD**
MTH66	unknown	C	**C**
Russula acrifolia	C	SD	**C**
Russula ochroleuca	C	C	**C**
Russula romellii	C	C	**C**
Russula vinosobrunnea	C	NA	**C**
Sebacina sp. 1	SD	MD	**SD**
Sebacina sp. 2	SD	MD	**SD**
Sebacina sp. 3	SD	SD	**SD**
Tarzetta sp.	unknown	SD	**SD**
Thelephoraceae	unknown	MD	**MD**
Thelephoraceae	unknown	MD	**MD**
Tomentella castanea	SD	SD	**SD**
Tomentella cinerascens	SD	SD	**SD**
Tomentella coerulea	SD	SD	**SD**
Tomentella ramosissima	SD	SD	**SD**
Tomentella sp. (possibly *coerulea*)	C	C	**C**
Tomentella sp. 1	SD	SD	**SD**
Tomentella sp. 2	SD	SD	**SD**
Tomentella sp. 3	SD	SD	**SD**
Tomentella sp. 4	C	C	**C**
Tomentella subtestacea	C	C	**C**
Tricholoma sciodes	MD	MD	**MD**
uncultured ectomycorrhiza (Helotiales sp.)	unknown	C	**C**
Uncultured ectomycorrhiza (Leotiomycetes)	unknown	MD	**MD**
Uncultured ectomycorrhiza (Pezizacea) 1	unknown	MD	**MD**
Uncultured ectomycorrhiza (Pezizacea)c1	unknown	SD	**SD**
Uncultured ectomycorrhiza 1	unknown	NA	**NA**
Uncultured ectomycorrhiza 2	unknown	LD	**LD**
Xerocomus chrysenteron	LD	C	**LD**
Xerocomus porosporus	LD	MD-LD	**LD**
Xerocomus pruinatus	LD	C	**LD**

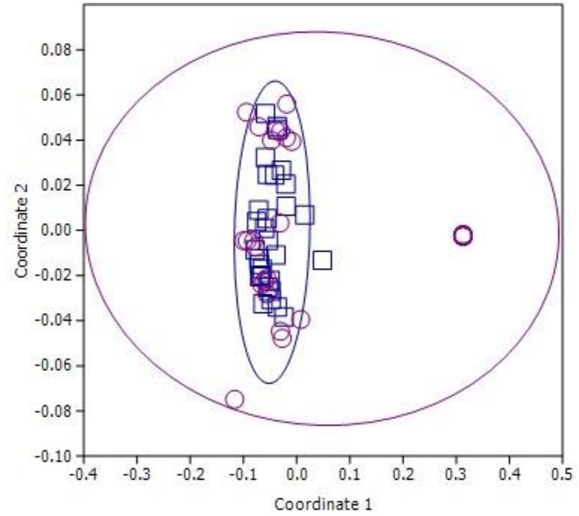
Supplementary Figure S2: Soil moisture at 10 cm below surface [% (percentage of volumetric water content)] and soil temperature at 10 cm below surface (°C) over the Experiment time period of 18 month (2011 and 2012). Data were provided by the BExIS and regional management Teams of the Biodiversity Exploratories:

Please see Supplementary Figure S2 on attached CD.

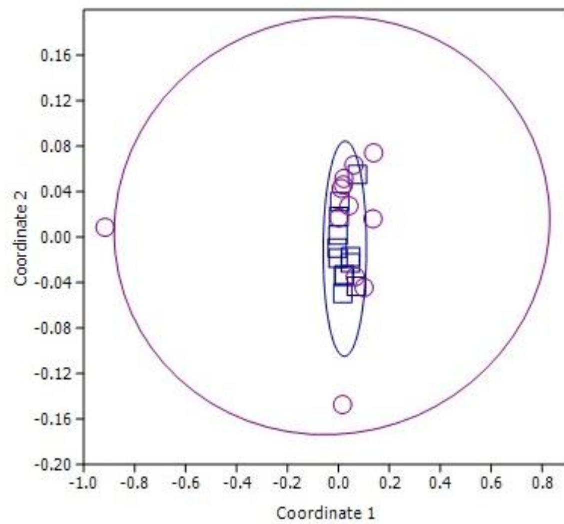
a) 3 months



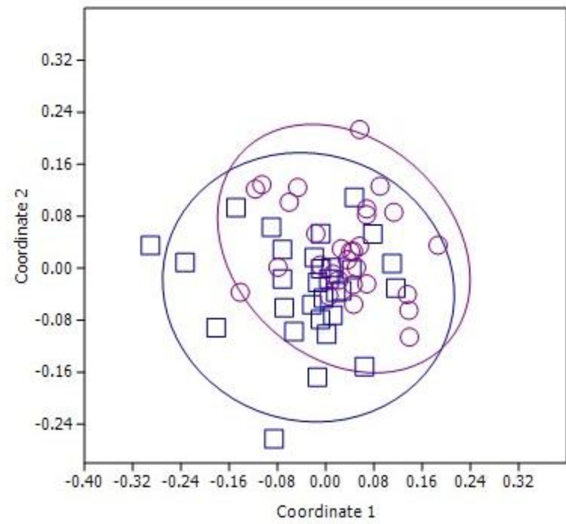
b) 6 months *



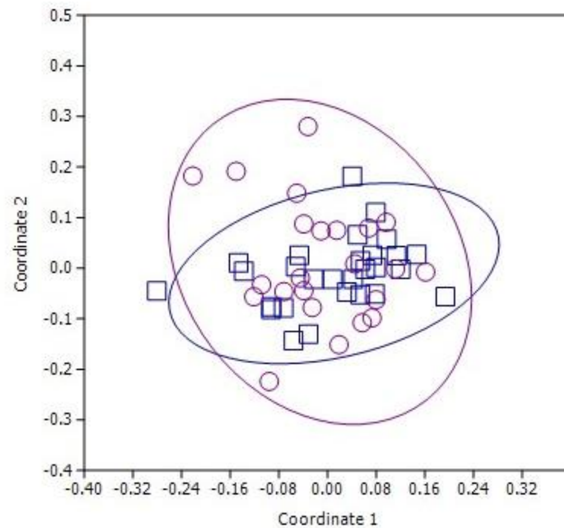
c) 12 months *



d) 15 months*



e) 18 months



NMDS Stress values:

Month after experiment installation	2D Stress
3	0.391
6	0.346
12	0.301
15	0.296
18	0.214

Supplementary Figure S3: NMDS with Bray Curtis Similarity for EM community in Ingrowth and Control cores for each harvest. Asterisks indicate for significant differences between the EM communities of Control and Ingrowth plots.a) 3 months, b) 6 months, c) 12 months, d) 15 months, e) 18 months

Supplementary Figure S4: Pictures of Morphotpyes found within the root Trenching experiment; caused by a severe computer problem, pictures are not true to scale. In addition a description sheet for EM is attached, with scematic schemes from and based on Agerer 1987-2001. Pictures were partly taken by Dr. Rodica Pena, Daryl Hughes, Markus Steckel and Otilia Mazilu.

Please see SupplementarFigure S4 on attached CD.

Supplementary Figure S5: NMDS of fungal communities, with ordisurf for different variables. A) All fungi b) EM c) saprophytes d) unknown

Please see SupplementarFigure S5 on attached CD.

Declarations of the authors own contributions

- All statistical analysis and graphics (despite the Biodiversity Exploratory map, which was dony by Kezia Goldmann[6])
- Morphotype- sequence corrections and blasting
- All pictures

Soil Sampling Campaign:

- Participation on soil/root sampling in Swabian Alb
- Processing of the roots (washing, freeze drying, milling)
- 454 Pyrosequencing:
  - Test of different DNA extraction methods (with help of Dr. Rodica Pena[1] and Thomas Klein[2])
  - DNA extraction and preparation of samples for 454 Pyrosequencing (with help of Benjamin Faust[1])
  - Processing of Sequences, Bioinformatical analysis, calculation of diversity indices, and NCBI data deposition: Dr. Bernd Wemheuer[5]
- Carbohydrate measurement
- Preparation of samples for CN measurements, measurements by Gisbert Langer-Kettner[1]
- Preparation of ICP samples by Christine Kettner[1]
- Morphotyping:
  - Washing of roots (with help of Marianne Smiatacz[1])
  - Morphotyping
  - DNA extraction and preparation for Sanger-Sequencing of Morphotypes

Soil Trenching Experiment:

- Installation and 0 month harvest by Dr. Rodica Pena
- Organization and harvesting of soil samples of all (3-18 month) harvests, with the help for sampling from Dr. Rodica Pena, Dr. Dennis Janz[1], Dr. Martin Leberecht[1], Yan Nan[1], Rainer Schulz[2], Markus Steckel, Daryl Hughes and Michael Reichel[3]

- Morphotyping: some samples from the 0 months harvest, morphotyping of all samples from the 3 months harvest and supervision of two Bachelor students for their thesis (Daryl Hughes and Markus Steckel) for the 6 and 12 months harvest respectively, 0 and 15 months harvest was morphotyped by Dr. Rodica Pena and 18 months by the Bachelor student Otilia Mazilu; washing of roots were respectively (with help of Marianne Smiatacz[1])
- Collaboration of DNA extraction and preparation for Sanger Sequencing of the Morphotypes (with help of Dr. Rodica Pena and Thomas Klein)
- Partly: pH measurements, soil moisture, root dryweights
- Sequence deposition to NCBI by Dr. Rodica Pena

In addition:

Setup of a large scale experiment to investigate the influence of the genetic diversity of beech on the EM diversity:

- Collection of beech nuts (with help of Dr. Sarah Seifert[4], Dr. Rodica Pena and Daryl Hughes)
- Germination of beech nuts (with help of Dr. Sarah Seifert and Dr. Rodica Pena)
- Growing of beech seedlings (with help of Marianne Smiatacz)
- Rating before planting (with help of Dr. Rodica Pena, Silke Ammerschubert[1], Merle Fastenrath[1] and Marianne Smiatacz)
- Organization and realization of the planting of over 3240 beech seedlings to the three Exploratories (with help for planting from Johannes Persch and Nils Böddingmeier)
- Rating of Hainich beech seedlings 2013 (with help of Michael Reichel)

[1] Büsgen Institut department of Forest Botany and Tree Physiology, Georg-August-University Göttingen (former and present members)

[2] LARI: Laboratory for Radioactive Isotopes, Georg-August-University Göttingen

[3] AG Chemistry and process technology of composite material, Georg-August-University Göttingen

[4] Forest Genetics and Forest Tree Breeding, Georg-August-University Göttingen (former member)

[5] Genomic and Applied Microbiology, Georg-August-University Göttingen

[6] UFZ-Helmholtz-Centre for Environmental Research, Department of Soil Ecology, Halle

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Curriculum vitae

Personal

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Education/Work experience

07.2014-present	Project management of German-Czech encounter at the "Ökologische Bildungsstätte Burg Hohenberg e.V."
05.2011 – 04.2014	Doctoral student at the Georg –August-Univeristy Göttingen within the doctoral program "Biological diversity and ecology", part of the research staff of "Büsgen Institut – Department of tree physiology and forest botany" Title of the doctoral thesis: „Functional diversity of mycorrhiza in relation to land-use changes and ecosystem functions"
10.2008 - 04.2011	Master of Science „Biodiversity and Ecology" at the University of Bayreuth Topic of the Masters Thesis: „Root-associated fungi of the Bohemian Gentian (*Gentianella bohemica*)"
10.2005 - 09.2008	Bachelor of Science, Biology with ecological focusing at the University of Bayreuth Topic of the Bachelor Thesis: „Mapping of the silting vegetation at the Environment- and Informationcentre Lindenhof of the LBV (Bavarian Society for the protection of birds), impact of cow grazing"
09.1996 - 06.2005	Luisenburggymnasium as Secundary school in Wunsiedel *Abitur* (general qualification for university entrance) with intensive courses in biology and physics
09.1992 – 08.1996	Elementary School Tröstau-Nagel in Nagel

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