Influence of mycorrhiza on nutrient physiology of trees in mixed and mono-specific stands along climatic and land use gradients

Dissertation

to attain degree Doctor of Philosophy (Ph. D.)

of the Faculty of Forest Sciences and Forest Ecology

Georg-August-Universität Göttingen

Submitted by

Likulunga Emmanuel Likulunga

born in Sesheke, Zambia

Göttingen, 2022

1st Referee: Prof. Dr. Andrea Polle

2nd Referee: Prof. Dr. Christian Ammer

Date of oral examination: May 16, 2022

Table of Contents

SUMMARY

Global change drivers such as climate change, land-use change, anthropogenic N deposition, deteriorating P nutrition and other calamities jeopardize temperate forest ecosystem functions. Essential components for the maintenance of ecosystem functions are fine roots and soil microbial communities because they are important for nutrient provision and water uptake of trees. Fungi contribute substantially to nutrient cycling since saprotrophic fungi drive the decomposition of organic material, while symbiotrophic fungi, mainly ectomycorrhizal fungi (EMF) facilitate nutrient uptake through symbiotic associations with tree roots. Understanding factors that influence fine root biomass and soil- or root-associated microbes is highly relevant for sustainable forest management under global change. To enhance knowledge on belowground functions, the responses of roots and fungi to seasonal changes in nutrient input by litter, nutrient input by deposition and different climatic conditions have to be investigated in different forest types. The main forest tree species in Germany, European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies,* Karst.) are susceptible to drought. Since drought periods are expected to increase in a future climate, the cultivation of more tolerant, non-native species such as Douglas-fir (*Pseudotsuga menziesii*) may be an option for future forest management. Yet, little is known about the influence of Douglas-fir on local soil fungal composition and diversity in pure and beech mixed tree stands.

In this study, our first goal was to investigate which factors contribute to seasonal trends of European beech fine root biomass along a climatic and soil P gradient. Field experiments were carried out in study sites characterized by low, medium and high soil P content. The study sites comprise beech dominated plots unfertilized or fertilized with either N, P or combined N and P. These study sites were also used to investigate the impact of nutrient dynamics on EMF composition and diversity as our second research goal. Fine root biomass, soil and root elements were analyzed from soil cores collected in spring and fall. We found an increase in fine root biomass only at the P-low forest site in fall under P fertilization. Higher fine root biomass was observed in fall than in spring only at the P-medium and P-high forest sites contrary to the P-low forest site. We constructed a general model incorporating all measured variables [site (P-low, P-medium, P-high), fertilizer treatment (unfertilized, N, P, P+N), season (spring,

fall), climate (temperature, precipitation), soil and root elements (total and soluble P, C, N, C/N ratio, K, Na, Ca, Mg, Mg, Fe, S), soil pH and water content)] and subjected it to stepwise regression. The variables retained by the model with lowest Akaike Information Criterion were used to partition the variance of fine root biomass. Soil chemistry, including soil water content, explained a high fraction of variation of fine root biomass in both organic layer and mineral soil while climate explained high proportion of root biomass variation in the mineral soil. The seasonal patterns in fine root biomass were therefore, attributed to changes in nutrient dynamics and climate but dependent on soil layer and site. Little biomass variation was explained by root resources in the organic layer, suggesting that in the organic layer soil resources dominate because of high biological activity and microbial interactions. We further analyzed the impact of nutrient dynamics on EMF composition and diversity at these study sites by morphotyping and ITS sequencing. Our results revealed that neither EMF composition nor richness and diversity were influenced by fertilization treatment. This was contrary to our expectation and implies that EMF show stability in response to moderate nutrient additions. This finding supports the notion of EMF relative resistance to N addition in beech forests. However, when analyzing fungi by DNA barcoding at the levels of orders, our collaborative study uncovered negative and positive responses of Russulales and Boletales, respectively, following P and P+N addition. Across all sites studied, we found that Russulales were enriched in soils with high N content. These results imply that fungal structures are driven by nutrient availability.

To address our third goal, the impact of introduction of Douglas-fir either in pure stands or in mixture with beech on soil fungal structures, field experiments were conducted in a dry and nutrient poor region and in a humid and nutrient rich region. In each region, four sites were used, which contained plots with either pure (beech, spruce, Douglas-fir) or mixed beech-conifer stands (beech-spruce, beech-Douglas-fir). Soil fungal community analyses (0-10 cm depth after removal of non-decomposed litter) were conducted by barcoding of the ITS region and Illumina sequencing. Soil elements (C, N, P, K, Na, Ca, Mg, Mn, Fe, S), C/N ratio, soil pH and soil relative water content were determined and used as explanatory variables for fungal composition. Our results showed distinct differences in fungal assemblages between nutrient-rich and nutrient-poor sites and among the stand types. Fungal communities were separated according

tree species composition and explained by soil chemistry. Fungal compositions in spruce and Douglas-fir forests were similar. Intermediate fungal compositions between pure beech and pure spruce (or pure Douglas-fir) stands were observed in mixed beech-conifer tree stands. Forest types did not influence mycorrhizal species richness. However, the relative abundance of symbiotrophs decreased in pure Douglas-fir and beech-Douglas-fir mixtures compared with native species. The relative abundance of the saprotrophic fungal orders Tremellales and Hymenochaetales increased in conifer tree stands while the abundance of other fungal orders was dependent on tree species composition and site properties. The similarity of fungal assemblages observed in non-native Douglas-fir and native Norway spruce implies that Douglas-fir can accommodate native fungi. However, pure Douglas-fir and mixed beech-Douglas-fir favored the relative abundance of saprotrophic fungi. Whether the shifted mycorrhiza/saprotroph ratio affects nutrient turnover, remains to be studied in the future.

Overall, our study shows that responses of fungi and root biomass are mainly climate and nutrient dependent. The seasonal cycling of fine root biomass between fall and spring (i.e. higher in spring than fall in organic layer at the P-low site and vice versa at P-high site) indicates plasticity of fine root biomass to nutrient availability. Apparently, beech can adjust its root system to cope with changes in nutrients. Whether the seasonal response of beech fine root biomass observed along climatic and soil P gradient in our study can be extrapolated to beech-conifer tree stands needs investigation. This is because in beech-conifer mixed stands, changes in nutrients attributable to different litter inputs as well as changes in microbial communities are expected. Moreover, we observed differences among fungal communities in pure beech and conifer stands and intermediate pattern in mixed beech-conifer stands in the top layer of the forest floor. How the tree species will influence fungal communities in deeper soil horizons due to variations in nutrient availability and microbial activity is yet unknown. The relative stability responses of EMF to nutrient additions implies that beech forests can still rely on microbial interactions for nutrient uptake but more information is needed how environmental fluctuation and anthropogenic deposition, different types of litter input and different root distribution influence forest nutrition. Our study highlights that root biomass, soil and root fungi are important for tree adaptation

to changing environmental conditions, contributing to knowledge on sustainable forest management.

ZUSAMMENFASSUNG

Globale Veränderungen wie Klimawandel, Landnutzungsänderungen, anthropogene Stickstoffeinträge, eine Verschlechterung des Stickstoffgehalts und andere Katastrophen gefährden die Ökosystemfunktionen gemäßigter Wälder. Wesentliche Komponenten für die Aufrechterhaltung der Ökosystemfunktionen sind Feinwurzeln und mikrobielle Bodengemeinschaften, da sie für die Nährstoffversorgung und Wasseraufnahme der Bäume wichtig sind. Pilze tragen wesentlich zum Nährstoffkreislauf bei, da saprotrophe Pilze die Zersetzung von organischem Material vorantreiben, während symbiotrophe Pilze, vor allem Ektomykorrhizapilze (EMP), die Nährstoffaufnahme durch symbiotische Verbindungen mit Baumwurzeln erleichtern. Grundlegende Erkenntnisse über Faktoren, welche die Feinwurzelbiomasse und die boden- oder wurzelassoziierten Mikroben beeinflussen, sind für eine nachhaltige Waldbewirtschaftung im Zuge des globalen Wandels von großer Bedeutung. Um das Wissen über unterirdische Funktionen zu erweitern, müssen die Reaktionen von Wurzeln und Pilzen auf saisonale Veränderungen des Nährstoffeintrags durch Streu oder Deposition und durch unterschiedliche klimatische Bedingungen in unterschiedlichen Waldtypen untersucht werden. Die wichtigsten Waldbaumarten in Deutschland, die Rotbuche (*Fagus sylvatica* L.) und die Gemeine Fichte (*Picea abies*, Karst.), sind anfällig für Trockenheit. Da in einem zukünftigen Klima mit der Zunahme von Trockenperioden zu rechnen ist, könnte der Anbau von trocken-toleranteren, nicht-einheimischen Arten wie Douglasie (*Pseudotsuga menziesii*) eine Option für die zukünftige Waldbewirtschaftung sein. Über den Einfluss der Douglasie auf die lokale Zusammensetzung und Vielfalt der Bodenpilze in Rein- und Buchenmischbeständen ist jedoch wenig bekannt.

In dieser Studie wurde zunächst untersucht, welche Faktoren die saisonalen Änderungen der Feinwurzelbiomasse der Rotbuche entlang eines Klima- und Boden-P-Gradienten erklären können. Die Feldversuche wurden an Untersuchungsstandorten mit niedrigem, mittlerem und hohem P-Gehalt im Boden durchgeführt. Die Versuchsflächen bestehen aus buchendominierten Parzellen, die entweder ungedüngt, mit N, mit P oder einer Kombination aus N und P gedüngt wurden. Diese Versuchsflächen wurden auch genutzt, um die Auswirkungen der Nährstoffdynamik auf die Zusammensetzung und Vielfalt der Feinwurzelbiomasse zu

untersuchen. Feinwurzelbiomasse, Boden- und Wurzelelemente wurden anhand von im Frühjahr und Herbst entnommenen Bodenkernen analysiert. Es wurde festgestellt, dass die Feinwurzelbiomasse nur am Standort mit niedrigem P-Gehalt im Herbst unter P-Düngung zunahm. Eine höhere Feinwurzelbiomasse im Herbst als im Frühjahr wurde nur an den Standorten mit mittlerem und hohem P-Gehalt beobachtet, nicht aber am Standort mit niedrigem P-Gehalt. Es wurde ein allgemeines Modell, das alle gemessenen Variablen [Standort (P-niedrig, P-mittel, P-hoch), Düngemittelbehandlung (ungedüngt, N, P, P+N), Jahreszeit (Frühling, Herbst), Klima (Temperatur, Niederschlag), Boden- und Wurzelelemente (gesamtes und lösliches P, C, N, C/N-Verhältnis, K, Na, Ca, Mg, Mg, Fe, S), Boden-pH und Wassergehalt)] enthält, erstellt und einer schrittweisen Regression unterzogen. Die Variablen, die in dem Modell mit dem niedrigsten Akaike-Informationskriterium enthalten waren, wurden zur Aufteilung der Varianz der Feinwurzelbiomasse verwendet. Die Bodenchemie, einschließlich des Bodenwassergehalts, erklärte einen großen Teil der Variation der Feinwurzelbiomasse sowohl in der organischen Schicht als auch im Mineralboden, während das Klima einen großen Teil der Variation der Wurzelbiomasse im Mineralboden erklärte. Die saisonalen Muster der Feinwurzelbiomasse wurden daher auf Veränderungen der Nährstoffdynamik und des Klimas zurückgeführt, waren jedoch von der Bodenschicht und dem Standort abhängig. Geringe Schwankungen der Biomasse wurden durch Wurzelressourcen in der organischen Schicht erklärt, was darauf hindeutet, dass in der organischen Schicht die Bodenressourcen aufgrund der hohen biologischen Aktivität und der mikrobiellen Interaktionen dominieren. Des Weiteren wurden die Auswirkungen der Nährstoffdynamik auf die Zusammensetzung und Vielfalt der EMP an diesen Untersuchungsstandorten durch Morphotypisierung und ITS-Sequenzierung untersucht. Die Ergebnisse zeigten, dass weder die Zusammensetzung noch der Reichtum und die Vielfalt der EMP durch die Düngemaßnahmen beeinflusst wurden. Dies stand im Gegensatz zu den Erwartungen und deutet darauf hin, dass die EMP als Reaktion auf eine moderate Nährstoffzufuhr stabil sind. Dieses Ergebnis stützt die Annahme, dass EMP in Buchenwäldern relativ resistent gegenüber N-Zugaben sind. Die Analyse von Pilzen mittels DNA-Barcoding auf Ordnungsebene ergab jedoch negative und positive Reaktionen von Russulales und Boletales auf P- bzw. P+N-Zugaben. Über alle untersuchten Standorte hinweg wurde festgestellt, dass Russulales in Böden mit hohem N-Gehalt angereichert waren. Diese Ergebnisse

deuten darauf hin, dass die Pilzstrukturen durch die Verfügbarkeit von Nährstoffen beeinflusst werden.

Um die Auswirkungen der Einführung von Douglasie entweder in Reinbeständen oder in Mischung mit Buche auf die Bodenpilzstrukturen zu untersuchen, wurden Feldversuche in einer trockenen und nährstoffarmen Region und in einer feuchten und nährstoffreichen Region durchgeführt. In jeder Region wurden vier Standorte genutzt, die entweder Parzellen mit Reinbeständen (Buche, Fichte, Douglasie) oder Mischbeständen aus Buche und Nadelbaum (Buche-Fichte, Buche-Douglasie) enthielten. Die Analyse der Pilzgemeinschaft im Boden (0-10 cm Tiefe nach Entfernung der nicht zersetzten Streuschicht) erfolgte durch Barcoding der ITS-Region und Illumina-Sequenzierung. Bodenelemente (C, N, P, K, Na, Ca, Mg, Mn, Fe, S), das C/N-Verhältnis, der pH-Wert des Bodens und der relative Wassergehalt des Bodens wurden bestimmt und als erklärende Variablen für die Pilzzusammensetzung verwendet. Die Ergebnisse zeigten deutliche Unterschiede in den Pilzgemeinschaften zwischen nährstoffreichen und nährstoffarmen Standorten sowie zwischen den Beständen. Die Pilzgemeinschaften wurden nach der Zusammensetzung der Baumarten getrennt und durch die Bodenchemie erklärt. Die Pilzzusammensetzung in Fichten- und Douglasienwäldern war ähnlich. Zwischen reinen Buchen- und reinen Fichten- (oder reinen Douglasien-) Beständen wurden in gemischten Buchen-Nadelholz-Beständen intermediäre Pilzzusammensetzungen beobachtet. Die Waldtypen hatten keinen Einfluss auf den Artenreichtum der Mykorrhizapilze. Allerdings nahm die relative Häufigkeit der Symbiotrophen in reinen Douglasien- und Buchen-Douglasien-Mischbeständen im Vergleich zu den einheimischen Arten ab. Die relative Häufigkeit der saprotrophen Pilzordnungen Tremellales und Hymenochaetales nahm in Nadelbaumbeständen zu, während die Häufigkeit anderer Pilzordnungen von der Baumartenzusammensetzung und den Standorteigenschaften abhing. Die Ähnlichkeit der Pilzarten, die in nicht einheimischer Douglasie und einheimischer Fichte beobachtet wurden, deutet darauf hin, dass Douglasie einheimische Pilze beherbergen kann. Reine Douglasie und gemischte Buchen-Douglasien begünstigten jedoch die relative Häufigkeit von saprotrophen Pilzen. Ob sich das veränderte Verhältnis zwischen Mykorrhiza und Saprotrophen auf den Nährstoffumsatz auswirkt, muss in Zukunft noch untersucht werden.

Insgesamt zeigt diese Arbeit, dass die Reaktionen von Pilzen und Wurzelbiomasse hauptsächlich vom Klima und den Nährstoffen abhängen. Der jahreszeitliche Wechsel der Feinwurzelbiomasse zwischen Herbst und Frühjahr (d. h. im Frühjahr höher als im Herbst in der organischen Schicht am Standort mit niedrigem P-Gehalt und umgekehrt am Standort mit hohem P-Gehalt) weist auf die Plastizität der Feinwurzelbiomasse in Abhängigkeit von der Nährstoffverfügbarkeit hin. Offensichtlich kann die Buche ihr Wurzelsystem anpassen, um mit Veränderungen der Nährstoffversorgung fertig zu werden. Ob die in dieser Studie beobachtete jahreszeitliche Reaktion der Feinwurzelbiomasse der Buche entlang des Klima- und Boden-P-Gradienten auf Buchen-Nadelbaumbestände extrapoliert werden kann, muss untersucht werden. Denn in Buchen-Nadelbaum-Mischbeständen sind Veränderungen der Nährstoffe, die auf unterschiedliche Streueinträge zurückzuführen sind, sowie Veränderungen der mikrobiellen Gemeinschaften zu erwarten. Darüber hinaus beobachteten wir Unterschiede zwischen den Pilzgemeinschaften in reinen Buchen- und Nadelbaumbeständen und intermediäre Muster in Buchen-Nadel-Mischbeständen in der obersten Schicht des Waldbodens. Wie die Baumarten die Pilzgemeinschaften in tieferen Bodenhorizonten aufgrund von Schwankungen in der Nährstoffverfügbarkeit und der mikrobiellen Aktivität beeinflussen werden, ist noch unbekannt. Die relativ stabile Reaktion von EMP auf Nährstoffzugaben deutet darauf hin, dass Buchenwälder bei der Nährstoffaufnahme immer noch auf mikrobielle Interaktionen angewiesen sind, doch sind weitere Informationen darüber erforderlich, wie Umweltschwankungen und anthropogene Ablagerungen, verschiedene Arten von Streueintrag und unterschiedliche Wurzelverteilung die Ernährung des Waldes beeinflussen. Diese Studie unterstreicht, dass Wurzelbiomasse, Boden und Wurzelpilze für die Anpassung der Bäume an sich verändernde Umweltbedingungen wichtig sind und einen Beitrag zum Wissen über eine nachhaltige Waldbewirtschaftung leisten.

CHAPTER 1: GENERAL INTRODUCTION

1.1. Temperate forests and global change

Forest ecosystems are essential components of the biosphere and provide ecosystem functions and services, which include climate regulation, provision of food, timber, shelter and fostering cultural and recreational activities (Bauhus et al. 2010; Gilliam, 2016; Mori, 2017, Simons et al. 2021). Thus, dependence of society on ecosystem services and functions underpins the value of nature (Cardinale, 2012; Braat and De Groot, 2012). For example, temperate forests contribute substantially to Earth 's carbon budget through their net primary productivity of about 8 Pg C yr^{-1}, highlighting their importance for carbon sequestration (Saugier et al. 2002). Temperate forest biomes are located between 23.5°N and 66.5° S latitudes with dominant distribution in the Northern Hemisphere (North America, Eastern Asia, Central and Western Europe) and less occurrence in the Southern Hemisphere (Chile, Australia, New Zealand, Tasmania) (Gilliam, 2016).

In Central Europe, temperate forests are composed of broadleaved deciduous and conifer evergreen species, mainly beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies*) (Leuschner and Ellenberg, 2017). European beech and Norway spruce are important ecological and economical species in large parts of Europe (Leuschner et al. 2006; Leuschner and Ellenberg, 2017). Beech forests have a wide distribution, occurring on a broad range of soil types (Härdtle et al. 2004; Leuschner et al. 2006). Beech and spruce forests are susceptible to climate change and biotic calamities (Schlyter et al. 2006; Gessler et al. 2007; Bolte et al. 2010). Climate projections point to rising temperatures, altered precipitation patterns and increased extreme weather events (IPCC, 2013), which may affect forest ecosystems. Other factors jeopardizing forest ecosystems in Europe include land-use intensification, drought, windstorms and bark beetle attack (Gessler et al. 2007; Seibold et al. 2019; Biedermann et al. 2019). In addition, increases in anthropogenic N deposition (Galloway et al. 2008) may negatively impact forest ecosystem functions and biodiversity (Sala et al. 2000; Calvo-Fernandez et al. 2017). Anthropogenic N depositions in N-limited forests can stimulate growth (Du and De Vries, 2018) consequently enhancing P requirements of the forests. If the enhanced P demand is not met tree show an imbalance in their N:P stoichiometry (Vitousek et al. 2010). Plant growth and productivity in terrestrial

ecosystems are determined by N and P (Elser et al. 2007; Vitousek et al. 2010). Therefore, knowledge on the impact of nutrient dynamics on ecosystem functions, to which belowground biota contribute substantially (Baldrian, 2017; Brundrett and Tedersoo, 2018), is required. On basis of this knowledge, the management of forest biomes can be improved with a sustainable approach.

In consideration of the negative impacts of global drivers such as climate change, anthropogenic N deposition and other calamities on beech and spruce forests (Schlyter et al. 2006; Gessler et al. 2007; Bolte et al. 2010; Sala et al. 2000), future forests should have higher resistance to these threats. One solution suggested for Germany forestry is to expand the cultivation of non-native tree species such as Douglas-fir (*Pseudotsuga menziesii*). Douglas-fir, a species native to North America (Hermann, 1987), is considered a favorable species for European forestry due to its desirable growth and wood characteristics (Sicard et al. 2006; von Lüpke, 2009; Isaac-Renton et al. 2014) and the potential to coexist with other broadleaved and coniferous tree species (Rothe and Binkley, 2001). However, more information is needed to unravel the impact of Douglas-fir on native forest ecosystem functions and belowground biota, in pure and mixed tree stands along nutrient and climate gradients.

1.2. Climate and tree P nutrition

Phosphorus (P) is an essential element for plant growth in temperate forests (Elser, 2007; Vitousek et al. 2010; Du et al. 2020). P is involved in metabolic processes such as nucleic acid synthesis, photosynthesis, cellular structure synthesis and enzyme activity regulation (Raghothama, 1999; Elser et al. 2007). P availability is driven by soil type and parent material (Augusto et al. 2017; Wardle, 2004). Various fractions of P in soil are unavailable to plants since P is bound in macromolecules as P-esters (Cairney, 2011; Plassard et al. 2011) or forms precipitates with elements such as calcium, iron or aluminum (Jones and Oburger, 2011; Plassard et al. 2011). Further, P availability to plants is limited by P fixation to soil particles and low P solubility (Holford, 1997). Microbial communities in soil contribute substantially to render P plant-available through degradation of macromolecules, solubilization and mycorrhizal associations with tree roots (Becquer et al. 2014; Clausing and Polle, 2020).

Decreasing patterns of foliar P concentrations in beech forests across Europe have been reported (Talkner et al. 2015), an indication of P limitation in forest sites (Talkner

et al. 2015; Jonard et al. 2015). Further, anthropogenic N depositions, soil acidification and climate change have been reported to affect P nutrition of forest trees (Gradowski and Thomas, 2008; Prietzel and Stetter, 2010). Low P concentrations in forests and increasing N/P ratio are of great concern as they reduce forest growth (Yang et al. 2016; Prietzel and Stetter, 2010). Therefore, understanding the impact of P and interaction with N on root biomass and fungal communities is needed for sustainable management of forest ecosystems.

1.3. Fine root biomass in temperate forests

Tree fine roots (< 2 mm) are important organs for water and nutrient uptake, enabling plants to thrive in terrestrial ecosystems (Harper et al. 1991). Although fine roots constitute a small proportion of biomass in temperate and boreal forests (about 2-3%) (Vogt et al. 1996; Hertel and Leuschner, 2002), they contribute substantially to carbon and nutrient cycling (Hendrick and Pregitzer, 1993; Helmisaari et al. 2002). However, factors affecting root dynamics and the role of seasonal nutrient dynamics in this regard are still poorly understood.

Across temporal and spatial scales, tree root production, mortality and biomass are highly dynamic (Brassard et al. 2009). In addition, fine root production exhibits plasticity in response to water and nutrient availability (Nadelhoffer 2000; Brassard et al. 2009; Delpierre et al. 2016; Leuschner, 2020). Therefore, extensive studies focus on understanding root responses to changes in climate and resource availability (Norby and Jackson, 2000; Peng et al. 2017). Generally, plants shift nutrient allocation from below-to aboveground under luxurious resource availability, a reflection of resource optimization (Ågren and Franklin, 2003). Studies by several groups (Högberg, 2007; Meyer-Grünefeldt et al. 2015; Dziedek et al. 2017) have demonstrated that increases in N culminate in increased shoot-root ratios of the trees or heath plants. Consequently, trees with an increased shoot-to-root ratio may become more drought susceptible due to reduced fine root biomass (Högberg et al. 1993). However, under moderate drought, increases in fine root production have also been reported (Gaul et al. 2008). Although increasing N deposition may enhance aboveground productivity, the consequences of drought susceptibility or limitation of nutrient uptake, especially P supply are not entirely clear.

An option to mitigate negative effects of weather and climate fluctuations on temperate forest ecosystem functions is transforming monocultures into mixed tree stands. However, understanding seasonal effects on nutrients and fine root biomass along climatic and nutrient gradients requires investigation. Lwila et al. (2021) demonstrated that fine root biomass is dependent on site and climate factors in pure and beech-conifer tree stands. Further, fine root biomass is dependent on soil depth (Lang et al. 2017; Meller et al. 2020). Beech and Douglas-fir penetrates deeper soil layers than spruce (Calvaruso et al. 2011). These differences in root distribution may influence the biological and physico-chemical characteristics of respective rhizospheres (Hinsinger et al. 2005). Foltran et al. (2020) demonstrated that in contrast to pure stands, beech-conifer mixed tree stands reduces soil base cation depletion.

As postulated by the “Law of Minimum” by Justus von Liebig (1840), growth is influenced by limiting resources (Salisbury and Ross, 1992). According to this law, plants exhibiting deficiency in one of the essential nutrients exhibits poor growth. Further to this law, the theoretical concepts by Bridgham et al. (1995) elaborated resource use efficiency. Under this theoretical concept, biomass production increases following addition of the limiting resource until a particular threshold is attained where further addition of the limiting reduces biomass production. Changes in mineral nutrients and climate can impacts fine root biomass (Vogt et al. 1996; Gao et al. 2021) but whether seasonal effects contribute to modulate root biomass patterns along soil fertility gradients needs investigation.

Building on the “Law of Minimum” and resource-use efficiency concepts, we investigated the impact of nutrients and season along climatic and soil nutrient gradients on fine root biomass. We selected three study sites in Germany (Figure 1.1) showing variation in soil P contents and climate, dominated by European beech (*Fagus sylvatica* L.) tree species (Lang et al. 2017). To investigate whether nutrient limitations could be rescued by P or N addition, we conducted a full factorial experiment, adding N fertilizer in five applications of 30 kg ha^{-1} during 2 years and P (one application of 50 kg ha^{-1} and the combination of N and P fertilizes to plots in each of the forest locations. We used this design in addition to non-fertilized plots to investigate. These study sites were also used to investigate changes in soil and root fungi following P and N fertilization.

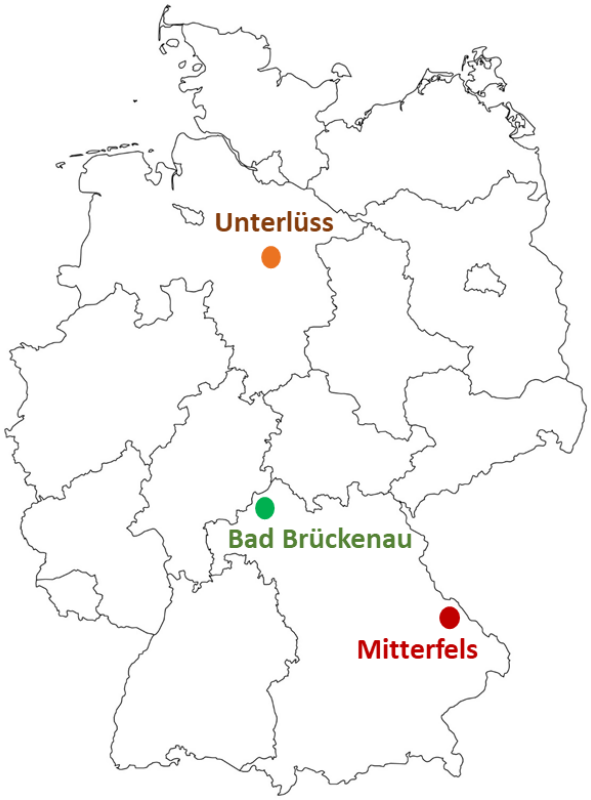


Figure 1.1. Location of the study sites of the research project Ecosystem Nutrition (SPP1685). Each study site is composed of 12 plots (three replicates per treatment: Control = no fertilizer treatment, N = 5 x 30 kg ha^{-1} nitrogen fertilizer, P = 50 kg ha^{-1} phosphorus fertilizer, N+P = combined N and P fertilizer treatment) in dominant European beech (*Fagus sylvatica* L.) stands. (map copyright: Vemaps.com).

1.4. Soil and root fungi

Fungi are a group of highly diverse eukaryotic microorganisms, which have successfully populated terrestrial ecosystems (Hawksworth, 2001; Tedersoo et al. 2014; Frąc et al. 2018). Soil and root fungi comprises major phyla such as Ascomycota and Basidiomycota (Kernaghan, 2013; Peršoh, 2015). Fungi are involved in many fundamental ecosystem functions such as nutrient cycling, water retention, soil carbon balance, and causes of or protection against plant diseases (Bardgett and Wardle, 2010; Baldrian, 2017; Brundrett and Tedersoo, 2018). Furthermore, belowground fungal communities together with other soil microbes contribute substantially to production of food, air and clean water, thereby, impacting human wellbeing (Wall et al. 2015). Based on their ecological functions, mycobiomes can be differentiated into three main categories: saprotrophs, symbiotrophs and pathotrophs (Schmit and Mueller, 2007; Nguyen et al. 2016). Saprotrophic fungi are free living and mainly involved in the decomposition of plant dead material (Rayner and Boddy, 1988), while

pathotrophic fungi negatively impact the health of plant communities, thereby, shaping vegetation composition (García-Guzmán and Heil, 2014). In temperate and boreal forests, symbiotrophs mainly comprise ectomycorrhizal fungi (EMF) (Tedersoo et al. 2014). EMF form mutualistic associations with tree roots and enhance nutrient and water acquisition by plants in exchange for carbon (Smith and Read, 1997). EMF symbiotic associations are characterized by the presence of the mantle, Hartig net (hyphal net) and external mycelium (Agerer, 1991). Based on the characteristics of emanating hyphae, EMF have been categorized into contact, short-distance, medium-distance and long-distance exploration types (Agerer, 2001) enabling nutrient acquisition from different distances. EMF are common in temperate forest soils, colonizing the majority of tree roots (Buée et al. 2009; Lang et al. 2011; Lang and Polle, 2011, Pena et al. 2010; Tedersoo et al. 2014).

The impact of European tree species and soil nutrients on soil and root associated fungal communities has been reported in previous studies (Zavišić et al. 2016; Goldmann et al. 2016; Bahnmann et al. 2018). However, we know little about the influence of Douglas fir on the soil mycobiome in German forests. There is concern that non-native species may have negative effects on soil diversity (Schmid et al. 2014). To assess sustainable forest management options, it is important to understand whether changes in tree composition introducing non-native tee species, both in pure and mixed stands impact fungal communities and their functional composition.

To gain knowledge if Douglas-fir causes shifts in soil fungal communities compared with the major German forest tree species, beech and spruce, mature forests composed of either the pure species or their mixtures (beech-Douglas-fir, beech-spruce) were selected in eight sites (Figure 1.2). The sites differ in soil conditions and climate (Foltran et al. 2020; Ammer et al. 2020). Lwila et al. (2021) also reported variations in fine root biomass between the categories of the sites (i.e. higher fine root biomass at nutrient-poor and dry sites than nutrient-rich and humid sites). Therefore, these forests were chosen to investigate the influence of tree species composition and soil properties on soil fungal composition and associated functional guilds.

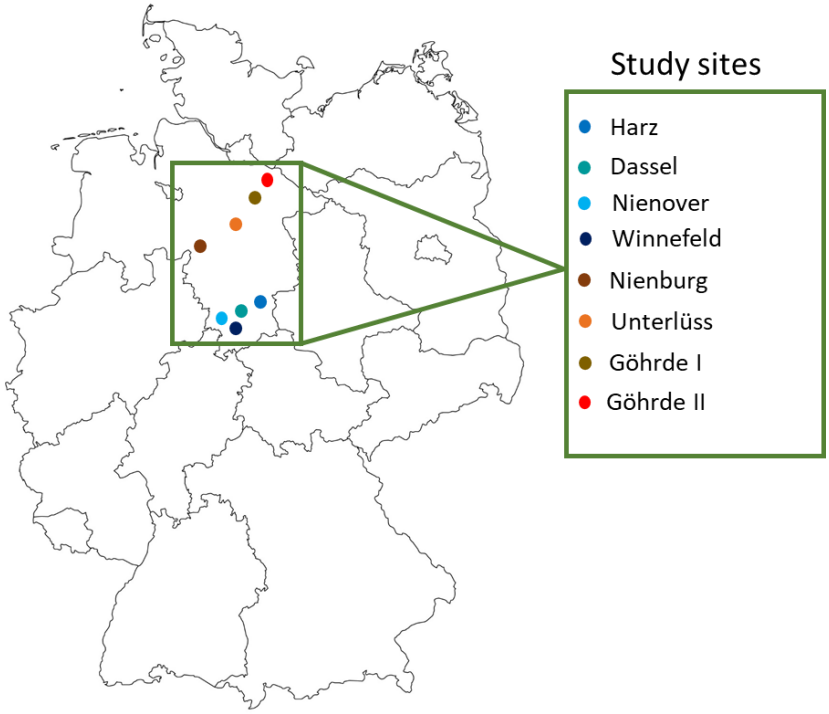


Figure 1.2. Location of the study sites of the Research Training Group 2300 (RTG2300) showing the northern (Nienburg, Unterlüss, Göhrde I and II) and southern (Harz, Dassel, Nienover and Winnefeld) sites. Each site is composed of 5 plots representing forest stands (pure stands: beech, Norway spruce and pure Douglas-fir; mixtures: beech-spruce and beech-Douglas-fir). (map copyright: Vemaps.com).

1.5. Goal of the research study

The main goals of this research were to elucidate the changes in fine root biomass and fungal composition under different forms of forest management (species mixture and nutrient addition) in temperate forests. The following specific goals were addressed:

to determine the impact of nutrients, season and climate on fine root biomass in European beech (*Fagus sylvatica* L.) dominated tree stands (**Chapter 2**)

to assess changes in EMF composition under P and N fertilization along a nutrient and climate gradient in European beech dominated tree stands (**Chapter 3**)

to determine the impact of tree species composition and soil properties on fungal communities in pure and beech-conifer mixed stands along a climatic gradient. This specific goal focused on changes in soil fungal communities when European beech (*Fagus sylvatica* L.) was mixed with native (*Picea abies*) and non-native (*Pseudotsuga menziesii*) conifers along nutrient and climatic gradients (**Chapter 4**)

1.6. References

Agerer, R., 1991. Characterization of ectomycorrhiza. Methods in Microbiology 23, 26-73. doi: 10.1016/S0580-9517(08)70172-7

Agerer, R., 2001. Exploration types of ectomycorrhizae. Mycorrhiza 11, 107–114. doi: 10.1007/ s005720100 108

Ågren, G.I., Franklin, O., 2003. Root: shoot ratios, optimization and nitrogen productivity. Annals of Botany 92, 795e800. doi: 10.1093/aob/mcg203

Ammer, C., Annighöfer, P., Balkenhol, N., Hertel, D., Leuschner, C., Polle, A., Lamersdorf, N., Scheu, S., Glatthorn, J., 2020. RTG 2300-study design, location, topography and climatic conditions of research plots in 2020. PANGAEA. doi: pangaea.de/10.1594/PANGAEA.923125

Augusto, L., Achat, D. L., Jonard, M., Vidal, D., and Ringeval, B., 2017. Soil parent material – A major driver of plant nutrient limitations in terrestrial ecosystems, Global Change Biology 23, 3808–3824. doi: 10.1111/gcb.13691, 2017

Bahnmann, B., Mašínová, T., Halvorsen, R., Davey, M.L., Sedlák, P., Tomšovský, M., Baldrian, P., 2018. Effects of oak, beech and spruce on the distribution and community structure of fungi in litter and soils across a temperate forest. Soil Biology and Biochemistry 119, 162–173. doi: 10.1016/j.soilbio.2018.01.021

Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiology Reviews 41, 109–130. doi: 10.1093/femsre/fuw040

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

Bauhus, J., van der Meer, P., Kanninen, M., 2010. Ecosystem Goods and Services from Plantation Forests. Earthscan, London, UK. ISBN: 978-1-84971-168-5

Becquer, A., Trap, J., Irshad, U., Ali, M.A., Claude, P., 2014. From soil to plant, the journey of P through trophic relationships and ectomycorrhizal association. Frontiers in Plant Science 5, 548. doi:10.3389/fpls.2014.00548

Biedermann, P.H.W., Müller, J., Grégoire, J.C., Gruppe, A., Hagge, J., Hammerbacher, A., Hofstetter, R.W., Kandasamy, D., Kolarik, M., Kostovcik,

M., Krokene, P., Sallé, A., Six, D.L., Turrini, T., Vanderpool, D., Wingfield, M.J., Bässler, C., 2019. Bark beetle population dynamics in the Anthropocene: Challenges and solutions. Trends in Ecology and Evolution 34, 914–924. doi: 10.1016/j.tree.2019.06.002

Bolte, A., Hilbrig, L., Grundmann, B., Kampf, F., Brunet, J., Roloff, A., 2010. Climate change impacts on stand structure and competitive interactions in a southern Swedish spruce–beech forest. European Journal of Forest Research 129 (3), 261–276. doi: 10.1007/s10342-009-0323-1

Braat, L.C., De Groot, R.S., 2012. The ecosystem services agenda: bridging the worlds of natural science and economics, conservation and development, and public and private policy. Ecosystem Services 1 (1), 4–15. doi: 10.1016/j.ecoser.2012.07.011

Brassard, B.W., Chen, H.Y.H., Bergeron, Y., 2009. Influence of environmental variability on root dynamics in Northern forests. Critical Reviews in Plant Sciences 28(3), 179-197, doi: 10.1080/07352680902776572

Bridgham, S.D., Pastor, J., McClaugherty, C.A., Richardson, C.J., 1995. Nutrient-use efficiency: a litterfall index, a model, and a test along a nutrient-availability gradient in North Carolina peatlands. The American Naturalist 145, 1–21

Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220 (4), 1108–1115. doi: 10.1111/nph.14976

Buée, M., Boer, W., Martin, F., Overbeek, L., Jurkevitch, E., 2009. The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant and Soil 321, 189–212. doi: 10.1007/s11104-009-9991-3

Cairney, J.W.G., 2011. Ectomycorrhizal fungi: the symbiotic route to the root for phosphorus in forest soils. Plant and Soil 344, 51-71. doi: 10.1007/s11104-011-0731-0

Calvaruso, C., N'Dira, V., Turpault, M.P., 2011. Impact of common European tree species and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) on the

physicochemical properties of the rhizosphere. Plant and Soil 342, 469–480. doi: 10.1007/s11104-010-0710-x

Calvo-Fernandez, J., Marcos, E., Calvo, L., 2017. Bulk deposition of atmospheric inorganic nitrogen in mountainous heathland ecosystems in North-Western Spain. Atmospheric Research 183, 237-244. doi: 10.1016/j.atmosres.2016.09.006

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67. doi: 10.1038/nature11148

Clausing, S., Polle, A., 2020. Mycorrhizal Phosphorus Efficiencies and Microbial Competition Drive Root P Uptake. Frontiers in Forests and Global Change 3, 54. doi: 10.3389/ffgc.2020.00054

Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., Rutishauser, T., Rathgeber, C.B.K., 2016. Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. Annals of Forest Science 73, 5–25. doi: 10.1007/s13595-015-0477-6

Du, E., De Vries, W., 2018. Nitrogen-induced new net primary production and carbon sequestration in global forests. Environmental Pollution 242, 1476–1487. doi: org/ 10.1016/j.envpol.2018.08.041

Du, E., Terrer, C., Pellegrini, A.F., Ahlström, A., van Lissa, C.J., Zhao, X., Xia, N., Wu, X., Jackson, R.B., 2020. Global patterns of terrestrial nitrogen and phosphorus limitation. Nature Geoscience 13 (3), 221–226. doi: 10.1038/s41561-019-0530-4

Dziedek, C., Fichtner, A., Calvo, L., Marcos, E., Jansen, K., Kunz, M., Walmsley, D., von Oheimb, G., Härdtle, W., 2017. Phenotypic plasticity explains response patterns of European beech (*Fagus sylvatica* L.) saplings to nitrogen fertilization and drought events. Forests 8, 91. doi:10.3390/f8030091

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10, 1135–1142. doi: 10.1111/j.1461- 0248.2007.01113.x

Foltran, C.E., Ammer, C., Lamersdorf, N., 2020. Douglas fir and Norway spruce admixtures to European beech along a site gradient in Northern Germany – Are soil nutrient conditions affected? bioRxiv. doi: 10.1101/2020.09.25.313213

Frąc, M., Hannula, S.E., Bełka, M., Jędryczka, M., 2018. Fungal biodiversity and their role in soil health. Frontiers in Microbiology 9, 707. doi: 10.3389/fmicb.2018.00707

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., et al., 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320, 889–892. doi: 10.2307/20054730

Gao, G., Liu, Z., Wang, Y., Wang, S., Ju, C., Gu, J., 2021. Tamm Review: Fine root biomass in the organic (O) horizon in forest ecosystems: Global patterns and controlling factors. Forest Ecology and Management 491, 119208. doi: 10.1016/j.foreco.2021.119208

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

Gaul, D., Hertel, D., Borken, W., Matzner, E., Leuschner, C., 2008. Effects of experimental drought on the fine root system of mature Norway spruce. Forest Ecology and Management 256 (5), 1151-1159. doi: 10.1016/j.foreco.2008.06.016

Gessler, A., Keitel, C., Kreuzwieser, J., Matyssek, R., Seiler, W., Rennenberg, H., 2007. Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. Trees 21 (1), 1–11. doi: 10.1007/s00468-006-0107-x

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

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

Gradowski, T., Thomas, S.C., 2006. Phosphorus limitation of sugar maple growth in Central Ontario. Forest Ecology and Management 226, 104–109. doi: 10.1016/j.foreco.2005.12.062

Härdtle, W., Von Oheimb, G., Friedel, A., Meyer, H., Westphal, C., 2004. Relationship between pH-values and nutrient availability in forest soils – the consequences for the use of ecograms in forest ecology. Flora 199,134–142

Harper, J. L., Jones, M., Sackville Hamilton, N. R., 1991. The evolution of roots and the problems of analyzing their behaviour. In: Plant Root Growth, an Ecological Perspective. pp. 3–22. Atkinson, D., Ed., Blackwell, London

Hawksworth, D.L., 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological Research 105, 1422–1432. doi: 10.1017/S0953756201004725

Helmisaari, H. S., Makkonen, K., Kellomäki, S., Valtonen, E., and Mälkönen, E., 2002. Below- and above-ground biomass, production and nitrogen use in Scots pine stands in eastern Finland. Forest Ecology and Management 165, 317–326

Hendrick, R. L., Pregitzer, K. S., 1993. Patterns of fine root mortality in two sugar maple forests. Nature 361, 59–61. doi: 10.1038/361059a0

Hermann, R.K., 1987. North American tree species in Europe. J. Forest. 85 (12), 27–32

Hertel, D., Leuschner, C., 2002. A comparison of four different fine root production estimates with ecosystem carbon balance data in a Fagus-Quercus mixed forest. Plant and Soil 239, 237–251. doi: 10.1023/A:1015030320845

Hinsinger, P., Gobran, G.R., Gregory, P.J., 2005. Rhizosphere geometry and heterogeneity arising from root mediated physical and chemical processes. New Phytologist 68, 293–303. doi: 10.1111/j.1469-8137.2005.01512.x

Högberg, P., 2007. Nitrogen impacts on forest carbon. Nature 447, 781-782

Högberg, P., Johannisson, C., Hällgren, J.E., 1993. Studies of 13C in the foliage reveal interactions between nutrients and water in forest fertilization experiments. Plant and Soil 152 (2), 207-214. doi: 10.1007/bf00029090

Holford, I.C.R., 1997. Soil phosphorus: its measurement, and its uptake by plants. Australian Journal of Soil Research 35, 227–239

IPCC., 2013. Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA

Isaac-Renton, M.G., Roberts, D.R., Hamann, A., Spiecker, H., 2014. Douglas-fir plantations in Europe: a retrospective test of assisted migration to address climate change. Glob. Change Biol. 20 (8), 2607–2617. doi: 10.1111/gcb.2014.20.issue-810.1111/gcb.12604

Jonard, M., Fürst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., Waldner, P., Benham, S., Hansen, K., Merilä, P., Ponette, Q., de la Cruz, A.C., Roskams, P., Nicolas, M., Croisé, L., Ingerslev, M., Matteucci, G., Decinti, B., Bascietto, M., Rautio, P., 2015. Tree mineral nutrition is deteriorating in Europe. Global Change Biology 21, 418–430. doi: 10.1111/gcb.12657

Jones, D.L., Oburger, E., 2011. Solubilization of phosphorus by soil microorganisms. In: Büneman, E. (Ed.), Phosphorus in Action. Springer, Berlin Heiderlberg, 169-198. doi: 10.1007/978-3-642-15271-9_7

Kernaghan, G., 2013. Functional diversity and resource partitioning in fungi associated with the fine feeder roots of forest trees. Symbiosis 61, 113–123

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

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

Lang, F., Krüger, J., Amelung, W., Willbold, S., Frossard, E., Bünemann, E. K., et al., 2017. Soil phosphorus supply controls P nutrition strategies of beech forest

ecosystems in Central Europe. Biogeochemistry 136, 5–29. doi: 10.1007/s10533017-0375-0

Leuschner, C., 2020. Drought response of European beech (*Fagus sylvatica* L.) – a review. Perspectives in Plant Ecology, Evolution and Systematics 47, 125576. doi: doi: 10.1016/j.ppees.2020.125576

Leuschner, C., Ellenberg, H., 2017. Ecology of Central European forests. Springer Verlag, Cham Switzerland

Leuschner, C., Meier, I.C., Hertel, D., 2006. On the niche breadth of Fagus sylvatica: soil nutrient status in 50 Central European beech stands on a broad range of bedrock types. Annals of Forest Science 63, 335–368. doi: 10.1051/forest:2006016

Lwila, A.S., Mund, M., Ammer, C., Glatthorn, J., 2021. Site conditions more than species identity drive fine root biomass, morphology and spatial distribution in temperate pure and mixed forests. Forest Ecology and Management 499, 119581. doi: 10.1016/j.foreco.2021.119581

Meller, S., Frossard, E., Spohn, M., Luster, J., 2020. Plant nutritional status explains the modifying effect of provenance on the response of beech sapling root traits to differences in soil nutrient supply. Frontiers in Forests and Global Change. 3, 535117. doi: 10.3389/ffgc.2020.535117

Meyer-Grünefeldt, M., Friedrich, U., Klotz, M., von Oheimb, G., Härdtle, W., 2015. Nitrogen deposition and drought events have non-additive effects on plant growth - Evidence from greenhouse experiments. Plant Biosystems 149, 424e432. doi: 10.1080/11263504.2013.853699

Mori, A.S., 2017. Biodiversity and ecosystem services in forests: management and restoration founded on ecological theory. Journal of Applied Ecology 54(1), 7–11. doi: 10.1111/1365-2664.12854

Nadelhoffer, K.J., 2000. The potential effects of nitrogen deposition on fine-root production in forest ecosystems. New Phytologist 147, 131-139

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing

fungal community datasets by ecological guild. Fungal Ecology 20, 241–248. doi: 10.1016/j.funeco.2015.06.006

Norby, R.J., Jackson, R.B., 2000. Root dynamics and global change seeking an ecosystem perspective. New Phytologist 147, 3–12. doi: 10.1046/j.1469-8137.2000.00676.x

Pena, R., Offermann, C., Simon, J., Naumann, P.S., Gessler, A., Holst, J., Dannenmann, M., Mayer, H., Kögel-Knabner, I., Rennenberg, H., Polle, A., 2010. Girdling affects ectomycorrhizal fungal (EMF) diversity and reveals functional differences in EMF community composition in a beech forest. Applied and Environmental Microbiology 76, 1831-1841. doi: 10.1128/AEM.01703-09

Peng, Y., Guo, D., Yang, Y., 2017. Global patterns of root dynamics under nitrogen enrichment. Global Ecology and Biogeography 26,102–114. doi: 10.1111/geb.12508

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., Janssens, I.A., 2013. Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nature Communications 4, 2934. doi: 10.1038/ncomms3934

Peršoh, D., 2015. Plant-associated fungal communities in the light of meta'omics. Fungal Divers 75, 1–25. doi: 10.1007/s13225-015-0334-9

Plassard, C., Louche, J., Ali, M.A., Duchemin, M., Legname, E., Cloutier-Hurteau, B., 2011. Diversity in phosphorus mobilisation and uptake in ectomycorrhizal fungi. Annals of Forest Science 68, 33-43. doi: 10.1007/s13595-010-0005-7

Prietzel, J., Stetter, U., 2010. Long-term trends of phosphorus nutrition and topsoil phosphorus stocks in unfertilized and fertilized Scots pine (*Pinus sylvestris*) stands at two sites in Southern Germany. Forest Ecology and Management 259, 1141-1150. doi: 10.1016/j.foreco.2009.12.030

Raghothama, K.G., Karthikeyan, A. S., 2005. Phosphate acquisition. Plant and Soil 274, 37. doi: 10.1007/s11104-004-2005-6

Rayner, A.D.M., Boddy, L., 1988. Fungal decomposition of wood, its biology and ecology. John Wiley & Sons Ltd, Chichester, UK

Rothe, A., Binkley, D., 2001. Nutritional interactions in mixed species forests: A synthesis. Can. J. For. Res. 31 (11), 1855–1870. doi: 10.1139/x01-120

Sala, O.E., Chapin, F.S III., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770–1774. doi: 10.1126/science.287.5459.1770

Salisbury, F.B., Ross, C.W., 1992. Plant Physiology, Hormones and Plant Regulators: Auxins and Gibberellins. 4th Edition, Wadsworth Publishing, Belmont, 357-381

Saugier, B.G-J., Roy, J., Mooney, H.A., 2002. Terrestrial global productivity. Annals of Botany, 89(6), 797. doi: 10.1093/aob/mcf106

Schlyter, P., Stjernquist, I., Bärring, L., Jönsson, A.M., Nilsson, C., 2006. Assessment of the impacts of climate change and weather extremes on boreal forests in northern Europe, focusing on Norway spruce. Climate Research 31, 75–84. doi: 10.3354/cr031075

Schmid, M., Pautasso, M., Holdenrieder, O., 2014. Ecological consequences of Douglas fir (*Pseudotsuga menziesii*) cultivation in Europe. European Journal of Forest Research 133, 13—29. doi: 10.1007/s10342-013-0745-7

Schmit, J.P., Mueller, G.M., 2007. An estimate of the lower limit of global fungal diversity. Biodiversity and Conservation 16 (1), 99–111. doi: 10.1007/s10531-006-9129-3

Seibold, S., Gossner, M.M., Simons, N.K., Blüthgen, N., Müller, J., Ambarlı, D., Ammer, C., Bauhus, J., Fischer, M., Habel, J.C., Linsenmair, K.E., Nauss, T., Penone, C., Prati, D., Schall, P., Schulze, E.D., Vogt, J., Wöllauer, S., Weisser, W.W., 2019. Arthropod decline in grasslands and forests is associated with landscape-level drivers. Nature 574, 671–674. doi: 10.1038/s41586-019-1684-3

Sicard, C., Saint-Andre, L., Gelhaye, D., Ranger, J., 2006. Effect of initial fertilization on biomass and nutrient content of Norway spruce and Douglas-fir plantations at the same site. Trees 20, 229–246. doi: 10.1007/s00468-005-0030-6

Simons, N. K., Felipe-Lucia, M. R., Schall, P., Ammer, C., Bauhus, J., Blüthgen, N., Boch, S., Buscot, F., Fischer, M., Goldmann, K., Gossner, M.M., Hänsel, F., Jung, K., Manning, P., Nauss, T., Oelmann, Y., Pena, R., Polle, A., Renner, S.C., Schloter, M., Schöning, I., Schulze, E-D., Solly, E.F., Sorkau, E., Stempfhuber, B., Wubet, T., Müller, J., Seibold, S., Weisser, W. W., 2021. National forest inventories capture the multifunctionality of managed forests in Germany. Forest Ecosystems 8 (5), 19. doi: 10.1186/s40663-021-00280-5

Smith, S.E., Read, D.J., 1997. Mycorrhizal symbiosis. USA Academic Press Inc., San Diego, CA

Talkner, U., Meiwes, K., Potočič, N., Seletković, I., Cools, N., De Vos, B., Rautio, P., 2015. Phosphorus nutrition of beech (*Fagus sylvatica* L.) is decreasing in Europe. Annals of Forest Science 72, 919-928. doi: 10.1007/s13595-015-0459-8

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

Vitousek, P. M., Porder, S., Houlton, B. Z., Chadwick, O. A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecological Applications 20, 5–15. doi: 10.1890/08-0127.1

Vogt, K.A., Vogt, D.J., Palmiotto, P.A., Boon, P., O'Hara, J., Asbjornsen, H., 1996. Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. Plant and Soil 187, 159-219. Doi: 10.1007/BF00017088

von Lüpke, B., 2009. Überlegungen zur Baumartenwahl und Verjüngungsverfahren bei fortschreitender Klima¨anderung in Deutschland. Forstarchiv 80, 67–75

Wall, D. H., Nielsen, U. N., Six, J., 2015. Soil biodiversity and human health. Nature 528, 69–76. doi: 10.1038/nature15744

Wardle, D.A., 2004. Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305, 509–513. doi: 10. 1126/science.10987 78

Yang, N., Zavišić, A., Pena, R., Polle, A., 2016. Phenology, photosynthesis, and phosphorus in European beech (*Fagus sylvatica* L.) in two forest soils with contrasting P contents. Journal of Plant Nutrition and Soil Science 179, 151-158. doi: 10.1002/jpln.201500539

Zavišić, A., Nassal, P., Yang, N., Heuck, C., Spohn, M., Marhan, S., Pena, R., Kandeler, E., Polle, A., 2016. Phosphorus availabilities in beech (*Fagus sylvatica* L.) forests impose habitat filtering on ectomycorrhizal communities and impact tree nutrition. Soil Biology and Biochemistry 98, 127–137. doi: 10.1016/j.soilbio.2016.04.006

CHAPTER 2: FINE ROOT BIOMASS OF EUROPEAN BEECH TREES IN DIFFERENT SOIL LAYERS SHOW DIFFERENT RESPONSES TO SEASON, CLIMATE AND SOIL NUTRIENTS

2.1. Introduction

In undisturbed temperate forest ecosystems, nitrogen (N) and phosphorus (P) are major nutrients that often limit tree growth (Vitousek et al. 2010; Du et al. 2020). Increased anthropogenic N deposition (Galloway et al. 2008) can stimulate net primary productivity in N-limited forests (Du and De Vries, 2018). Enhanced productivity requires enhanced supply with mineral nutrients and thus, can shift tree nutrition from N to P limitation (Jonard et al. 2015; Peñuelas et al. 2013). Therefore, deterioration of P nutrition might be a consequence of enhanced N availabilities (Talkner et al. 2015). Increased availabilities of both P and N often had synergistic, positive effects on tree species, while effects of single nutrient applications on tree growth were less clear (Elser et al. 2007).

Tree fine roots play a central role in nutrient and water uptake (Vogt et al. 1996; McCormack et al. 2015). Fine roots exhibit distinct seasonal trends of production and mortality (Brassard et al. 2009). Further, root production is highly plastic, responding to soil nutrient and water availabilities (Nadelhoffer 2000; Brassard et al. 2009; Delpierre et al. 2016; Leuschner, 2020). In general, increases in nutrient availability result in higher resource allocation to above- than to belowground tissues, a behavior that has been hypothesized to reflect resource optimization (Ågren and Franklin, 2003). Field observations in northern forests and greenhouse studies with tree seedlings showed that an increase in N decreased the root-to-shoot ratio (Högberg, 2007; Meyer-Grünefeldt et al. 2015; Dziedek et al. 2017), specifically due to decreases in the fine root biomass (Li et al. 2016). Since fine roots are key to soil foraging, the decrease in fine root biomass along with increased aboveground biomass is thought to increase drought sensitivity of trees (Högberg et al. 1993). However, some tree species may compensate this risk by enhanced fine root production under drought stress (Leuschner, 2020). Thus, climate variables and nutrients are both important drivers of root biomass (Vogt et al. 1996; Gao et al. 2021) but the relative importance of these factors in soils with strongly different nutrient pools is not yet fully understood.

In Central Europe, European beech (*Fagus sylvatica* L.) is a dominant forest tree species stocking on a vast range of soil types (Leuschner, 2020). In beech forests, fine root biomass and turnover are highly variable, depending on the environmental conditions and soil depth (Kirfel et al. 2019, Meller et al. 2020). For example, Lwila et al. (2021) found higher fine root biomass at drier and nutrient poor sites than in humid and nutrient rich beech stands. Lang et al. (2017) reported increasing fine root biomass of beech in the forest floor with decreasing soil P stocks in the mineral horizon. Clausing and Polle (2020) showed that organic and mineral soil layers played crucial but divergent roles for P uptake under high and low P availabilities. In P poor soil, high fine root biomass and efficient mycorrhizal associations in the organic layer governed P uptake, whereas in P rich soil, P foraging was mainly taking place in the mineral top soil (Clausing and Polle, 2020). Although inverse relationships of soil P contents and root biomass have often been observed (Schneider et al. 2001; Lang et al. 2017), the importance of soil P, soil N or other mineral elements relative to climate and season for the dynamics of fine root biomass in temperate beech forests remains unknown. Since roots also constitute a significant carbon sink (Godbold and Brunner, 2007; Robinson, 2007, Whitehead, 2011; Reich et al. 2014), it is highly relevant to understand which variables are the major drivers for root biomass in beech forests stocking on different soil types.

In this study, we investigated temporal variation of P, N, and fine root biomass in the organic layer and mineral top soil of three beech forests differing in soil P stocks and climate. To assess the impact of enhanced P and N availabilities, we used P, N or N+P fertilized as well as unfertilized plots in each forest (Clausing et al., 2021). Our previous study showed that the fertilizer treatments had only small effects on soil and root-associated fungal community composition (Clausing et al. 2021). Here, we focused on seasonal nutrient and root dynamics after fertilization. According to theoretical concepts (Bridgham et al. 1995), very low resource availability limits biomass production; with increasing, yet limiting resources, biomass increases up to a certain threshold level; further resource increase causes declining resource efficiencies and thus, the increase in biomass production levels off. Very high nutrient availability will result in decreased fine root biomass. Consequently, we expected that P fertilization could increase root biomass, when soil P availability was a limiting factor, while under high soil P availabilities, N might be a limiting resource. We hypothesized:

(i) fertilization increases fine root biomass, if the added resource was severely growth-limiting and decreases root biomass under luxurious resource availabilities. (ii) The effects of soil P or N resources on root biomass are higher in fall than in spring because biomass allocation is seasonally shifting from above- to belowground (Brassard et al. 2009; Heid et al. 2018). (iii) Climate has a dominant effect on fine root biomass in the organic and soil nutrient resources in the mineral soil because the organic layer is more subjected to fluctuations in precipitation and temperature than mineral soil. To test our hypotheses, we determined the trajectories of fine root biomass, soil and root nutrients by sequential soil coring in spring and fall for 2.5 years and dissected the contributions of climate, fertilization, soil and root chemistry for fine root biomass by variance partitioning.

2.2. Materials and methods

2.2.1. Site description

This study was carried out in European beech (*Fagus sylvatica* L.) dominated forests that exhibit variation in soil P stocks and parent material (Lang et al. 2017). The P-low site (Unterlüss) is located in North German Plain (52°50'21.7"N, 10°16'2.3"E) on an elevation of 115 m above sea level (a.s.l) with mean annual temperature and precipitation of 8.0 ℃ and 779 mm respectively (Table 2.1). The beech forests at the P-low site are about 132 years old. The soil is sandy with sandy till parent material. The P-medium site (Mitterfels) in Bavarian Forest (48°58'34.1"N, 12°52'46.7"E) is located at an elevation of 1023 m above sea level (a.s.l). The average beech stand age is 131 years. The soil type is loamy sand with paragneiss parent material (Table 2.1). The P-medium site has mean annual temperature and precipitation of 4.9 ℃ and 1200 mm respectively. The P-high site (Bad Brückenau) is situated in “Bayerische Rhön” biosphere reservation (50°21'7.2"N, 9°55'44.5"E) on basalt parent material with silt clay soils. The elevation, mean annual temperature and precipitation at the P-high site are 809 m (a.s.l), 5.8 ℃ and 1031 mm respectively. The beech trees on the P-rich site have an age of 137 years (Table 2.1). Further climate, stand and soil characteristics of the study sites have been compiled in Table 2.1.

In each forest site, we set up an N, P, and P+N fertilization experiment (details: Clausing et al., 2021). Briefly, 12 study plots, each with an area of 400 m^2 were established in each study site in summer 2016. The plots were treated with either nitrogen (N), phosphorous (P), combined nitrogen and phosphorus (P+N) or were kept

as control (Con). Each treatment was replicated three times per study site, yielding a total of 36 plots. P was applied once in the late summer 2016 in form of KH_2PO_4 (50 kg P ha^{-1}) to the P and P+N treatments. N was applied as NH_4NO_3 (30 kg N ha^{-1}) to the N and P+N treatments at five time points (August 2016, May 2017, September to October 2017, April to May 2018 and September to October 2018). KCl was applied to the Con and N treatments to account for the K in the KH_2PO_4 fertilizer applied to the P and P+N treatments. The fertilizer was dissolved in tap water and sprayed on the soil surface of the plots.

2.2.2. Sampling

In each plot, soil samples were collected in fall 2016, spring 2017, fall 2017, spring 2018, and fall 2018 as reported by (Clausing et al. 2021). Since we were interested in the long-term effects of the fertilizer, we did not sample directly after fertilizer application to avoid the immediate impacts of P or N addition. Twelve soil cores (21 cm depth x 5.5 cm diameter) were randomly taken and separated into organic layer (OL) and mineral top soil (ML). Then, the 12 samples of the OL were pooled resulting in one sample per plot. The ML samples were also pooled. The samples were sieved (4 mm mesh size) and divided into bulk soil, fine roots (<2 mm) and coarse roots (>2 mm). Bulk soil, fine roots and coarse roots were transported in cooling boxes (approximately 4 ℃) to the laboratory for analysis.

Table 2.1. Description of the climate (MAT: mean annual temperature; MAP: sum of annual precipitation), altitude (ALT), soil and stand characteristics of the study sites (Unterlüss: P-low; Mitterfels: P-medium; Bad Brückenau: P-high). Data were taken from Haußmann and Lux (1997), Lang et al. (2017) and Clausing et al. (2021).

Variables	**P-low**	**P-medium**	**P-high**
Coordinates	52°50'21.7"N, 10°16'2.3"E	48°58'34.1"N, 12°52'46.7"E	50°21'7.2"N, 9°55'44.5"E
ALT (m.a.s.l)	115	1023	809
MAT (°C)	8.0	4.9	5.8
MAP (mm)	779	1200	1031
Soil type	Hyperdystric folic Cambisol	Hyperdystric chromic folic cambisol	Dystric skeletic cambisol
Soil parent material	Sandy till	Paragneiss	Basalt
Organic layer thickness (cm)	Oi: 4; Oe: 5 ;Oa: 4	Oi: 4; Oe: 4	Oi: 3; Oe: 12
Humus form	Mor-like Moder	Moder	Mull-like Moder
Soil texture	Topsoil: Loamy sand Subsoil: Sand	Loam (topsoil) Sandy loam (subsoil)	Silty clay loam (topsoil) Loam (subsoil)
pH (H_20) (A horizon: 0-5 cm)	3.5 (0.08)	3.6 (0.1)	3.8 (0.15)
Total soil P (mg kg^{-1}) (A horizon: 0-5 cm)	195 (15)	1375 (34)	2966 (45)
Extractable soil P (mg kg^{-1}) (A horizon: 0-5 cm)	11 (3)	70 (6)	116 (9)
Stand age (years)	132	131	137
Tree species composition (%)	*Fagus sylvatica* (91) *Quercus petraea* (9)	*Fagus sylvatica* (96) *Picea abies* (2) *Abies alba* (2)	*Fagus sylvatica* (99) *Acer pseudoplatanus* (1)
Natural vegetation	Luzulo-Fagetum	Dryopteris-Fagetum	Hordelymo-Fagetum
Mean height of beech trees (m)	27.3	20.8	26.8
Diameter at breast height of beech trees (cm)	27.5	37.6	36.8
Basal area of beech trees(m^2 ha^{-1})	36.7	28.1	35.6
Number of beech trees (ha^{-1})	480	252	335

2.2.3. Root biomass and soil water content

The weight of the fresh soil and root samples was determined. The samples were oven-dried (40 ℃) and weighed. The water content in the soil (WC_s) was calculated as:

$$WC\_s\ (g\ g^{-1}\ DW) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Dry weight}}$$

The fine root biomass (FR) in the mineral soil was determined as:

$$FR\ (mg\ g^{-1}\ DW\ soil) = \frac{\text{Total dry weight of FR in mineral soil}}{\text{Total dry weight of soil in mineral soil}}$$

The fine root biomass in the organic layer was determined correspondingly.

2.2.4. Soil and root chemistry

Dry soil samples were mixed with deionized water (1:2.5 ratio of soil to water) and shaking (1 hour at 200 rpm). After sedimentation of particles, pH was measured with a pH meter (WTW, Weilheim, Germany).

To determine C and N contents aliquots of dry soil and root samples were milled using a ball mill (MN400, Retsch GmbH, Haan, Germany). Aliquots of about 2 to 12 mg (soil) and 1.5 mg (fine roots) were weighed into 4 × 6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) on a microbalance (Model: Cubis MSA 2.7S-000-DM, Sartorius, Göttingen, Germany). Variation in C contents between the soil layers and among soil types led to overflow of the measuring unit in the spectrometer when the same sample weights were used for analyses. Therefore, we used different weights for the soil samples. We used the CN analyzer (vario MICRO cube CN analyzer, Elementar Analysensysteme, GmbH, Langenselbold Germany) or a mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany) at the Kompetenzzentrum für Stabile Isotope (KOSI, Göttingen. Germany) to measure the C and N concentrations. Acetanilide (10.36 % N, 71.09% C) was used as the standard.

To measure the contents of total P (P_t) and other elements (K, Ca, Na, Mg, Mn, Fe, Al and S), 50 mg of each sample (roots, soil) was extracted in 65 % HNO_3 (25 ml) for 12 hours at 160 ℃ (Heinrichs et al., 1986). Labile P (P_s) was determined by extracting 100 mg of sample in 150 ml of Bray-1 solution (0.03 N NH_4F, 0.025 N HCl) (Bray and Kurtz, 1945). The resulting extracts were subjected to filtration using phosphate free

filter paper (MN 280 ¼, Macherey-Nagel, Düren, Germany) after which elemental analysis by inductively coupled plasma–optical emission spectroscopy (ICP-OES) (iCAP 7000 Series ICP–OES, Thermo Fisher Scientific, Dreieich, Germany) was done at the following wavelengths (nm) (Na: 589.592; K: 766.490; Ca: 317.933; Mg: 285.213; Mn: 260.569; Fe: 238.204; Al: 308.215; S: 182.034; P: 185.942). The calibration was performed using concentrations of element standards (0.1 mg l^{-1} to 20 mg l^{-1}) (Einzelstandards, Bernd Kraft, Duisburg, Germany). Data for soil and root nutrients in 2018 were downloaded from the dryad repository (Clausing et al. 2020).

2.2.5. climate and weather

Data for climate and weather were obtained from http://www.wetterzentrale.de. Mean temperature (T) for each sampling date was calculated as mean temperature of three months (month of sampling and two months before sampling). Similarly, precipitation (Prec) was calculated as the sum of the precipitation for the month of sampling and two months before sampling. We calculated the deviation from the long-term climatic conditions (dfc) as the difference between monthly mean temperature of the sampling month and the long-term mean of the temperature in the corresponding months for the period 1981-2010. The deviation from the long-term precipitation was calculated as the difference between the monthly sum of precipitation in the sampling month and the long-term mean of the sum of precipitation in the sampling month. The data shown in supplementary Table S2.1.

2.2.6. Statistical analysis

The statistical analyses were performed with R software (R Core Team, 2020). The data were checked for normal distribution and homogeneity of variances using linear model residuals (function “lm” from R package “lme4”) and the Shapiro-Wilk test (Shapiro and Wilk, 1965). The data were log or square root transformed if the normality assumption was violated. Non-parametric test (Kruskal-Wallis test) was used if the normality assumption was not met after data transformation. When the normality assumption was met, we used linear mixed effect models (function: “lmer”, R package: “lme4”), analysis of variance (ANOVA) and a post hoc Tukey HSD test for comparison of means (package: “multcomp”) to test for the effect of site, fertilizer treatment and season on fine root biomass, on P and N in the soil and fine roots. We used plot (n = 3 per site) as random factor in the mixed models. Differences of the means at $p \leq 0.05$ were considered to indicate significant effects.

In addition to P and N, we also analyzed other elements in roots and soil from each forest site, soil layer, and season. An overview on the nutrient data for soil and roots has been compiled in supplementary Table S2.2.

To determine the contribution of explanatory variables to fine root mass, we constructed a linear model incorporating all variables [site (P-rich, P-medium, P-poor), treatment (Con, N, P, P+N), season (spring, fall), climate (Temperature (T, T dfc), precipitation (Prec, Prec dfc)), soil properties (C/N ratio, C, N, P_t, P_s, Na, K, Ca, Mg, Mn, Fe, S, WC_s and pH) and root chemistry (C/N ratio, C, N, P_t, P_s, Na, K, Ca, Mg, Mn, Fe, S)]. To obtain a model with good fit (lowest AIC), the constructed model was subjected to backward stepwise regression by using the “stepAIC” function from R “MASS” package (Venables and Ripley, 2002). The variables retained by stepwise regression were divided into five categories: site, climate, season, soil properties (mineral elements and WC_s) and root chemistry (mineral elements). Factors in each of the four categories for the organic (site, soil properties, root chemistry) and mineral (Season, soil properties, root chemistry and climate) soil layers were retained (Table 2.2). We partitioned the variance of fine root biomass explained by each category using “calc.relimp” function from R “Relaimpo” package (Grömping, 2006) based on the lmg method (averaging sequential sum of squares over all orderings) developed by Lindeman et al. (1980).

Table 2.2. Linear regression models for variation in fine root biomass in the organic and mineral soil. Data show the final regression model obtained by stepwise backward selection of variables for soil properties and root chemistry (WC: water content, C/N: C/N ratio, C, N, Na, K, Ca, Mg, Mn, Fe, S, Ps: soluble phosphorus, Pt: total phosphorus), and climate (temperature (T, T dfc), precipitation (Prec, Prec dfc)) and the categorical factors "site" and season. The models with lowest AIC were used. Soil and root variables are indicated with lowercase letters "s" and "r" respectively.

Organic layer				**Mineral layer**			
Category	Variables	Estimate (SE)	*p*-value	Category	Variables	Estimate (SE)	*p*-value
Soil	WC_s	19.23 (5.65)	**< 0.001**	Soil	WC_s	1.85 (0.66)	**0.006**
	C_s	0.20 (0.13)	0.124		K_s	-0.28 (0.09)	**0.003**
	N_s	-3.83 (2.58)	0.139		Mg_s	-0.16 (0.03)	**< 0.001**
Root	Pt_r	31.10 (8.59)	**< 0.001**		S_s	3.10 (0.61)	**< 0.001**
Climate	Prec	-0.41 (0.09)	**< 0.001**		Pt_s	0.61 (0.26)	**0.022**
	Prec dfc	0.39 (0.10)	**< 0.001**		Ps_s	-9.21 (2.20)	**< 0.001**
	T	3.67 (0.84)	**< 0.001**	Root	CN_r	0.03 (0.01)	**0.016**
Site	P-low	9.20 (11.12)	0.409		Na_r	-2.86 (0.92)	**0.002**
	P-medium	90.94 (10.66)	**< 0.001**		Ca_r	-0.40 (0.15)	**0.006**
					Mg_r	1.67 (0.38)	**< 0.001**
					Fe_r	-0.12 (0.04)	**0.007**
					S_r	0.62 (0.46)	0.181
					Pt_r	1.91 (0.82)	**0.021**
				Climate	Prec	0.02 (0.00)	**< 0.001**
					Prec dfc	-0.03 (0.01)	**< 0.001**
					T	0.37 (0.12)	**0.003**
					T dfc	-0.66 (0.32)	**0.040**
				Season	Spring	3.25 (1.10)	**0.004**
Parameters of the regression model							
Start AIC		1298.00				61.18	
Stop AIC		1261.48				37.14	
F		46.11				20.84	
p-value		**< 0.001**				**< 0.001**	
Adjusted R^2		0.69				0.67	

2.3. Results

2.3.1. Fertilizer treatment and season affect phosphorus in soil

P application caused significant increases in total (p = 0.039) and soluble P (p = 0.008) in the mineral soil of the P-low forest in spring but not in fall (Fig. 2.1A,B). We did not find any significant effects of P or N treatment or the combination of both on the P contents in the organic layer. No significant fertilization effects were found for the total or soluble P contents in the P-medium or P-rich forest soils (Fig. 2.1A,B).

Irrespective of the fertilizer treatments, total P contents were higher in the organic layer in spring than in fall, indicating seasonal cycling at each of the three studied forest sites (Fig. 2.1A). In the mineral soil, seasonal increases in total P in spring were also significant in the P-medium (p = 0.002) and P-high forest (p = 0.001) but not in the P-low forest (p = 0.103, Fig. 2.1A). In the P-low forest, soluble P contents were higher in spring than in fall in both soil layers studied (p < 0.010, Fig. 2.1B). A significant increase in soluble P in spring was also observed in the mineral soil of the P-high forest (p < 0.001, Fig. 2.1B).

The comparison of the P contents in the three forests confirmed significant differences among the sites in the order P-low < P-medium < P-rich forest (p < 0.001, Fig. 2.1) both soil layers, in agreement with previous investigations in these forests (Lang et al. 2017; Zavišić et al. 2016; Table 1).

2.3.2. Fertilizer treatment and season affect phosphorus in roots

P application as a single factor or in combination with N resulted in forest- and soil layer-specific effects on the P content of fine roots (Fig. 2.2). In the P-low forest, fine roots from the mineral layer contained increased total (p = 0.014) and soluble P contents (p = 0.003) in spring after combined fertilization with P and N (Fig. 2.2A). In the P-low forest, the soluble P contents roots from the mineral soil was also enhanced after P (p < 0.001) application (Fig. 2.2B). In fall, no fertilization effects were found in roots from the mineral soil and organic layer in the P-low forest and the total and soluble P contents were lower than in spring (Fig. 2.2A,B).

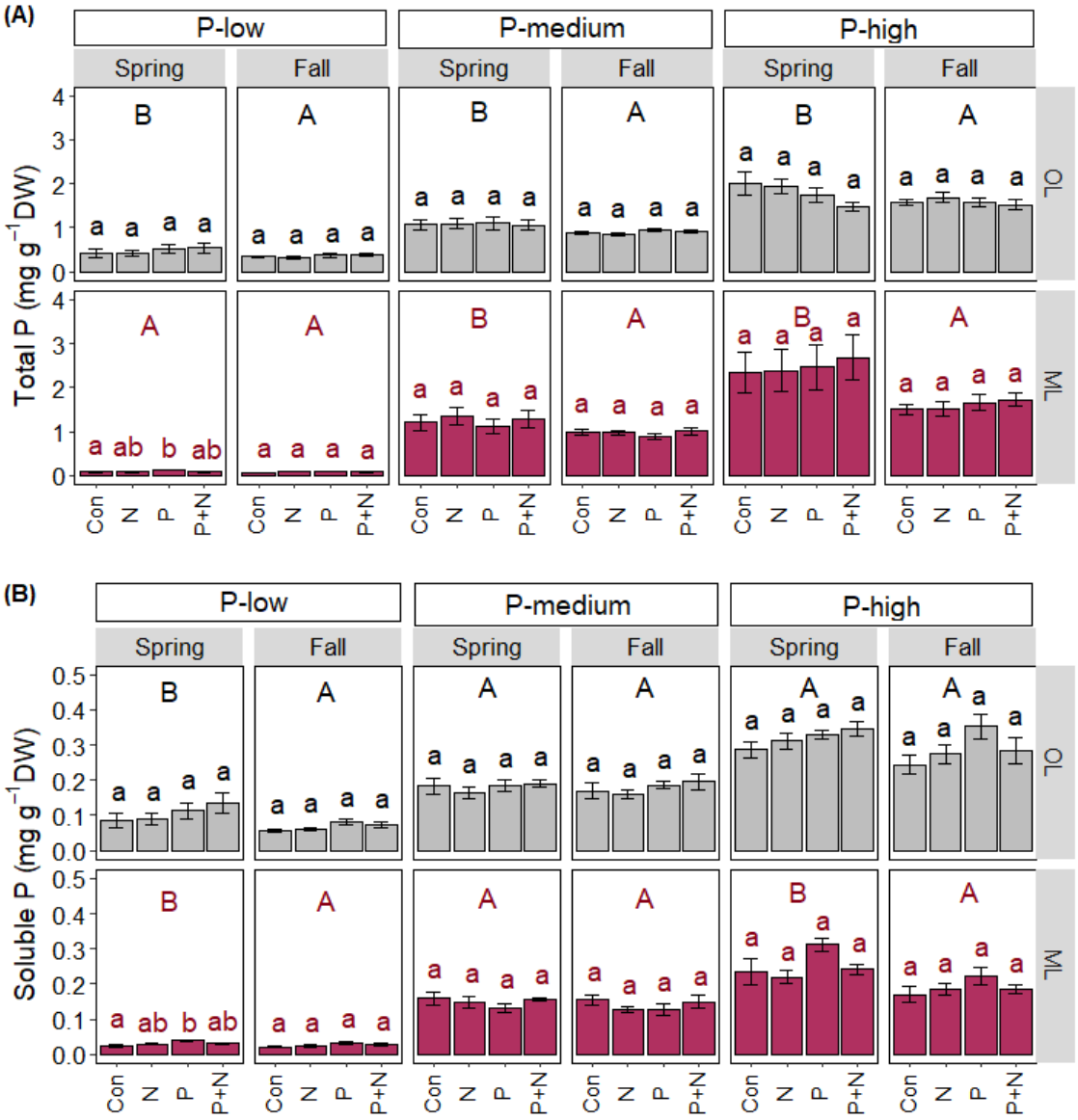


Figure 2.1. Total (A) and soluble (B) phosphorus content in the organic (OL) and mineral (ML) soil in beech (*Fagus sylvatica*) forests characterized by low, medium and high P contents. The study was conducted at the forest sites Unterlüss (P-low), Mitterfels (P-medium) and Bad Brückenau (P-high) using 3 plots per treatment (Con = control = no fertilizer treatment, N = 5 x 30 kg ha^{-1} nitrogen fertilizer, P = 50 kg ha^{-1} phosphorus fertilizer, P+N = combined N and P fertilizer treatment). Samples were collected in fall 2016, 2017, 2018 and in spring 2017 and 2018. Data show means per season and treatment (spring: n = 6, fall: n = 9, ± SE). Significant differences at $p \leq 0.05$ are indicated for the fertilizer treatment (lowercase letters) and season (uppercase letters) for each forest site.

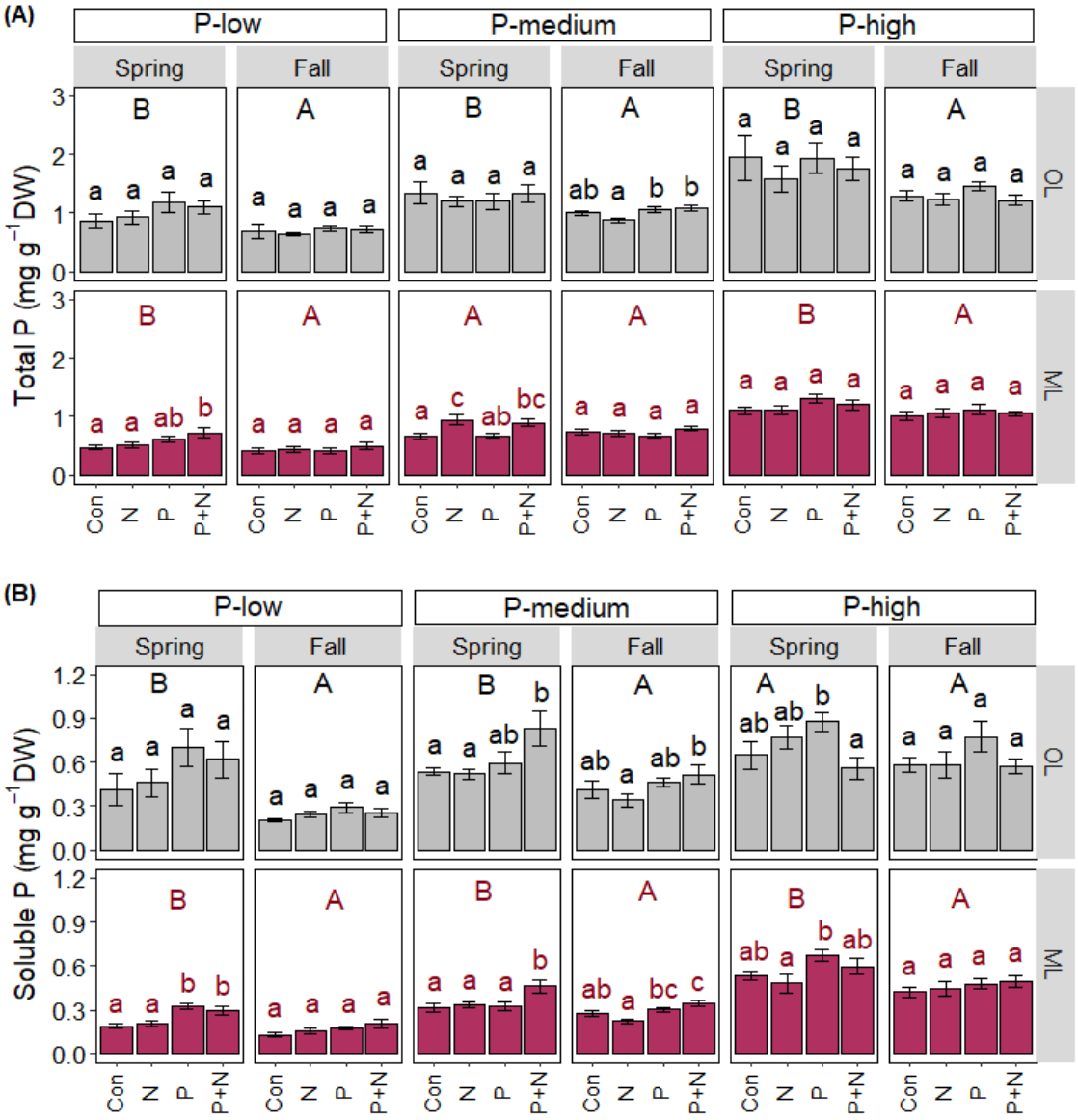


Figure 2.2. Total (A) and soluble (B) phosphorus contents of beech (*Fagus sylvatica*) fine roots in the organic (OL) and mineral (ML) soil layer. The study was conducted at the forest sites Unterlüss (P-low), Mitterfels (P-medium) and Bad Brückenau (P-high) using 3 plots per treatment (Con = control = no fertilizer treatment, N = 5 x 30 kg ha^{-1} nitrogen fertilizer, P = 50 kg ha^{-1} phosphorus fertilizer, P+N = combined N and P fertilizer treatment). Samples were collected in fall 2016, 2017, 2018 and in spring 2017 and 2018. Data indicate means for each season and treatment (spring: n = 6, fall: n = 9, ± SE). Significant differences at $p \leq 0.05$ are indicated for the fertilizer treatment (lowercase letters) and season (uppercase letters) in each forest site.

The total P contents in roots from the P-high forest did not respond to any fertilization treatment (Fig. 2.2A) but the soluble P levels in roots from the organic layer and from the mineral soil were enhanced in spring in the P-high forest after P fertilization (Fig. 2.2B).

In the P-medium forest, root total P contents increased in spring under N or combined N and P fertilization but only in roots from mineral soil (Fig. 2.2A). Combined N and P treatment also caused increased soluble P contents in these roots in spring ($p = 0.020$, Fig. 2.2B). Roots from the P-medium forest further showed P or combined N and P treatment effects in fall, resulting in enhanced total P contents in roots from the organic layer and higher soluble P contents in roots from both soil layers ($p = 0.012$, Fig. 2.2B).

In addition to fertilization effects, the total P contents in roots were higher in spring than in fall in the organic layer and in the mineral soil, with the exception of roots in the mineral soil at the P-medium site (Fig. 2.2A). The soluble P contents in roots from both the organic layer and the mineral soil also were higher in spring than in fall, with the exception of roots in the organic layer at the P-high forest (Fig. 2.2B).

We also found differences for the root P contents among the forest sites: total P contents in roots increased in the order P-low < P-medium < P-high in both seasons and soil layers ($p < 0.001$). Soluble P in roots from the mineral layer showed a pattern similar to that of total P ($p < 0.001$), whereas roots from the organic layer showed this pattern only in spring ($p = 0.006$).

2.3.3. Season but not fertilizer treatment affect nitrogen in soil and roots

In addition to P, we investigated the impact of fertilizer application and season on soil and root N contents (Fig. 2.3). We did not observe any changes in soil or root N in response to N, P or the combination of N and P application, neither in the organic layer nor in the mineral soil (Fig. 2.3 A,B).

The organic layer contained higher N contents in spring than in fall in each forest site (Fig. 2.3A). In the organic layer in the P-low and P-rich forest, root N was also higher in spring than fall, while in the P-medium forest, root N was higher in the mineral soil in spring than in fall ($p = 0.043$, Fig. 2.3B).

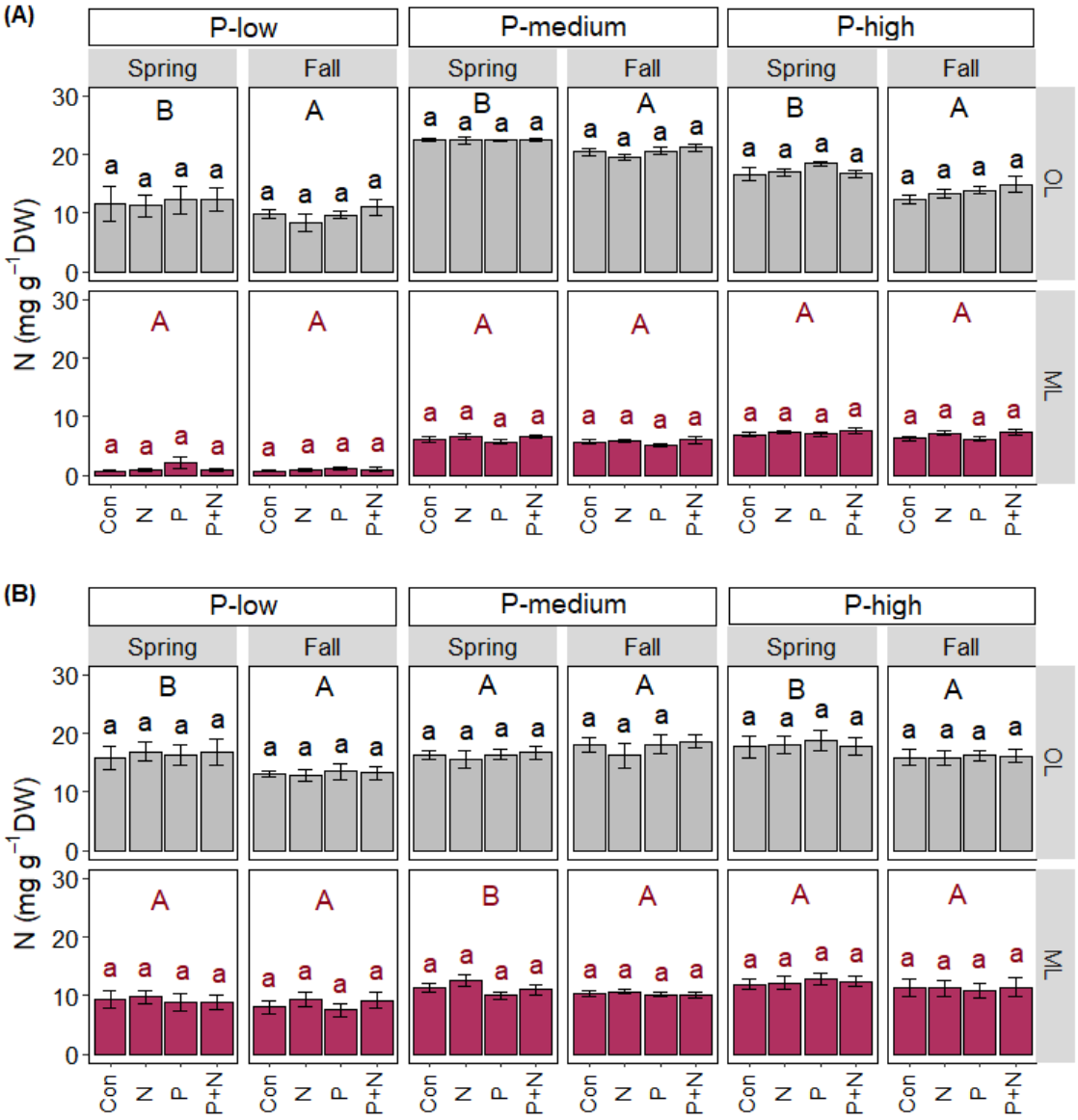


Figure 2.3. Soil (A) and fine root (B) nitrogen contents of beech (*Fagus sylvatica*) in the organic (OL) and mineral (ML) soil layer of forests characterized by low, medium and high soil P contents. The study was conducted at the forest sites Unterlüss (P-low), Mitterfels (P-medium) and Bad Brückenau (P-high) using 3 plots per treatment (Con = control = no fertilizer treatment, N = 5 x 30 kg ha^{-1} nitrogen fertilizer, P = 50 kg ha^{-1} phosphorus fertilizer, P+N = combined N and P fertilizer treatment). Samples were collected in fall 2016, 2017, 2018 and in spring 2017 and 2018. Data indicate means for each season and treatment (spring: n = 6, fall: n = 9, ± SE). Significant differences at $p \leq 0.05$ are indicated for season (uppercase letters) in each forest site.

2.3.4. Fine root biomass Is mainly controlled by multiple site conditions and marginally by P fertilization

A significant fertilizer effect on fine root biomass was not found in the organic layer (Fig. 2.4A) and only observed at the P-low site in the mineral layer in fall (Fig. 2.4), where root biomass was approximately 1.3-fold greater after P application than in the control plots ($p = 0.018$) (Fig. 2.4B).

Root biomass showed significant seasonal changes, with contrasting patterns in different forests (Fig. 2.4). In the organic layer, root mass was higher in spring than fall in the P-low forest ($p = 0.006$) and lower in spring than in fall in the P-high forest ($p < 0.001$). In the mineral soil, root mass was higher in fall than spring in the P-medium ($p < 0.001$) and P-high ($p < 0.001$) forests (Fig. 2.4).

Root biomass was further massively affected by the forest site, irrespective of season: in the organic layer, root mass increased in the order P-high < P-low < P-medium (spring: $p < 0.001$; fall: $p < 0.001$). In the mineral layer, root mass was lower in the P-low than in P-medium (spring: $p < 0.001$; fall: $p < 0.001$) and P-high forests (spring: $p < 0.001$; fall: $p < 0.001$).

To evaluate the impact of climate, season, site, fertilizer, soil properties and root chemistry on fine root biomass, we used a total of 33 variables (specified under materials and methods) and determined those retained by stepwise linear regression (Table 2.2). The model for root biomass in the organic layer ($p < 0.001$, adjusted $R^2 = 0.69$) contained parameters for the following categories: soil properties (water, carbon and nitrogen contents), root chemistry (total P), climate (temperature and precipitation) and site (Table 2.2). The model for root biomass in mineral soil ($p < 0.001$, adjusted $R^2 = 0.67$) contained parameters for the following categories: soil properties (water, potassium, magnesium, sulfur, and soluble and total P contents), root chemistry (C/N ratio, sodium, calcium, magnesium, iron and total P contents), climate (temperature and precipitation), and season (Table 2.2).

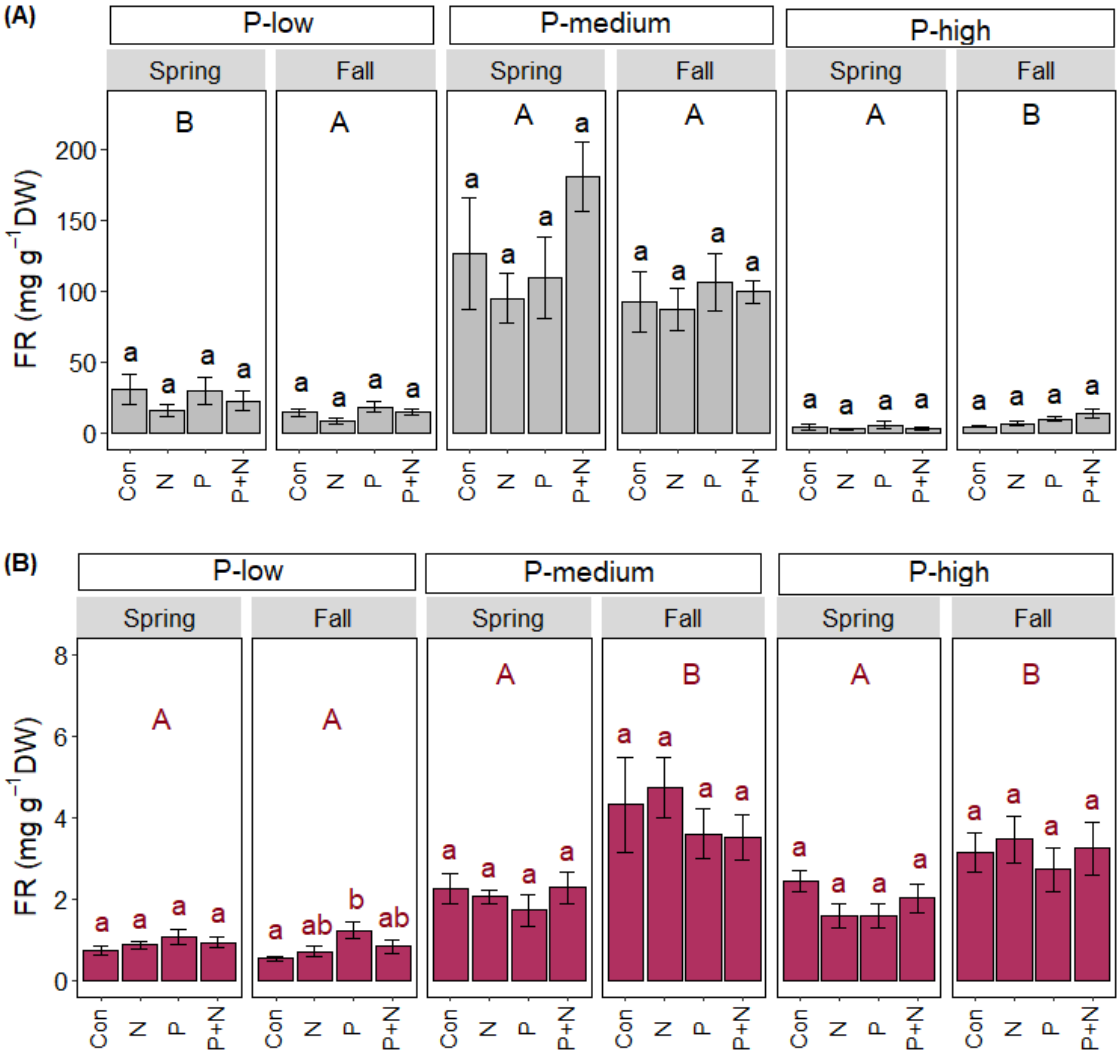


Figure 2.4. Beech (*Fagus sylvatica*) fine root mass in the organic (A) and mineral (B) soil layer of forests characterized by low, medium and high soil P contents. The study was conducted at the forest sites Unterlüss (P-low), Mitterfels (P-medium) and Bad Brückenau (P-high) using 3 plots per treatment (Con = control = no fertilizer treatment, N = 5 x 30 kg ha^{-1} nitrogen fertilizer, P = 50 kg ha^{-1} phosphorus fertilizer, P+N = combined N and P fertilizer treatment). Samples were collected in fall 2016, 2017, 2018 and in spring 2017 and 2018. Data indicate means for each season and treatment (spring: n = 6, fall: n = 9, ± SE). Significant differences at $p \leq 0.05$ are indicated for the fertilizer treatment (lowercase letters) and season (uppercase letters) in each forest site.

To determine the contribution of each category, we partitioned the variance that explained fine root biomass (Fig. 2.5). In the organic layer, the variation of fine root biomass was mainly explained by soil properties (37%) and site (27%), whereas root chemistry and climate only explained little variation of fine root mass (1% and 5% respectively). In the mineral layer, fine root biomass variation was attributed to soil properties (30%), root chemistry (20%) and climate (16%). In both soil layers, we observed a high contribution of soil properties to fine root biomass variation. A major difference between the factors explaining root biomass variation were higher contributions of root chemistry and climate in mineral soil than in the organic layer.

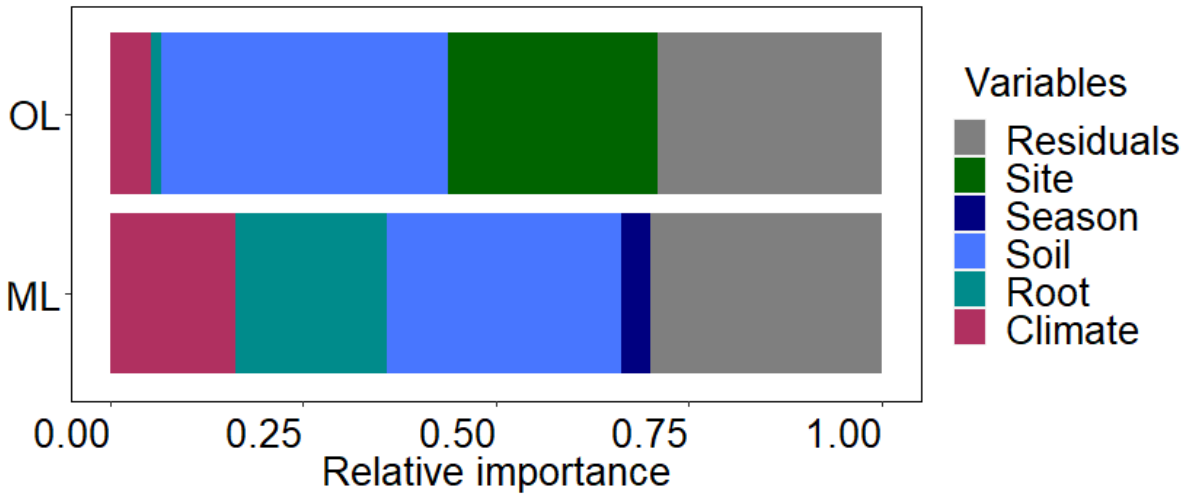


Figure 2.5. Proportion of fine root biomass variance explained by site, treatment, season, soil, root and climate variables in the organic (OL) and mineral (ML) soil layer. The variables within each category were retained by stepwise regressing model (provided in Table 2.2) and explain 71% (OL) and 70% (ML) of the variation of fine root biomass. Residuals refers to unexplained proportion of fine root biomass variance.

2.4. Discussion

2.4.1. Soil and root P contents show seasonal dynamics and site-specific fertilization effects

In this study, we investigated key factors that determine root biomass in temperate forests such as temperature, nutrient and water availability (Pregitzer et al. 1993; Eissenstat et al. 2000; Hertel et al. 2013). A novel aspect was that we also included root chemistry because we reasoned that seasonal allocation shifts in nutrients may also influence root biomass. Several studies have shown that beech trees mobilize internal P resources in spring from stem and roots to support leaf flush (Netzer et al. 2017; Zavišić and Polle, 2018), while root growth is favored later in the growth season (this study, Zavišić and Polle, 2018; Brassard et al. 2009). Internal P re-

allocation in spring involves transformation of organic P into labile P forms, their utilization for growth and replenishment of plant storage pools in late summer and fall (Netzer et al. 2017; Spohn et al. 2018; Zavišić and Polle, 2018). Mesocosm experiments with ^{33}P tracers showed that P uptake by beech trees is low in spring, despite high P demand and internal resource allocation to leaf production, while P uptake is high in late summer and fall (Spohn et al. 2018; Zavišić and Polle, 2018). These results appear counterintuitive to the present results, showing increased P contents in soil and fine roots in spring and decreased P contents in these compartments in fall. However, these patterns most likely reflect physiological acclimation of P transport systems, which are highly sensitive to P availabilities, resulting in decreased P uptake capacities under high and increased P uptake capacities under low P supply (Kavka et al. 2016; 2021), thus, complying with seasonal fluctuation of P contents in our study.

The observed seasonal trajectory occurred on top of the differences in soil and root P contents due to the P fertility gradient of our study sites (Lang et al. 2017). Because of the site-independence of the nutrient dynamics, we assume that leaf shedding in late fall (after our fall sampling) and litter degradation, resulting in nutrient release and leaching over the summer (Fetzer et al. 2021; Fetzer et al. 2022) (after our spring sampling), caused cycling between higher and lower P and N contents in the organic layer. The P respective N inputs by annual litter fall amount approximately (P/N) 0.16/2.9 g m^{-2} at the P-low, 0.21/3.6 g m^{-2} at the P-medium and 0.23/3.2 g m^{-2} at the P-high site (Lang et al. 2017). Our results mirror this pattern in the organic layer in spring. Overall, our results support that tree phenology promotes nutrient cycling via litter feedback (Augusto et al. 2002; Hobbie, 2015).

An interesting observation was that seasonal P but not N variations (with the exception of the P-medium site) were also found in mineral soil and roots from mineral soil across the P-fertility gradient. The divergent behavior of P and N can be ascribed to different mineralization rates of N and P from degradation of organic compounds in leaf litter (Brödlin et al. 2019a), differences in mobility and sorption in forest soil (Kaiser et al. 2003; Fetzer et al. 2022), seasonal P loss due to drying and re-wetting cycles (Brödlin et al. 2019b), and other abiotic or biotic factors (Augusto et al. 2002). Here, N fertilization rarely affected root nutrient contents but a decrease in root P after N fertilization at the P-medium site (with the highest N contents)

suggests that excess N inputs may have triggered imbalance of the N:P ratio (Peñuelas and Sardans, 2022). Unexpectedly P or P+N fertilization also caused increases in labile P in roots at the P-medium and P-high sites (mineral soil), revealing that added P was captured in the ecosystem, irrespective of the soil P contents. Since the fertilizer induced P increments were small, the contribution of trees to P retention was apparently masked, if only (much higher) total P contents of tissues were studied. Surprisingly, the increments occurred in most cases only in spring and not in fall implying that P added to forest soil enters the phenological P cycle of the trees.

2.4.2. Impact of nutrient resources and climate on root mass varies between organic and mineral soil

A central question of our study was if shifts in resource dynamics and climatic factors affected fine root biomass. In agreement with our first hypothesis, we observed an increase in fine root biomass following P addition in the mineral layer at the P-low site, i.e., only in soils with the least P availabilities (Lang et al. 2017). This result supports the conceptual theory of enhanced resource use efficiency under low resource availabilities (Bridgham et al. 1995). Accordingly, we found neither positive synergistic effects of P+N (Elser et al. 2007) nor negative effects of N (van Dijk et al. 1990; Clemensson-Lindell and Persson, 1995; Nadelhoffer, 2000) on root biomass. Similar to our findings, Majdi and Nylund (1996) reported neutral effects of N addition on root biomass in Norway spruce. In our study, the variation in fine root biomass was not explained by fertilization treatment. Whether minute positive root biomass responses due to P fertilization could counteract P deterioration in European forests (Jonard et al. 2015; Talkner et al. 2015) remains a matter of debate. However, in agreement with other studies (Finér et al. 2007), site fertility had profound influence beech root biomass.

Tree nutrition depends not only on root biomass but also on plant-microbial interactions, which show stratification between the organic and mineral soil horizons (Baldrian, 2017; Brabcová et al. 2018; Khokon et al. 2021). In the forests studied here, fertilization did not strongly influence the fungal community composition but P application enhanced the abundance of mycorrhizal species from the family of Boletaceae, which have increased capacities for P retention (Clausing et al. 2021).

Thereby, higher P uptake and allocation to aboveground sinks may have happened in all fertilized plots without drastic shifts in root biomass.

Enhanced root biomass occurred in most cases in fall. These results agree with other studies (López et al. 2001; Montagnoli et al. 2012) but exceptions have also been reported, showing higher fine root biomass in spring (our study: organic layer at the P-low site; Grier et al. 1981; Burton et al. 2000). These findings highlight root plasticity, most likely reflecting that plants invest in organs that are essential for acquisition of the least available resources (Bridgham et al. 1995; Niklas, 2004). Despite cycling of fine root biomass and P resources between spring and fall, season was only minor factor explaining root biomass variation. We suspect that root biomass directly responded to the consequences of phenological changes, i.e., the changes in internal and external nutrient availabilities and therefore, not strongly to "season" *per se*. For example, among the soil and root nutrients tested, our regression model kept basic cations in the soil and roots as important factors. Studies in beech forests showed that rhizosphere bacteria were involved in seasonal modulation of cations leading to higher resource availability in spring than in fall (Collignon et al. 2011; Calvaruso et al. 2014).

Our regression model also elucidated important differences between the impact of P on the variation of root mass in the organic and mineral soil. In the organic layer, soil P was not among the nutrients that could explain fine root biomass variations, despite the importance of the organic layer for P mobilization and tree nutrition (Hauenstein et al. 2018; Heuck and Spohn, 2016; Zederer and Talkner, 2018). In contrast to the organic layer, the labile P fraction was the most important explanatory variable for root biomass in mineral soil. Although the difference in fine root biomass variation explained by "soil properties" between the soil horizons was minimal (about 6%), the relevant explanatory factors differed between the soil compartments. Therefore, an important insight of our study was that fine roots along vertical profiles obviously responded autonomously to differences in the surrounding environment. This idea had further support because "root chemistry", mainly attributable to root P and root basic cation contents, explained a remarkable fraction of root biomass variation in mineral soil (19%) and but very little in the organic layer (1%).

A further category that explained root biomass variations was climate but in contrast to our initial hypothesis, we found stronger impact of climate in the lower (16%) than in the upper soil horizon (5%). A likely explanation is that the soil water content (category “soil”) was a stronger factor in the organic than in the mineral layer. This finding suggests higher sensitivity of fine root biomass close to the surface of the forest floor to changes in the actual water availability, whereas fluctuations in water content in deeper soil layers are buffered by the water holding capacity of the soil structure, thus, leading to a stronger impact of precipitation. Other studies also reported the importance of climatic factors for standing fine root biomass and root turnover (Meier and Leuschner 2010; Hertel et al. 2013, Gao et al. 2021). Since the organic layer is especially important for tree P nutrition in the P-low site (Lang et al. 2017, Hauenstein et al. 2018, Clausing and Polle, 2020), lower water contents and higher temperatures may put root biomass at this site at a higher risk of failure than in the other forests of our study.

2.5. Conclusion

In agreement with our first hypothesis, we show that P-fertilization led to increased fine root biomass only in the mineral soil with the least P availability, while soil or root P contents increased in all soil types. This result supports the resource optimization hypothesis. Our study further sustained the second hypothesis that root biomass allocation shows seasonal shifts. These shifts were associated with pheno-seasonal changes in soil and root nutrient levels and were more important in mineral soil than in the organic layer. We propose that higher importance of soil and root nutrients for root biomass in the mineral as compared to the organic layer may be attributable to differences in microbial nutrient cycling. These processes are dominant in the organic layer and may have overruled the impact of soil properties or root chemistry. In contrast to our third hypothesis, root biomass variation was driven to a much higher extent by climate in the mineral than in the organic layer. Therefore, our results imply that climate change with long-term warmer and drier conditions is likely to endanger root systems below the organic layer. Therefore, negative climate effects may not only be expected for forests on nutrient-poor parent material that rely on the organic layer for nutrient supply but also for forests on more fertile sites, where roots forage in the mineral horizon.

2.6. References

Ågren, G.I., Franklin, O., 2003. Root: shoot ratios, optimization and nitrogen productivity. Annals of Botany 92, 795e800. doi: 10.1093/aob/mcg203

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

Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiology Reviews 41, 109–130. doi: 10.1093/femsre/fuw040

Brabcová, V., Štursová, M., Baldrian, P., 2018. Nutrient content affects the turnover of fungal biomass in forest topsoil and the composition of associated microbial communities. Soil Biology and Biochemistry 118, 187–198. doi: 10.1016/j.soilbio.2017.12.012

Brassard, B.W., Chen, H.Y.H., Bergeron, Y., 2009. Influence of environmental variability on root dynamics in Northern forests. Critical Reviews in Plant Sciences 28(3), 179-197, doi: 10.1080/07352680902776572

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Science 59, 39–46. doi:10.1097/ 00010 694-19450 1000- 00006

Bridgham, S.D., Pastor, J., McClaugherty, C.A., Richardson, C.J., 1995. Nutrient-use efficiency: a litterfall index, a model, and a test along a nutrient-availability gradient in North Carolina peatlands. The American Naturalist 145, 1–21

Brödlin, D., Kaiser, K., Hagedorn, F., 2019a. Divergent patterns of carbon, nitrogen, and phosphorus mobilization in forest soils. Frontiers in Forests and Global Change 2, 66. doi: 10.3389/ffgc.2019.00066

Brödlin, D., Kaiser, K., Kessler, A., Hagedorn, F., 2019b. Drying and rewetting foster phosphorus depletion of forest soils. Soil Biology and Biochemistry 128, 22-34. doi: 10.1016/j.soilbio.2018.10.001

Burton, A.J, Pregitzer, K.S., Hendrick, R.L., 2000. Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. Oecologia 125, 389–399

Calvaruso, C., Collignon, C., Kies, A., Turpault, M-P., 2014. Seasonal evolution of the rhizosphere effect on major and trace elements in soil solutions of Norway spruce (*Picea abies* Karst) and beech (*Fagus sylvatica*) in an acidic forest soil. Open Journal of Soil Science 4, 323–336. doi: 10.4236/ojss.2014.49034

Clausing, S., Likulunga, E.L., Janz, D., Feng, H., Schneider, D., Daniel, R., Krüger, J., Lang, F., Polle, A., 2021. Impact of nitrogen and phosphorus addition on resident soil and root mycobiomes in beech forests. Biology and Fertility of Soils. doi: 10.1007/s00374-021-01593-x

Clausing, S., Polle, A., 2020. Mycorrhizal Phosphorus Efficiencies and Microbial Competition Drive Root P Uptake. Frontiers in Forests and Global Change 3, 54. doi: 10.3389/ffgc.2020.00054

Clemensson-Lindell, A., Persson, H., 1995. Fine root vitality in a Norway spruce stand subjected to various nutrient supplies. Plant and Soil. 168-169, 167-172. doi: 10.1007/BF00029325

Collignon, C., Uroz, S., Turpault, M.P., Frey-Klett, P., 2011. Seasons differently impact the structure of mineral weathering bacterial communities in beech and spruce stands. Soil Biology and Biochemistry 43 (10), 2012-2022. doi: 10.1016/j.soilbio.2011.05.008

Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., Rutishauser, T., Rathgeber, C.B.K., 2016. Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. Annals of Forest Science 73, 5–25. doi: 10.1007/s13595-015-0477-6

Du, E., De Vries, W., 2018. Nitrogen-induced new net primary production and carbon sequestration in global forests. Environmental Pollution 242, 1476–1487. doi: org/ 10.1016/j.envpol.2018.08.041

Du, E., Terrer, C., Pellegrini, A.F., Ahlström, A., van Lissa, C.J., Zhao, X., Xia, N., Wu, X., Jackson, R.B., 2020. Global patterns of terrestrial nitrogen and phosphorus limitation. Nature Geoscience 13 (3), 221–226. doi: 10.1038/s41561-019-0530-4

Dziedek, C., Fichtner, A., Calvo, L., Marcos, E., Jansen, K., Kunz, M., Walmsley, D., von Oheimb, G., Härdtle, W., 2017. Phenotypic plasticity explains response patterns of European beech (*Fagus sylvatica* L.) saplings to nitrogen fertilization and drought events. Forests 8, 91. doi:10.3390/f8030091

Eissenstat, D.M., Wells, C.E., Yanai, R.D., Whitbeck, J.L. 2000. Building roots in a changing environment: implications for root longevity. *The New Phytologist* 147: 33–42. doi: 10.1046/j.1469-8137.2000.00686.x

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10, 1135–1142. doi: 10.1111/j.1461-0248.2007.01113.x

Fetzer, J., Frossard, E., Kaiser, K., Hagedorn, F., 2022. Leaching of inorganic and organic phosphorus and nitrogen in contrasting beech forest soils – seasonal patterns and effects of fertilization. Biogeosciences 19, 1527-1546. doi: 10.5194/bg-19-1527-2022

Fetzer, J., Loeppmann, S., Frossard, E., Manzoor, A., Brödlin, D., Kaiser, K., and Hagedorn, F., 2021. Leaching of Phosphomonoesterase activities in beech forest soils: consequences for phosphorus forms and mobility. Frontiers in Forests and Global Change 4, 684069. doi: 10.3389/ffgc.2021.684069

Finér, L., Helmisaari, H. S., Lõhmus, K., Majdi, H., Brunner, I., Børja, I., Eldhuset, T., Godbold, D., Grebenc, T., Konôpka, B., Kraigher, H., Möttönen, M. R., Ohashi, M., Oleksyn, J., Ostonen, I., Uri, V., Vanguelova, E., 2007. Variation in fine root biomass of three European tree species: Beech (*Fagus sylvatica* L.), Norway spruce (*Picea abies* L. Karst.), and Scots pine (*Pinus sylvestris* L.). Plant Biosystems, 141(3), 394-405. doi: 10.1080/11263500701625897

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., et al., 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320, 889–892. doi: 10.2307/20054730

Gao, G., Liu, Z., wang, Y., Wang, S., Ju, C., Gu, J., 2021. Tamm Review: Fine root biomass in the organic (O) horizon in forest ecosystems: Global patterns and

controlling factors. Forest Ecology and Management 491, 119208. doi: 10.1016/j.foreco.2021.119208

Godbold, D.L., Brunner, I., 2007. The platform for European root science, COST action E38: An introduction and overview. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 141(3) 390-393. doi: 10.1080/11263500701625715

Grier, C.C., Vogt, K.A., Keyes, M.R., Edmonds, R.L., 1981. Biomass distribution and above- and below-ground production in young and mature *Abies amabilis* zone ecosystems of the Washington Cascades. Canadian Journal of Forest Research 11, 155–167

Grömping, U., 2006. Relative Importance for Linear Regression in R: The Package Relaimpo. Journal of Statistical Software 17 (1), 1-27. doi: 10.18637/jss.v017.i01

Hauenstein, S., Neidhardt, H., Lang, F., Krüger, J., Hofmann, D., Pütz, T., Oelmann, Y., 2018. Organic layers favor phosphorus storage and uptake by young beech trees (*Fagus sylvatica* L.) at nutrient poor ecosystems. Plant Soil 432, 289–301. doi: 10.1007/s11104-018-3804-5

Haußmann, T., Lux, W., 1997. Dauerbeobachtungsfl€achen zur Umweltkontrolle im Wald: Level II. BMELF. Erste Ergebnisse, Bonn, p. 148

Heid, L., Calvaruso, C., Andrianantenaina, A., Granier, A., Conil, S., Rathgeber, C.B.K., Turpault, M-P., Longdoz, B., 2018. Seasonal time-course of the above ground biomass production efficiency in beech trees (*Fagus sylvatica* L.). Annals of Forest Science 75, 31. doi: 10.1007/s13595-018-0707-9

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

Hertel, D., Strecker, T., Müller-Haubold, H., Leuschner, C. 2013. Fine root biomass and dynamics in beech forests across a precipitation gradient – is optimal

resource partitioning theory applicable to water-limited mature trees? Journal of Ecology 101, 1183-1200. doi: 10.1111/1365-2745.12124

Heuck, C., Spohn, M., 2016. Carbon, nitrogen and phosphorus net mineralization in organic horizons of temperate forests: stoichiometry and relations to organic matter quality. Biogeochemistry 131, 229–242. doi: 10.1007/s10533-016-0276-7

Hobbie, S.E., 2015. Plant species effects on nutrient cycling: revisiting litter feedbacks. Trends in Ecology and Evolution 30, 357–363. doi: 10.1016/j.tree.2015.03.015

Högberg, P., 2007. Nitrogen impacts on forest carbon. Nature 447, 781-782

Högberg, P., Johannisson, C., Hällgren, J.E., 1993. Studies of 13C in the foliage reveal interactions between nutrients and water in forest fertilization experiments. Plant and Soil 152 (2), 207-214. doi: 10.1007/bf00029090

Jonard, M., Fürst A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., Waldner, P., Benham, S., Hansen, K., Merilä, P., Ponette, Q., de la Cruz, A.C., Roskams, P., Nicolas, M., Croisé, L., Ingerslev, M., Matteucci, G., Decinti, B., Bascietto, M., Rautio, P., 2015. Tree mineral nutrition is deteriorating in Europe. Global Change Biology 21, 418–430. doi: 10.1111/gcb.12657

Kaiser, K., Guggenberger, G., Haumaier, L., 2003. Organic phosphorus in soil water under a European beech (*Fagus sylvatica* L.) stand in northeastern Bavaria, Germany: seasonal variability and changes with soil depth. Biogeochemistry 66, 287–310

Kavka, M., Majcherczyk, A., Kües, U., Polle, A., 2021. Phylogeny, tissue-specific expression, and activities of root-secreted purple acid phosphatases for P uptake from ATP in P starved poplar. Plant Science 307, 110906. doi: 10.1016/j.plantsci.2021.110906

Kavka, M., Polle, A., 2016. Phosphate uptake kinetics and tissue-specific transporter expression profiles in poplar (*Populus* x *canescens*) at different phosphorus availabilities. BMC Plant Biology 16, 206. doi: 10.1186/s12870-016-0892-3

Khokon, A.M., Schneider, D., Daniel, R., Polle, A., 2021. Soil layers matter: vertical stratification of root-associated fungal assemblages in temperate forests

Reveals differences in habitat colonization. Microorganisms 9, 2131. doi: 10.3390/microorganisms9102131

Kirfel, K., Heinze, S., Hertel, D., Leuschner, C., 2019. Effects of bedrock type and soil chemistry on the fine roots of European beech – A study on the belowground plasticity of trees. Forest Ecology and Management 444, 256-268 doi: 10.1016/j.foreco.2019.04.022

Lang, F., Krüger, J., Amelung, W., Willbold, S., Frossard, E., Bünemann, E. K., et al., 2017. Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. Biogeochemistry 136, 5–29. doi: 10.1007/s10533017-0375-0

Leuschner, C., 2020. Drought response of European beech (Fagus sylvatica L.) – a review. Perspectives in Plant Ecology, Evolution and Systematics 47, 125576. doi: doi: 10.1016/j.ppees.2020.125576

Li, Y., Niu, S., Yu, G., 2016. Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. Global Change Biology 22, 934–943. doi: 10.1111/gcb.13125

Lindeman, R.H., Merenda, P.F., Gold, R.Z., 1980. Introduction to Bivariate and Multivariate Analysis. Scott, Foresman and company, Glenview, IL.

López, B., Sabaté, S., Gracia, C., 2001. Fine-root longevity of *Quercus ilex*. New Phytologist 151, 437–441. doi: 10.1046/j.0028-646x.2001.00189.x

Lwila, A.S., Mund, M., Ammer, C., Glatthorn, J., 2021. Site conditions more than species identity drive fine root biomass, morphology and spatial distribution in temperate pure and mixed forests. Forest Ecology and Management 499, 119581. doi: 10.1016/j.foreco.2021.119581

Majdi, H., Nylund, j.e., 1996. Does liquid fertilisation affect life span of mycorrhizal short roots and fine root dynamics? Plant Soil185, 305–309. doi: 10.1007/BF02257536

McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., et al., 2015. Redefining fine roots improves understanding of below-

ground contributions to terrestrial biosphere processes. New Phytologist 207, 505–518. doi: 10.1111/nph.13363

Meier, I.C., Leuschner, C., 2010. Variation of soil and biomass carbon pools in beech forests across a precipitation gradient. Global Change Biology 16, 1035-1045. doi: 10.1111/j.1365-2486.2009.02074.x

Meller, S., Frossard, E., Spohn, M., Luster, J., 2020. Plant nutritional status explains the modifying effect of provenance on the response of beech sapling root traits to differences in soil nutrient supply. Frontiers in Forests and Global Change. 3, 535117. doi: 10.3389/ffgc.2020.535117

Meyer-Grünefeldt, M., Friedrich, U., Klotz, M., von Oheimb, G., Härdtle, W., 2015. Nitrogen deposition and drought events have non-additive effects on plant growth - Evidence from greenhouse experiments. Plant Biosystems. 149, 424e432. doi: 10.1080/11263504.2013.853699

Montagnoli, A., Terzaghi, M., Di Iorio, A., Scippa, G.S., Chiatante, D., 2012b. Fine-root seasonal pattern, production and turnover rate of European beech (*Fagus sylvatica* L.) stands in Italy Prealps: possible implications of coppice conversion to high forest. Plant Biosystems 146, 1012–1022. doi: 10.1080/11263504.2012.741626

Nadelhoffer, K.J., 2000. The potential effects of nitrogen deposition on fine-root production in forest ecosystems. New Phytologist 147, 131-139

Netzer, F., Schmid, C., Herschbach, C., Rennenberg, H., 2017. Phosphorus nutrition of European beech (*Fagus sylvatica* L.) during annual growth depends on tree age and P-availability in the soil. Environmental and Experimental Botany 137, 194–207. doi: 10.1016/j.envexpbot.2017.02.009

Niklas, K.J., 2004. Plant allometry: is there a grand unifying theory? Biological Reviews 79, 871–889. doi: 10.1017/s1464793104006499

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., Janssens, I.A., 2013. Human-induced nitrogen–phosphorus imbalances alter

natural and managed ecosystems across the globe. Nature Communications 4, 2934. doi: 10.1038/ncomms3934

Peñuelas, J., Sardans, J., 2022. The global nitrogen-phosphorus imbalance. Science 375, 266–267. doi: 10.1126/science.abl4827

Pregitzer, K.S., Hendrick, R.L., Fogel, R., 1993. The demography of fine roots in response to patches of water and nitrogen. New Phytologist 125, 575–580. doi: 10.1111/j.1469-8137.1993.tb03905.x

R Core Development Team., 2020. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna

Reich, P.B., Luo, Y.J., Bradforde, J.B., Poorter, H., Perry, C.H., Oleksyn, J., 2014. Temperature drives global patterns in forest biomass distribution in leaves, stems, and roots. Proceedings of the National Academy of Sciences of the United States of America 111 (38), 13721–13726. doi: 10.1073/pnas.1216053111

Schneider, K., Turrion, M-B., Grieson, P.F., Gallardo, J.F., 2001. Phosphatase activity, microbial phosphorus, and fine root growth in forest soils in the Sierra de Gata, western central Spain. Biology and Fertility of Soils 34, 151–155. doi: 10.1007/s003740100387

Shapiro, S.S., Wil, M.B., 1965. An analysis of variance test for normality (complete samples). Biometrika, 52(3–4), pp.591–611. doi: 10.1093/biomet/52.3-4.591

Spohn, M., Zavišić, A., Nassal, P., Bergkemper, F., Schulz, S., Marhan, S., Schloter, M., Kandeler, E., Polle, A., 2018. Temporal variations of phosphorus uptake by soil microbial biomass and young beech trees in two forest soils with contrasting phosphorus stocks. Soil Biology and Biochemistry 117, 191-202. doi: 10.1016/j.soilbio.2017.10.019

Talkner, U., Meiwes, K., Potočič, N., Seletković, I., Cools, N., De Vos, B., Rautio, P., 2015. Phosphorus nutrition of beech (*Fagus sylvatica* L.) is decreasing in Europe. Annals of Forest Science 72, 919-928. doi: 10.1007/s13595-015-0459-8

Van Dijk, H.G., De Louw, M.H.J., Roelofs, J.G.M., Verburgh, J.J.,1990. Impact of artificial, ammonium-enriched rainwater on soils and young coniferous tress in a greenhouse. Part II - Effects on the trees. Environmental Pollution 63, 41- 59. doi: 10.1016/0269-7491(90)90102-i

Venables, W.N., Ripley, B.D., 2002. Modern applied statistics with S, 4th edn. Springer, New York

Vitousek, P. M., Porder, S., Houlton, B. Z., Chadwick, O. A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecological Applications 20, 5–15. doi: 10.1890/08-0127.1

Vogt, K.A., Vogt, D.J., Palmiotto, P.A., Boon, P., O'Hara, J., Asbjornsen, H., 1996. Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. Plant and Soil 187, 159-219. Doi: 10.1007/BF00017088

Whitehead, D., 2011. Forests as carbon sinks—benefits and consequences. Tree Physiology 31 (9), 893–902. doi: 10.1093/treephys/tpr063

Zavišić, A., Nassal, P., Yang, N., Heuck, C., Spohn, M., Marhan, S., Pena, R., Kandeler, E., Polle, A., 2016. Phosphorus availabilities in beech (*Fagus sylvatica* L.) forests impose habitat filtering on ectomycorrhizal communities and impact tree nutrition. Soil Biology and Biochemistry 98, 127–137. doi: 10.1016/j.soilbio.2016.04.006

Zavišić, A., Polle, A., 2018. Dynamics of phosphorus nutrition, allocation and growth of young beech (*Fagus sylvatica* L.) trees in P-rich and P-poor forest soil. Tree Physiology 38, 37-51. doi: 10.1093/treephys/tpx146

Zederer, D. P., Talkner, U., 2018. Organic P in temperate forest mineral soils as affected by humus form and mineralogical characteristics and its relationship to the foliar P content of European beech. Geoderma 325, 162–171. doi: 10.1016/j.geoderma.2018.03.033

Supplement Table S2.1. Climate and weather data for the different forest types and sampling time (T0: fall 2016; T1: spring 2017; T2: fall 2017; T3: spring 2018; T4: fall 2018). The data were obtained from www.wetterzentrale.de. The temperature (T) data represent mean values for the two months before sampling and during sampling. Precipitation (Prec) is provided as sum of precipitation for two months before and the sampling month. The deviations from long-term climatic conditions (dfc) were determined as the difference of the monthly mean temperature or sum of precipitation of three months (two months before sampling and month of sampling) and the corresponding long-term mean temperature or sum of precipitation for the period 1981-2010.

Forest	Sampling date	T (℃)	T dfc (℃)	Prec (mm)	Prec dfc (mm)
P-low	T0	18.2	0.5	132.5	-61.9
	T1	9.5	0.2	127.9	-23.5
	T2	15.8	-1.2	296.2	105.6
	T3	5.5	0.2	56.5	-88.7
	T4	18.9	1.9	113.1	-77.5
P-medium	T0	16.8	-0.3	376.0	85.5
	T1	8.5	0.2	227.2	36.7
	T2	12.9	0.1	198.0	-65.3
	T3	9.7	1.4	77.7	-62.2
	T4	14.6	1.8	175.1	-88.2
P-high	T0	17.3	0.9	166.3	-38.8
	T1	9.4	1.6	156.5	-53.3
	T2	15.6	0.0	271.2	59.2
	T3	3.9	0.3	134.0	-77.7
	T4	18.8	3.2	94.7	-117.3

Supplement Table S2.2. Mean values for fine root mass (FR) and other parameters (total P: Pt, soluble P: Ps, C, N, CN, Na, K, Ca, Mg, Mn, Fe, S, Al, pH and water content: WC) analyzed from the soil (denoted with lowercase letter s) and fine roots (denoted with lowercase letter r). The soil samples were collected from P-low (Unterluess), P-medium (Mitterfels) and P-high (Bad Bruckenau) forest sites. The mean values are provided with respective standard errors (n = 6 for spring season; n = 9 for fall season) for each soil layer (OL: organic; ML: mineral) according to sites and fertilizer treatment. The mean for elements and FR values are given in mg g^{-1} DW. WC is given in g g^{-1} DW. The mean values for soil dry mass (g) were determined from the pooled soil cores (volume: 5987.04 cm^3) fractionated into organic and mineral layers. Supplement Table S2.2 is available as a separate data sheet.

Soil variables																		
P-low																		
Spring (OL)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	0.42	0.10	0.08	0.02	254.68	58.34	11.65	2.96	27.41	5.38	0.07	0.01	0.34	0.03	2.36	0.74	0.46	0.06
N	0.42	0.07	0.09	0.02	263.23	39.66	11.27	1.84	22.48	0.66	0.07	0.01	0.30	0.03	2.15	0.59	0.44	0.05
P	0.52	0.10	0.11	0.02	270.82	49.50	12.27	2.39	22.42	0.62	0.08	0.01	0.36	0.03	2.65	0.96	0.45	0.05
P+N	0.53	0.10	0.14	0.03	257.61	36.90	12.33	1.98	21.23	0.51	0.07	0.00	0.34	0.03	3.30	0.98	0.52	0.08

	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW	
Con	0.65	0.39	3.69	0.54	0.99	0.21	3.00	0.62	0.42	0.06	4.52	0.11	158.83	32.56
N	0.63	0.33	4.51	0.58	1.00	0.17	3.09	0.19	0.44	0.05	4.49	0.09	141.35	35.46
P	0.66	0.40	3.73	0.69	1.11	0.16	2.79	0.42	0.43	0.06	4.35	0.10	144.91	25.74
P+N	0.87	0.41	4.02	0.66	1.08	0.13	2.75	0.26	0.44	0.05	4.46	0.10	143.93	31.33

Fall (OL)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	0.33	0.02	0.06	0.00	214.45	15.08	9.75	0.76	22.11	0.50	0.08	0.01	0.29	0.01	1.28	0.14	0.44	0.07
N	0.31	0.04	0.06	0.00	188.77	31.41	8.32	1.47	23.09	0.71	0.07	0.02	0.28	0.01	1.39	0.25	0.41	0.06
P	0.37	0.04	0.08	0.01	215.14	11.16	9.62	0.59	22.50	0.40	0.07	0.01	0.31	0.01	1.55	0.27	0.42	0.03
P+N	0.38	0.04	0.07	0.01	238.55	25.87	10.97	1.31	21.99	0.45	0.07	0.01	0.29	0.01	2.10	0.35	0.44	0.04

Continuation of supplement Table S2.2

Soil variables																		
P-low																		
Fall (OL)																		
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	0.14	0.02	3.99	0.17	0.95	0.08	3.49	0.25	0.59	0.04	4.44	0.12	240.52	29.57				
N	0.21	0.05	4.57	0.39	0.82	0.15	3.60	0.22	0.66	0.06	4.27	0.07	251.36	48.89				
P	0.24	0.09	3.94	0.45	0.97	0.12	3.44	0.27	0.59	0.04	4.31	0.09	186.56	35.91				
P+N	0.31	0.09	3.92	0.24	0.98	0.07	3.24	0.24	0.59	0.04	4.44	0.08	215.85	35.27				
Spring (ML)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	0.08	0.01	0.02	0.00	20.73	2.82	0.80	0.19	30.69	4.10	0.05	0.01	0.25	0.03	0.44	0.08	0.28	0.01
N	0.08	0.01	0.03	0.00	24.99	3.82	0.94	0.21	29.68	2.85	0.04	0.00	0.20	0.02	0.33	0.07	0.25	0.03
P	0.12	0.01	0.04	0.00	50.46	17.68	2.21	0.92	26.21	2.32	0.05	0.01	0.23	0.01	0.42	0.09	0.27	0.00
P+N	0.08	0.01	0.03	0.00	23.95	3.46	0.94	0.22	28.75	3.04	0.05	0.01	0.22	0.02	0.35	0.05	0.25	0.02
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
Con	0.05	0.01	5.05	0.33	0.12	0.02	4.83	0.27	0.85	0.02	4.15	0.08	1110.24	53.22				
N	0.05	0.01	5.24	0.57	0.11	0.01	4.53	0.34	0.86	0.01	4.09	0.05	998.63	50.86				
P	0.07	0.02	4.61	0.19	0.22	0.09	4.53	0.24	0.84	0.02	4.06	0.01	1007.69	44.77				
P+N	0.05	0.01	4.82	0.52	0.12	0.02	4.36	0.37	0.85	0.02	4.16	0.09	929.48	70.50				
Fall (ML)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	0.07	0.01	0.02	0.00	20.89	2.24	0.71	0.10	31.42	0.93	0.03	0.00	0.21	0.01	0.34	0.04	0.26	0.02
N	0.08	0.01	0.02	0.00	26.28	4.85	0.87	0.20	32.92	1.48	0.03	0.01	0.20	0.01	0.29	0.04	0.27	0.02
P	0.10	0.01	0.03	0.00	33.46	6.63	1.20	0.31	30.16	0.92	0.03	0.01	0.23	0.01	0.33	0.03	0.29	0.02
P+N	0.08	0.01	0.03	0.00	29.67	6.70	1.04	0.29	30.58	1.14	0.04	0.01	0.19	0.02	0.33	0.04	0.24	0.03

Continuation of supplement Table S2.2

Soil variables																		
P-low																		
Fall (ML)																		
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	0.05	0.00	4.09	0.30	0.09	0.01	4.29	0.27	0.91	0.01	4.14	0.06	1809.69	132.56				
N	0.05	0.01	4.98	0.52	0.10	0.02	4.56	0.31	0.91	0.02	4.06	0.03	1814.43	179.57				
P	0.04	0.00	4.75	0.26	0.13	0.03	4.90	0.35	0.90	0.01	4.08	0.08	1527.51	145.22				
P+N	0.04	0.01	3.67	0.31	0.12	0.03	3.91	0.41	0.90	0.02	4.11	0.06	1606.26	137.88				
P-medium																		
Spring (OL)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	1.06	0.10	0.18	0.02	465.86	1.47	22.50	0.34	20.73	0.30	22.50	0.34	0.57	0.05	2.37	0.24	0.79	0.11
N	1.09	0.12	0.16	0.02	463.52	5.21	22.39	0.66	20.77	0.44	22.39	0.66	0.60	0.07	2.94	0.40	0.89	0.13
P	1.10	0.15	0.18	0.02	464.48	2.07	22.39	0.22	20.76	0.19	22.39	0.22	0.59	0.09	2.81	0.41	0.89	0.14
P+N	1.06	0.11	0.19	0.01	465.04	3.99	22.45	0.26	20.72	0.15	22.45	0.26	0.62	0.08	2.28	0.29	0.80	0.14
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
Con	0.17	0.02	2.32	0.40	2.22	0.16	2.68	0.41	0.27	0.01	4.09	0.06	70.49	12.13				
N	0.22	0.02	2.53	0.33	2.25	0.27	2.94	0.31	0.28	0.01	4.02	0.04	62.54	11.20				
P	0.22	0.02	2.25	0.39	2.26	0.22	2.73	0.44	0.29	0.01	3.94	0.04	67.42	7.52				
P+N	0.17	0.02	2.11	0.47	2.06	0.18	2.43	0.48	0.27	0.01	4.08	0.06	74.47	8.78				
Fall (OL)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	0.89	0.04	0.17	0.02	425.32	13.43	20.42	0.55	20.84	0.41	20.42	0.55	0.68	0.09	1.54	0.21	1.24	0.21
N	0.84	0.03	0.16	0.01	407.82	12.36	19.53	0.50	20.90	0.50	19.53	0.50	0.79	0.12	1.56	0.21	1.71	0.28
P	0.94	0.04	0.19	0.01	420.06	13.92	20.60	0.67	20.45	0.56	20.60	0.67	0.71	0.09	1.61	0.24	1.35	0.22
P+N	0.91	0.05	0.20	0.02	427.42	11.71	21.16	0.71	20.28	0.50	21.16	0.71	0.64	0.08	1.49	0.16	1.30	0.15

Continuation of supplement Table S2.2.

Soil variables																		
P-low																		
Fall (OL)																		
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	0.15	0.02	5.82	1.02	1.85	0.09	7.87	1.55	0.34	0.04	4.07	0.05	50.87	6.13				
N	0.17	0.02	7.43	1.13	1.76	0.08	9.30	1.65	0.32	0.03	4.06	0.06	47.58	8.67				
P	0.15	0.02	5.89	1.05	1.89	0.09	7.92	1.39	0.32	0.03	3.99	0.04	48.23	4.53				
P+N	0.15	0.02	5.41	0.86	1.94	0.10	7.20	1.21	0.29	0.02	3.98	0.05	44.18	6.32				
P-medium																		
Spring (ML)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	1.21	0.18	0.16	0.02	109.69	7.71	6.18	0.47	17.80	0.16	6.18	0.47	2.95	0.75	1.24	0.35	6.02	0.93
N	1.35	0.19	0.15	0.02	117.05	6.02	6.62	0.41	17.76	0.37	6.62	0.41	3.46	0.89	1.14	0.25	8.14	1.26
P	1.12	0.16	0.13	0.01	100.83	5.91	5.74	0.33	17.58	0.26	5.74	0.33	3.76	0.78	0.84	0.31	7.81	1.14
P+N	1.29	0.19	0.16	0.00	114.69	5.75	6.64	0.28	17.24	0.23	6.64	0.28	3.96	0.97	0.99	0.34	8.36	1.40
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
Con	0.44	0.09	37.48	5.80	0.83	0.11	39.95	7.96	0.57	0.01	4.14	0.04	379.41	63.43				
N	0.45	0.08	42.60	6.64	0.98	0.11	47.20	8.82	0.57	0.01	4.21	0.05	420.07	42.44				
P	0.45	0.09	40.43	6.14	0.81	0.11	46.44	7.92	0.58	0.01	4.14	0.04	444.31	46.98				
P+N	0.53	0.11	43.01	6.77	0.90	0.11	49.84	9.59	0.58	0.01	4.20	0.04	362.73	36.56				
Fall (ML)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	0.98	0.06	0.15	0.01	103.88	8.87	5.76	0.48	17.93	0.13	5.76	0.48	2.27	0.21	0.83	0.17	5.23	0.53
N	0.97	0.06	0.13	0.01	103.48	4.23	5.95	0.27	17.42	0.29	5.95	0.27	2.51	0.19	0.69	0.12	6.27	0.33
P	0.89	0.06	0.13	0.02	88.61	3.77	5.14	0.19	17.16	0.28	5.14	0.19	2.78	0.26	0.64	0.10	6.42	0.57
P+N	1.01	0.07	0.15	0.02	104.42	9.81	6.08	0.63	17.25	0.28	6.08	0.63	2.75	0.22	0.76	0.18	6.12	0.56

Continuation of supplement Table S2.2.

Soil variables																		
P-medium																		
Fall (ML)																		
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	0.32	0.02	27.70	1.24	0.69	0.06	32.72	2.17	0.65	0.04	4.15	0.02	795.33	105.53				
N	0.32	0.01	29.71	0.63	0.73	0.05	35.65	1.59	0.60	0.02	4.21	0.08	678.79	83.07				
P	0.37	0.03	29.11	1.23	0.65	0.04	40.30	3.78	0.62	0.02	4.20	0.05	811.98	93.76				
P+N	0.32	0.03	29.10	1.09	0.73	0.09	36.78	1.83	0.59	0.01	4.22	0.04	782.43	130.14				
P-high																		
Spring (OL)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	2.01	0.27	0.29	0.02	336.22	29.31	16.57	1.12	20.12	0.52	16.57	1.12	0.99	0.12	12.00	3.73	8.10	3.67
N	1.94	0.17	0.31	0.02	332.09	10.17	16.92	0.55	19.66	0.45	16.92	0.55	1.06	0.12	10.31	2.15	5.77	1.30
P	1.75	0.17	0.33	0.01	361.30	9.33	18.42	0.47	19.63	0.27	18.42	0.47	0.91	0.11	7.76	2.08	3.39	0.89
P+N	1.47	0.09	0.34	0.02	322.70	19.32	16.66	0.60	19.31	0.70	16.66	0.60	0.75	0.04	8.12	1.14	6.76	1.90
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
Con	2.42	0.50	30.40	6.25	1.75	0.25	20.35	3.70	0.42	0.03	4.62	0.12	50.71	10.94				
N	2.07	0.36	26.22	3.43	1.61	0.16	19.35	2.26	0.47	0.05	4.75	0.08	42.84	8.77				
P	2.56	0.61	20.19	3.53	1.73	0.24	14.35	1.91	0.42	0.04	4.50	0.10	49.00	12.84				
P+N	1.67	0.23	22.77	3.85	1.34	0.09	15.64	1.85	0.46	0.04	4.72	0.08	79.36	13.17				
Fall (OL)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	1.58	0.07	0.24	0.03	208.72	15.06	12.25	0.78	16.97	0.34	12.25	0.78	1.09	0.12	5.50	1.22	6.88	2.02
N	1.69	0.11	0.27	0.03	215.75	16.67	13.24	0.78	16.20	0.48	13.24	0.78	1.12	0.09	6.13	0.45	7.28	0.69
P	1.58	0.11	0.35	0.03	234.00	12.72	13.89	0.61	16.82	0.37	13.89	0.61	1.11	0.10	3.50	0.40	4.45	0.65
P+N	1.52	0.12	0.28	0.04	257.20	32.11	14.81	1.39	17.02	0.72	14.81	1.39	0.86	0.09	4.99	0.38	6.55	0.96

Continuation of supplement Table S2.2.

Soil variables																		
P-high																		
Fall (OL)																		
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	1.16	0.16	34.70	3.68	1.13	0.06	26.22	2.25	0.54	0.04	4.49	0.09	165.89	20.81				
N	1.18	0.06	36.12	2.17	1.16	0.05	28.15	2.04	0.50	0.04	4.70	0.05	149.17	18.38				
P	1.04	0.13	33.79	3.70	1.23	0.07	25.77	2.21	0.52	0.05	4.40	0.09	140.84	16.03				
P+N	1.55	0.41	32.24	4.86	1.29	0.12	24.08	3.70	0.51	0.04	4.73	0.09	145.60	20.72				
Spring (ML)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	2.34	0.45	0.23	0.04	102.53	4.83	6.94	0.33	14.79	0.35	6.94	0.33	2.16	0.58	8.90	2.26	14.15	2.90
N	2.39	0.47	0.22	0.02	106.07	3.98	7.48	0.27	14.18	0.25	7.48	0.27	1.94	0.38	13.18	3.61	16.88	3.12
P	2.47	0.51	0.31	0.02	104.37	4.56	7.05	0.35	14.83	0.18	7.05	0.35	2.35	0.63	6.18	1.93	10.96	2.58
P+N	2.69	0.50	0.24	0.01	111.10	8.54	7.65	0.43	14.44	0.35	7.65	0.43	2.00	0.49	11.68	3.02	17.81	2.80
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
Con	2.77	0.61	68.80	9.51	0.98	0.14	62.61	12.48	0.55	0.01	4.54	0.04	330.17	31.27				
N	2.46	0.43	63.19	8.74	0.97	0.12	54.96	10.05	0.54	0.01	4.61	0.05	274.33	34.08				
P	2.49	0.55	68.85	11.03	0.97	0.13	62.76	12.81	0.62	0.06	4.43	0.04	458.74	78.41				
P+N	3.21	0.95	69.94	9.96	1.02	0.16	61.61	12.34	0.56	0.01	4.54	0.08	291.91	20.63				
Fall (ML)																		
	Pt_s		Ps_s		C_s		N_s		CN_s		Na_s		K_s		Ca_s		Mg	
Con	1.51	0.12	0.17	0.02	90.00	6.36	6.28	0.44	14.33	0.32	6.28	0.44	1.17	0.13	4.68	0.86	10.06	2.07
N	1.53	0.17	0.19	0.02	97.65	2.91	7.22	0.31	13.57	0.22	7.22	0.31	1.18	0.14	6.09	0.98	8.71	1.67
P	1.66	0.17	0.22	0.03	89.57	3.51	6.25	0.31	14.43	0.27	6.25	0.31	1.40	0.14	3.80	0.56	8.25	1.00
P+N	1.72	0.15	0.19	0.01	106.22	9.57	7.43	0.52	14.18	0.45	7.43	0.52	1.24	0.12	5.50	0.57	10.61	1.05

Continuation of supplement Table S2.2.

Soil variables																		
P-high																		
Fall (ML)																		
	Mn_s		Fe_s		S_s		Al_s		WC_s		pH		Soil DW					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	1.63	0.25	48.45	6.46	0.68	0.04	37.76	4.06	0.64	0.02	4.43	0.05	782.90	85.20				
N	1.33	0.21	37.31	6.20	0.75	0.06	31.98	5.26	0.61	0.02	4.58	0.04	613.14	69.10				
P	1.62	0.19	48.91	3.90	0.65	0.04	41.02	2.02	0.63	0.02	4.37	0.04	694.55	69.48				
P+N	2.20	0.39	49.65	3.24	0.71	0.04	41.15	1.87	0.61	0.02	4.54	0.05	637.47	72.84				
Root variables																		
P-low																		
Spring (OL)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
Con	0.85	0.12	0.41	0.11	485.78	3.23	15.79	1.99	32.94	3.52	0.18	0.02	1.05	0.27	3.84	0.43	0.72	0.08
N	0.92	0.12	0.46	0.10	488.34	5.41	16.88	1.67	30.30	2.79	0.22	0.04	1.10	0.27	4.10	0.55	0.79	0.07
P	1.18	0.18	0.70	0.13	478.49	8.96	16.28	1.83	31.05	3.01	0.19	0.01	1.20	0.33	3.44	0.24	0.74	0.07
P+N	1.10	0.12	0.62	0.12	485.71	3.67	16.82	2.23	31.22	3.56	0.15	0.02	0.98	0.21	4.66	0.52	0.79	0.09
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.27	0.06	0.59	0.13	1.20	0.07	0.51	0.13	30.60	10.46								
N	0.38	0.09	0.91	0.23	1.23	0.06	0.72	0.19	15.54	4.26								
P	0.25	0.07	0.77	0.20	1.20	0.07	0.64	0.21	30.17	9.49								
P+N	0.35	0.10	0.66	0.13	1.23	0.10	0.55	0.12	22.79	7.31								

Continuation of supplement Table S2.2.

Root variables																		
P-low																		
Fall (OL)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	0.68	0.12	0.21	0.01	473.54	9.96	13.02	0.55	36.75	1.34	0.15	0.02	0.77	0.08	3.78	0.43	0.81	0.12
N	0.63	0.02	0.24	0.02	469.50	15.83	12.74	0.97	37.95	2.21	0.17	0.01	0.75	0.07	4.01	0.34	0.77	0.04
P	0.73	0.05	0.29	0.03	505.36	26.25	13.43	1.27	38.91	2.11	0.13	0.01	0.79	0.09	3.85	0.40	0.71	0.04
P+N	0.72	0.07	0.25	0.03	472.41	14.23	13.20	1.08	38.40	4.11	0.14	0.01	0.67	0.06	4.54	0.44	0.77	0.07
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.17	0.02	2.16	0.68	1.14	0.11	1.20	0.34	14.47	2.56								
N	0.30	0.07	1.53	0.40	1.13	0.10	0.99	0.30	8.50	2.19								
P	0.21	0.05	1.46	0.30	1.18	0.08	0.94	0.13	18.40	3.55								
P+N	0.24	0.04	1.22	0.25	1.16	0.08	0.74	0.16	14.87	2.12								
Spring (ML)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
Con	0.47	0.04	0.19	0.01	449.56	14.36	9.43	1.53	53.04	6.87	0.22	0.05	0.95	0.06	2.83	0.44	0.62	0.05
N	0.51	0.04	0.21	0.02	449.27	14.56	9.79	1.19	48.82	5.05	0.21	0.04	0.84	0.11	2.56	0.48	0.66	0.03
P	0.62	0.05	0.33	0.02	442.22	21.59	8.94	1.52	54.80	6.39	0.17	0.04	0.86	0.08	2.57	0.39	0.63	0.05
P+N	0.72	0.09	0.30	0.03	425.33	22.42	8.87	1.32	51.84	5.61	0.22	0.04	1.01	0.23	2.93	0.45	0.77	0.08
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.17	0.05	2.92	0.82	0.81	0.09	2.39	0.54	0.74	0.12								
N	0.17	0.04	4.01	0.58	0.77	0.06	3.40	0.48	0.87	0.10								
P	0.13	0.03	3.35	0.95	0.80	0.07	2.88	0.62	1.07	0.19								
P+N	0.18	0.07	4.53	0.85	0.94	0.19	3.63	0.76	0.94	0.13								

Continuation of supplement Table S2.2.

Root variables																		
P-low																		
Fall (ML)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	0.42	0.05	0.13	0.02	406.68	29.71	8.02	1.07	54.74	4.23	0.17	0.03	0.64	0.08	2.50	0.40	0.67	0.11
N	0.45	0.05	0.16	0.02	447.18	10.52	9.36	1.17	52.63	4.58	0.20	0.04	0.62	0.06	2.62	0.28	0.62	0.05
P	0.42	0.05	0.18	0.01	410.61	23.68	7.53	1.03	59.23	4.35	0.13	0.02	0.63	0.08	2.49	0.39	0.57	0.05
P+N	0.50	0.06	0.21	0.03	416.33	20.39	9.18	1.32	51.05	4.93	0.16	0.02	0.67	0.10	2.61	0.33	0.63	0.05
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.09	0.02	4.92	1.06	0.78	0.10	3.00	0.24	0.54	0.06								
N	0.12	0.02	4.34	0.61	0.79	0.06	3.25	0.33	0.70	0.13								
P	0.07	0.01	4.19	0.79	0.74	0.06	2.62	0.22	1.24	0.19								
P+N	0.07	0.01	4.48	0.90	0.77	0.06	2.80	0.26	0.84	0.17								
P-medium																		
Spring (OL)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
Con	1.33	0.18	0.54	0.02	493.89	4.45	16.32	0.78	30.58	1.39	0.18	0.03	1.23	0.30	3.77	0.39	0.84	0.13
N	1.20	0.09	0.52	0.04	479.26	9.61	15.48	1.51	32.71	3.63	0.17	0.03	1.18	0.29	3.62	0.70	0.77	0.15
P	1.20	0.14	0.59	0.08	483.78	11.73	16.40	0.84	29.86	1.65	0.15	0.02	1.11	0.23	3.30	0.26	0.70	0.05
P+N	1.33	0.15	0.83	0.12	489.71	5.29	16.70	1.10	29.94	1.91	0.14	0.01	1.30	0.28	2.76	0.12	0.64	0.03
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.08	0.01	0.26	0.05	1.31	0.16	0.36	0.07	126.39	39.26								
N	0.10	0.02	0.22	0.05	1.28	0.16	0.33	0.06	94.91	17.19								
P	0.09	0.01	0.22	0.03	1.20	0.05	0.28	0.02	109.53	28.97								
P+N	0.07	0.01	0.15	0.02	1.08	0.03	0.22	0.03	180.65	24.67								

Continuation of supplement Table S2.2.

Root variables																		
P-medium																		
Fall (OL)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	1.00	0.05	0.41	0.06	492.56	3.06	18.05	1.20	28.20	1.77	0.14	0.02	0.94	0.10	3.24	0.39	0.75	0.04
Con	0.87	0.04	0.34	0.04	453.15	41.53	16.18	2.11	30.32	2.37	0.14	0.01	0.84	0.12	3.23	0.53	0.68	0.04
N	1.05	0.05	0.47	0.03	492.37	2.84	18.09	1.55	28.74	2.28	0.13	0.01	0.96	0.13	3.15	0.37	0.71	0.02
P	1.09	0.06	0.51	0.06	489.60	4.70	18.63	1.17	27.00	1.47	0.13	0.01	1.00	0.09	3.32	0.40	0.77	0.05
P+N	1.00	0.05	0.41	0.06	492.56	3.06	18.05	1.20	28.20	1.77	0.14	0.02	0.94	0.10	3.24	0.39	0.75	0.04
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.07	0.01	0.53	0.14	1.26	0.06	1.00	0.26	92.59	21.01								
N	0.06	0.00	0.41	0.09	1.19	0.07	0.65	0.13	87.10	15.17								
P	0.06	0.01	0.44	0.08	1.18	0.04	0.74	0.16	106.17	20.03								
P+N	0.07	0.01	0.44	0.11	1.29	0.10	0.74	0.20	99.63	7.94								
Spring (ML)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
Con	0.67	0.04	0.31	0.03	473.71	11.06	11.34	0.75	42.41	1.96	0.21	0.03	0.64	0.06	1.60	0.30	0.63	0.13
N	0.95	0.09	0.33	0.02	461.98	7.08	12.67	0.96	37.52	2.79	0.31	0.06	0.93	0.25	2.46	0.33	1.05	0.24
P	0.67	0.04	0.32	0.03	474.25	6.79	10.05	0.55	47.80	2.27	0.22	0.03	0.60	0.05	2.04	0.29	0.58	0.05
P+N	0.90	0.06	0.46	0.05	463.39	9.35	11.10	0.87	42.83	2.85	0.22	0.02	0.80	0.06	1.67	0.29	0.90	0.17
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.06	0.01	3.73	1.05	0.99	0.10	4.50	1.15	2.26	0.38								
N	0.06	0.02	6.06	1.80	1.27	0.22	7.37	2.17	2.06	0.16								
P	0.05	0.01	3.35	0.41	0.81	0.05	4.09	0.43	1.72	0.38								
P+N	0.05	0.01	4.70	0.95	0.97	0.06	5.75	1.12	2.27	0.39								

Continuation of supplement Table S2.2.

Root variables																		
P-medium																		
Fall (ML)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	0.74	0.04	0.27	0.02	450.43	10.48	10.33	0.50	44.19	1.55	0.23	0.02	0.89	0.09	1.79	0.19	1.18	0.18
N	0.71	0.05	0.22	0.01	445.68	7.49	10.74	0.46	42.10	1.30	0.18	0.01	0.83	0.06	1.76	0.27	1.24	0.13
P	0.68	0.04	0.30	0.02	452.35	7.09	10.15	0.39	45.07	1.51	0.20	0.02	0.86	0.08	1.93	0.33	1.17	0.18
P+N	0.79	0.04	0.35	0.02	447.05	9.25	10.12	0.62	45.39	2.22	0.21	0.02	0.93	0.07	2.08	0.23	1.23	0.19
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.08	0.01	7.01	1.02	0.92	0.04	8.03	1.17	4.31	1.17								
N	0.07	0.00	7.17	0.73	0.94	0.05	8.65	0.89	4.73	0.75								
P	0.06	0.01	6.00	0.84	0.85	0.05	7.86	0.79	3.60	0.61								
P+N	0.07	0.01	6.68	0.86	0.87	0.03	7.99	1.05	3.52	0.55								
P-high																		
Spring (OL)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
Con	1.95	0.38	0.65	0.09	454.13	8.75	17.65	1.85	27.16	2.50	0.27	0.03	2.00	0.52	6.00	0.49	1.53	0.26
N	1.58	0.22	0.77	0.08	433.57	20.29	18.01	1.48	25.12	2.27	0.32	0.07	1.80	0.35	5.62	0.66	1.38	0.20
P	1.94	0.26	0.88	0.07	450.85	8.89	18.81	1.73	25.07	2.16	0.26	0.03	1.94	0.50	5.20	0.64	1.23	0.14
P+N	1.74	0.20	0.56	0.08	455.11	7.10	17.78	1.58	26.73	2.16	0.28	0.06	1.55	0.36	5.98	0.44	1.44	0.16
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.61	0.08	4.37	1.04	1.76	0.29	3.34	0.77	4.13	2.43								
N	0.45	0.12	3.02	0.89	1.49	0.17	2.46	0.78	2.65	0.46								
P	0.47	0.06	2.96	1.16	1.65	0.15	2.57	0.91	5.73	2.33								
P+N	0.45	0.05	2.28	0.15	1.49	0.16	1.80	0.08	2.94	0.83								

Continuation of supplement Table S2.2.

Root variables																		
P-high																		
Fall (OL)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	1.29	0.09	0.58	0.05	440.63	9.38	15.91	1.28	29.01	2.19	0.26	0.04	1.17	0.08	5.07	0.70	1.33	0.12
N	1.24	0.09	0.58	0.09	420.01	25.80	15.84	1.21	27.44	2.42	0.23	0.01	1.38	0.30	3.90	0.42	1.51	0.29
P	1.46	0.08	0.77	0.10	438.37	10.10	16.23	0.83	27.60	1.59	0.21	0.03	1.29	0.17	4.22	0.50	1.41	0.13
P+N	1.22	0.08	0.57	0.05	438.58	10.97	16.10	1.05	28.18	1.87	0.18	0.01	0.88	0.06	4.79	0.46	1.49	0.20
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.37	0.04	6.40	1.31	1.35	0.04	5.37	1.04	4.76	0.72								
N	0.29	0.03	5.40	1.46	1.30	0.07	4.97	0.96	6.62	1.59								
P	0.41	0.05	6.74	1.76	1.31	0.04	5.95	1.51	9.92	1.79								
P+N	0.42	0.10	6.51	1.97	1.33	0.05	5.35	1.48	13.70	2.80								
Spring (ML)																		
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
Con	1.10	0.07	0.53	0.03	454.02	9.16	11.97	0.94	38.87	2.43	0.28	0.02	0.92	0.20	3.45	0.63	1.12	0.08
N	1.12	0.08	0.48	0.06	450.71	7.11	12.21	1.14	38.50	3.42	0.45	0.09	0.93	0.19	3.40	0.61	1.27	0.13
P	1.31	0.07	0.67	0.04	440.96	6.85	12.91	0.94	34.97	2.27	0.33	0.07	1.08	0.18	3.35	0.56	1.28	0.12
P+N	1.20	0.08	0.60	0.05	453.57	3.94	12.38	0.89	37.50	2.40	0.30	0.03	0.91	0.11	3.86	0.50	1.32	0.03
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.28	0.03	6.21	0.68	1.42	0.14	6.71	0.45	2.43	0.26								
N	0.25	0.01	5.71	0.61	1.32	0.08	6.26	0.36	1.59	0.31								
P	0.30	0.03	8.19	1.47	1.36	0.13	7.57	0.91	1.60	0.30								
P+N	0.23	0.03	5.13	0.61	1.26	0.07	5.53	0.57	2.01	0.37								

Continuation of supplement Table S2.2.

Root variables																	
P-high																	
Fall (ML)																	
	Pt_r		Ps_r		C_r		N_r		CN_r		Na_r		K_r		Ca_r		Mg_r	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Con	1.00	0.07	0.42	0.03	444.84	4.80	11.40	1.51	44.19	4.82	0.28	0.02	0.81	0.06	2.51	0.22	1.26	0.08
N	1.06	0.08	0.44	0.05	445.61	4.42	11.28	1.44	44.36	4.58	0.35	0.02	0.80	0.06	2.89	0.25	1.62	0.18
P	1.12	0.08	0.48	0.03	434.51	7.67	10.82	1.27	44.72	4.80	0.33	0.04	0.88	0.08	2.50	0.34	1.41	0.17
P+N	1.05	0.04	0.49	0.04	436.38	3.90	11.47	1.71	44.51	5.52	0.29	0.01	0.74	0.05	2.73	0.27	1.46	0.08
	Mn_r		Fe_r		S_r		Al_r		FR									
Con	0.23	0.02	7.71	0.81	1.29	0.11	8.51	0.45	3.13	0.48								
N	0.26	0.03	7.61	0.78	1.13	0.06	8.64	0.73	3.46	0.58								
P	0.34	0.04	9.90	1.43	1.25	0.10	9.61	0.84	2.72	0.54								
P+N	0.27	0.03	7.74	0.44	1.18	0.07	8.43	0.23	3.25	0.65								

CHAPTER 3: IMPACT OF NITROGEN AND PHOSPHORUS ADDITION ON RESIDENT SOIL AND ROOT MYCOBIOMES IN BEECH FORESTS

Simon Clausing, Likulunga Emmanuel Likulunga, Denis Janz, Huang Ying Feng, Dominik Schneider, Rolf Daniel, Jaane Krüger, Friederike Lang, Andrea Polle

Published in: Biology and Fertility of Soils, 2021 (57), 1031–1052

https://link.springer.com/article/10.1007/s00374-021-01593-x

Biology and Fertility of Soils (2021) 57:1031–1052
https://doi.org/10.1007/s00374-021-01593-x

ORIGINAL PAPER

Impact of nitrogen and phosphorus addition on resident soil and root mycobiomes in beech forests

S. Clausing[1] · L. E. Likulunga[1,2] · D. Janz[1] · H. Y. Feng[1,3] · D. Schneider[4] · R. Daniel[4] · J. Krüger[5] · F. Lang[5] · A. Polle[1]

Received: 29 December 2020 / Revised: 16 August 2021 / Accepted: 18 August 2021 / Published online: 15 September 2021

Abstract

N and P are essential macronutrients for all organisms. How shifts in the availability of N or P affect fungal communities in temperate forests is not well understood. Here, we conducted a factorial P × N fertilization experiment to disentangle the effects of nutrient availability on soil-residing, root-associated, and ectomycorrhizal fungi in beech (*Fagus sylvatica*) forests differing in P availability. We tested the hypotheses that in P-poor forests, P fertilization leads to enhanced fungal diversity in soil and roots, resulting in enhanced P nutrition of beech, and that N fertilization aggravates P shortages, shifting the fungal communities toward nitrophilic species. In response to fertilizer treatments (1×50 kg ha^{-1} P and 5×30 kg ha^{-1} N within 2 years), the labile P fractions increased in soil and roots, regardless of plant-available P in soil. Root total P decreased in response to N fertilization and root total P increased in response to P addition at the low P site. Ectomycorrhizal species richness was unaffected by fertilizer treatments, but the relative abundances of ectomycorrhizal fungi increased in response to P or N addition. At the taxon level, fungal assemblages were unaffected by fertilizer treatments, but at the order level, different response patterns for saprotrophic fungi among soil and ectomycorrhizal fungi on roots were found. Boletales increased in response to P, and Russulales decreased under N + P addition. Our results suggest that trait conservatism in related species afforded resistance of the resident mycobiome composition to nutritional imbalances.

Keywords Soil fertility · Mycorrhiza · Saprotrophic fungi · Stratification · Seasonality · Ecosystem nutrition

Introduction

Nitrogen (N) and phosphorus (P) are essential nutrients that determine plant growth and productivity in many terrestrial ecosystems (Elser et al. 2007; Vitousek et al. 2010).

✉ A. Polle
apolle@gwdg.de

1 Forest Botany and Tree Physiology, Georg-August Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

2 Biological Sciences Department, University of Zambia, Great East Road Campus, 32379 Lusaka, Zambia

3 Experimental Center of Forestry in Northern China, Chinese Academy of Forestry, Men Tougou District, Shuizha West Road 1, Beijing 102300, People's Republic of China

4 Genomic and Applied Microbiology & Göttingen Genomics Laboratory, Georg-August University Göttingen, Grisebachstr. 8, 37077 Göttingen, Germany

5 Soil Ecology, University of Freiburg, Bertoldstraße 17, 79085 Freiburg (i.Br.), Germany

The main natural sources of N and P are biological N fixation from atmospheric N_2 for N and rock weathering for P (Augusto et al. 2017; Wardle, 2004). Therefore, P availability is mainly dependent on the parent soil material and soil age (Augusto et al. 2017; Wardle, 2004), while N can also be influenced by anthropogenic inputs, for example, N emissions from agriculture and the burning of fossil fuels (Galloway et al. 2008). Forests are often naturally N-limited (Vitousek et al. 2010). Consequently, anthropogenic N deposition can change the nutrient regime and affect the P demand of forest ecosystems (Vitousek et al. 2010). N deposition into forest ecosystems has increased primary production in N-limited forest ecosystems (Du and De Vries, 2018; Schulte-Uebbing and De Vries, 2018). Increasing N:P ratios in plant tissues have therefore been suggested to indicate that many European forest ecosystems are transitioning from N to P limitation (Jonard et al. 2015; Peñuelas et al. 2013).

In temperate and boreal forest soils, large fractions of P and N are bound by organic matter and, thus, not directly available for uptake by trees (Lambers et al. 2008; van der Heijden et al. 2008). Trees benefit from P and

N mineralization by microbial decomposers (Baldrian, 2017; Schimel and Schaeffer, 2012). Soil fungi are generally more efficient degraders of complex plant compounds than other soil microbiota (López-Mondéjar et al. 2018; Štursová et al. 2012). Thus, the taxonomic diversity and functional composition of soil fungal microbiomes is of high relevance for forest P and N nutrition. Both saprotrophic and ectomycorrhizal fungi (EMF) contribute to N and P mobilization by secreting organic acids and producing hydrolytic and oxidative exoenzymes (Bödeker et al. 2014; Courty et al. 2010; Op De Beeck et al. 2018; Pritsch and Garbaye, 2011). In deciduous temperate forest soils, the fraction of EMF hyphal biomass is similar to or even higher than that of saprotrophs, suggesting that EMF have key functions in nutrient recycling in these ecosystems (Awad et al. 2019), though this is dependent strongly on the availability of nutrients (Högberg et al. 2017). Trees invest a higher proportion of carbon (C) into the symbiont when N is limiting, which stimulates fungal growth (Högberg et al. 2017; Näsholm et al. 2013). Therefore, the fungal N requirement increases, further decreasing N return to the plant. By this mechanism, the relative abundances of mycorrhizal and saprotrophic fungi may be shifted in favor of the former (Högberg et al. 2017). However, the impact of changes in N and P availability on these functional groups and the P nutrition of trees is still unknown.

Belowground fungal communities are affected by multiple abiotic and biotic habitat filters such as climate, geographic location, soil type, and vegetation that drive their composition (Bahnmann et al. 2018; Bahr et al. 2015; Goldmann et al. 2016; Kolaříková et al. 2017; Suz et al. 2014; Tedersoo et al. 2014; Wubet et al. 2012). Among these drivers, N is an important factor that affects fungi in soil (Almeida et al. 2019), fungi thriving on roots (Nguyen et al. 2020; Schröter et al. 2019), and fungal symbionts, i.e., EMF (Cox et al. 2010; de Witte et al. 2017; van der Linde et al. 2018). For example, Lilleskov et al. (2002) reported a shift in the EMF community in Alaska toward nitrophilic species and, thus, a loss in diversity along a gradient of increasing N deposition. In boreal spruce forests, N fertilization was shown to cause a significant turnover of soil fungal communities, decrease fungal biomass, and increase the N:P ratio of the needles (Allison et al. 2007; Almeida et al. 2019). Other studies reported only weak or no effect of N treatments on the fungal community composition (Maaroufi et al. 2019; Nicolás et al. 2017; Purahong et al. 2018), and relationships with P mobilization were not detected (Forsmark et al., 2021). Empirical studies and theoretical models suggest that EMF in temperate beech forests are less sensitive to N deposition than conifers (Lilleskov et al. 2019; Rotter et al. 2020; Taylor et al. 2000), but it is unknown how N fertilization affects EMF and other soil fungi when N availability is increased under P shortage.

Only a few studies have investigated fungal communities after P fertilization in forests. Almeida et al. (2019) reported significant community turnover and loss of fungal biomass after P fertilization in a spruce forest. However, along a natural P gradient in temperate beech forests, EMF diversity increased with increasing P availability (Zavišić et al. 2016). After the addition of superphosphate to P-limited young beech trees, the EMF community composition was altered, but microbial biomass was unaffected (Zavišić et al. 2018). These disparate observations indicate that the responses of EMF and other fungi to P inputs depend on the P supplied by the soil and likely the interaction of P and N supply (de Witte et al. 2017). Furthermore, different availabilities of carbon (C) for fungal communities colonizing the root surface and those living in soil (Clausing et al. 2021; Clemmensen et al. 2013) may lead to divergent responses of fungi to N and P availabilities in each of these habitats. A further important aspect underlying fungal responses to changes in nutrient resources is their phylogenetic relationship because fungal traits for nutrient acquisition are relatively conserved within a phylum or subphylum (Treseder and Lennon, 2015) and therefore shifts in phylogenetically related fungi in response to enhanced N or P availabilities might be expected. However, experiments addressing these ecological processes are scarce (Amend et al. 2016; Zanne et al. 2020).

The aim of our study was to investigate how shifts in N and P availability affect fungal assemblages and functional composition with different degrees of reliance on root and soil nutrient resources. To this end, we studied the richness, diversity, and taxonomic composition of EMF living in symbioses with root tips and conducted Illumina sequencing of whole, root-associated fungal (RAF), and soil-associated fungal (SAF) assemblages in beech forest plots fertilized with either P, N, or P + N and untreated plots (*Fagus sylvatica* L.). Since soil fertility affects fungal assemblages, we selected three forests differing strongly in P availability (Lang et al. 2017) and analyzed the responses of the fungal communities and root N and P contents to fertilization in the organic layer and mineral soil. Since the EMF community composition and richness in P-rich soils of our selected forest sites differed from that in P-poor soil (Zavišić et al., 2016), we hypothesized that P fertilization on P-poor soil results in increased P availability and shifts the composition of EMF and RAF toward those of fungal assemblages in P-rich soil. Thereby, the abundance of mycorrhizal fungi increases relative to that of saprotrophic fungi, leading to higher P nutrition of roots. Second, we hypothesized that P fertilization has no effects on EMF, RAF, or SAF richness, diversity, community composition, or root P contents in P-rich soil. Third, we proposed that N fertilization results in higher inorganic N availability, which shifts the mycorrhizal fungal community composition toward nitrophilic

fungal phyla in P-rich soil. Since these fungi are usually less species-rich and produce less mycelium (Lilleskov et al. 2019; Taylor et al. 2000), we expected a higher abundance of saprotrophic fungi relative to mycorrhizal fungi in the soil. Fourth, we hypothesized that in poor soil, N fertilization aggravates P shortages in roots and, therefore, EMF richness, diversity, and composition are unaffected or increase as trees maintain investment in mycorrhizas to counteract P deficiency.

Material and methods

Site characteristics and study plots

The N and P fertilization experiments were carried out in three beech (*F. sylvatica* L.) forests, differing in parent material and thus, soil P stocks. The high-P site (HP) Bad Brückenau is located in the biosphere reservation "Bayerische Rhön" on basalt, the medium-P site (MP) Mitterfels is situated in the Bavarian Forest on paragneiss and the low-P site (LP) Luess is located in the North German Plain on sandy till. P stocks in the A horizon (1 m soil depth) at the HP, MP, and LP site before fertilizer application are approximately 9.0, 6.8, and 1.6 t ha−1, respectively (Supplement Table S1, Lang et al. 2017). The pH of the soils ranged from 3.5 to 3.8 (Supplement Table S1). Information on the climate (1981 to 2010) and weather during sampling was obtained from www.wetterzentrale.de (Supplement Table 2).

For this study, 12 plots with an area of 400 m^2 each were installed in each forest in summer 2016 under 120- to 140-year-old beech trees (Supplement Table S1). One control (Con) and three different fertilizer treatments (N, P, P + N), each replicated three times per forest (= a total of 36 plots) and located about 300 m apart from each other, were treated as follows: P was applied only once in late summer 2016 as KH_2PO_4 at the dosage of 50 kg P ha^{-1} to the P and N + P plots. N was applied as NH_4NO_3 five times (late summer 2016, spring 2017, summer 2017, fall 2017, spring 2018) corresponding to a dosage of 30 kg N ha^{-1} per treatment on the N and N + P plots. To account for the K input in the P treatments, KCl was applied once in fall 2016 to the Con and N plots. The minerals were dissolved in tap water and applied with garden sprayers.

Harvest and processing of soil cores

Soil was sampled in the third year after the start of the treatments in spring (LP: 16.04.2018, HP: 23.04.2018, MP: 02.05.2018) and fall (LP: 17.09.2018, HP: 25.09.2018, MP: 01.10.2018). The weather conditions in the months of sampling and before as well as the long-term climate (1981 to 2010) are shown in the supplementary materials (Supplement Table S2). The sampling took place approximately 6 months after N addition. In each plot, 12 randomly distributed soil cores (depth 0.21 m, diameter 55 mm) were extracted after removal of surface litter. Each soil core was separated in organic (OL) and mineral topsoil (ML). The respective layers were pooled yielding one OL and one ML sample per plot. Each sample was fractionated into bulk soil, fine roots (< 2 mm), coarse roots (> 2 mm), and residual materials (fruits, twigs, and stones) in the field. Each subsample was directly divided into three aliquots: a fresh sample that was kept cool at 4 °C until use, a sample that was immediately frozen in liquid N (and stored at − 80 °C in the laboratory) and a sample that was dried (40 °C, 14 days). Bulk soil was sieved (mesh width: 4 mm) and the root samples were carefully washed before the aliquots were taken. All fractions were weighed in the laboratory. During the harvest in fall 2018, an additional soil core was collected in each plot. The sampling position was located adjacent to that of the soil cores for chemical analysis. The extra soil core was used to collect the roots for mycorrhizal morphotyping.

Determination of soil and root chemistry

To determine inorganic N, the concentrations of exchangeable ammonium (NH_4^+) and nitrate (NO_3^-) in soil, 20 g of fresh sieved bulk soil was extracted at the field site in 40 ml $CaCl_2$ solution for 60 min under shaking, subsequently filtered with phosphate free filter paper (MN 280 ¼, Macherey–Nagel, Düren, Germany) and kept cool. In the laboratory, the extracts were dried twice by cryodessication for 72 h (BETA I, Christ, Osterode am Harz, Germany) and dissolved in 1.5 ml ultra-pure water. The concentrated extracts were used to determine nitrate (# 109,713, Merck, Darmstadt, Germany) and exchangeable ammonium concentrations with kits (# 100,683, Merck) according to the manufacturer's instructions. The extinction of the assays was measured in an UV–Vis spectrophotometer (Shimadzu 1601, Hannover, Germany) at 690 nm for NH_4^+, and 340 nm for NO_3^-.

To determine soil pH values, 10 g of oven dried milled soil was suspended in 25 ml deionized water and shaken for 1 h at 200 rpm. After sedimentation of the particles, the pH was measured by a pH meter (WTW, Weilheim, Germany). After addition of 0.01 M $CaCl_2$ (1:5 soil-to-solution ratio) and 16 h equilibration, the samples were measured again (ISO10390, 2005).

To determine element contents, dry soil and root samples were milled (Retsch MN 400, Haan, Germany) to a fine powder before determining element contents. For the determination of total P (P_{tot}), 50 mg of the powder was weighed and extracted in 25 ml of 65% HNO_3 at 160 °C for 12 h according to Heinrichs et al. (1986). For the determination of the labile P (P_{lab}) fraction about 100 mg of soil or root powder was extracted in 150 ml of Bray-1 solution (0.03 N NH_4F, 0.025 N HCl) for 60 min on a shaker at 180 rpm (Bray and Kurtz 1945). The extracts were

filtered using phosphate free filter paper (MN 280 ¼, Macherey–Nagel) and used for elemental analysis by inductively coupled plasma–optical emission spectroscopy (ICP-OES) (iCAP 7000 Series ICP–OES, Thermo Fisher Scientific, Dreieich, Germany). P was measured at the wavelength of 185.942 nm (axial) and calibrated with a series of concentrations by element standards (P: 0.1 mg l^{-1} to 20 mg l^{-1}) (Einzelstandards, Bernd Kraft, Duisburg, Germany). In addition to P, we also determined K, Ca, Mg, Mn, Fe, Al, and S in the P_{tot} extracts.

To determine C and N, subsamples of 2 to 12 mg of soil or 1.5 mg of root powder were weighed into tin capsules (size of 4 × 6 mm, IVA Analysentechnik, Meerbusch, Germany) using a microbalance (Model MC5, Sartorius, Goettingen, Germany) before determining C and N contents. The range of weights for the soil samples was necessary to avoid overflow of the measuring unit of the mass spectrometer since the C concentrations in the OL and ML and between sand of other soil types varied drastically. The amounts of C and N of the soil and plant samples were measured at the KOSI (Kompetenzzentrum Stabile Isotope, Göttingen. Germany) using an isotope mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany) and acetanilide (10.36% N, 71.09% C) as the standard.

Determination of ectomycorrhizal fungal species by morphotyping and Sanger sequencing

Roots from the extra soil core collected in fall were separated according to OL and ML, and immediately processed after sampling. The beech roots were gently washed in 4 °C precooled tap water, spread in water in a glass dish, and categorized according to their visual appearance under a stereomicroscope (Leica M205 FA, Wetzlar, Germany) as vital ectomycorrhizal, vital non-mycorrhizal or dry. All root tips in each soil core were inspected and counted. Two soil cores did not contain any fine roots. Ectomycorrhizal colonization and root tip mortality were calculated as follows:

$$\text{Ecomycorrhizal colonization (\%)} = \frac{\text{(number of vital mycorrhizal root tips)} \times 100}{\text{(number of vital mycorrhizal root tips + number of vital nonmycorrhizal root tips}}$$

$$\text{Root tip mortality (\%)} = \frac{\text{number of dry root tips}}{\text{number of all counted root tips}} \times 100$$

The abundance of different morphotypes was determined under a stereomicroscope (Leica M205 FA, Wetzlar, Germany) using a simplified identification key (after Agerer 1987–2012). All root tips in each soil core were categorized and counted. For mycorrhizal species identification, all different morphotypes were collected, which comprised at least three root tips per sample. Samples with no ectomycorrhizal root tips were included as zero values. We distinguished 44 morphotypes and sequenced 19 morphotypes, which were most abundant and covered > 90% of the beech root ectomycorrhizal fungal community. We used the protocol of Pena et al. (2017) for DNA extraction, ITS sequencing, and species identification. We used the primers ITS1F (5′CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). Fungal sequences have been deposited in NCBI GenBank under the accession numbers MT859114 to MT859131 (Supplement Table S3). Relative abundance of EMF species was calculated as follows:

$$\text{Relative abundance of EMF species (\%)} = \frac{\text{(number of root tips colonized by a EMF species)} \times 100}{\text{number of all vital ectomycorrhizal root tips}}$$

DNA extraction and preparation of soil and root samples for Ilumina sequencing

Frozen soil and root samples that had been stored at − 80 °C were milled in a ball mill (Retsch GmbH, Haan, Germany) in liquid N_2. DNA was isolated from 250 mg soil or from 180 mg roots with the DNeasy® PowerSoil® Pro kit (Qiagen, Hilden, Germany) or innuPREP Plant DNA kit (Analytik Jena AG, Jena, Germany), following the manufacturer's recommendations. DNA was purified using the DNeasy® PowerClean® Pro Cleanup kit (Qiagen). The amount of isolated DNA was measured in a NanoDrop ND-1000 spectrophotometer (PEQLAB Biotechnologie GmbH, Erlangen, Germany). For each DNA extraction, a PCR was performed in a reaction volume of 50 µl using 0.3 µl of Phusion High-Fidelity DNA Polymerase (2 U μl^{-1}, New England Biolabs (NEB), Frankfurt, Germany), 6 µl of 5 × Phusion HF buffer (NEB), 0.09 µl of $MgCl_2$ (50 mM, NEB), 0.6 µl of dNTP mix (10 mM each, Thermo Fisher Scientific, Osterode am Harz, Germany), 0.6 µl of the forward (ITS3-KYO2) and reverse primer (ITS4) (10 mmol/l, Microsynth, Wolfurt, Austria), and about 250 (roots) to 1050 (soil) ng of template DNA in 5 µl. The primers ITS3-KYO2 (Toju et al. 2012) and ITS4 (White et al. 1990) included the adapters for MiSeq sequencing. The PCR reactions were performed in a Labcycler (SensoQuest, Göttingen, Germany). The cycling

parameters were 1 cycle of 98 °C for 30 s; 30 cycles of 98 °C for 10 s, 47 °C for 20 s, and 72 °C for 20 s; and a final extension at 72 °C for 5 min. The PCR products were subjected to electrophoresis in 2% agarose gels (Biozym LE Agarose, Biozym Scientific GmbH, Hessisch Oldendorf, Germany) using GelRed (10 000 ×, VWR, Darmstadt, Germany) to stain the 1 kb DNA ladder (NEB) that was used for the determination of the product size. The PCR products were visualized with an FLA-5100 Fluorescence Laser Scanner (Raytest GmbH, Straubenhardt, Germany) and an Aida Image Analyzer v. 4.27 (Raytest GmbH). All PCR reactions were performed in triplicate, pooled, and purified using the MagSi-NGS^{PREP} Plus Kit (Steinbrenner Laborsysteme, Wiesenbach, Germany). Quantification of the purified PCR products was performed with a Quant-iT dsDNA HS assay kit (Life Technologies GmbH, Darmstadt, Germany) in a Qubit fluorimeter (Life Technologies GmbH, Darmstadt, Germany) following the manufacturer's recommendations.

Amplicon sequencing and bioinformatic processing

Amplicon sequencing was conducted at Göttingen Genomics Laboratory on the MiSeq platform using the MiSeq Reagent Kit v3 (Illumina Inc., San Diego, USA). For amplicon sequence variant (ASV) assembly paired-end sequencing data from the Illumina MiSeq were quality-filtered with fastp (version 0.20.0) using default settings with the addition of an increased per base phred score of 20, base pair corrections by overlap (-c), as well as 5′- and 3′-end read trimming with a sliding window of 4, a mean quality of 20 and minimum sequence size of 50 bp (von Hoyningen-Huene et al. 2019). Subsequently, quality-filtered reads were merged using PEAR v.0.9.11 (Zhang et al. 2014) with default parameters. Primer sequences were clipped with cutadapt v.2.5 (Martin 2011). VSEARCH v.2.14.1 (Rognes et al. 2016) was used for size exclusion of reads < 140 bp, dereplication, denoising (UNOISE3, default settings), and chimera removal (de novo followed by reference-based chimera removal). ASVs were clustered at 97% sequence identity [corresponding to operational taxonomic units (OTUs), the usual threshold in most fungal studies] employing VSEARCH (–sortbysize and –cluster_size). Reads were mapped to OTUs and used to create a count table using VSEARCH (–usearch_global, -id 0.97).

OTUs were taxonomically classified using the BLAST algorithm against the UNITE + INSDC 8.2 public database (Kõljalg et al. 2013) with an identity cutoff of 90%. Unclassified and non-blast hit OTUs (< 90% identity) were aligned against the GenBank (nt, 2020–01-17) database (Geer et al. 2010) and only OTUs with a fungal classification were kept in the OTU table. The OTU count table was rarefied to the count number of 11,000 (minimum number reads in one sample) using the rrarefy() function of the package vegan v2.5.6 (Oksanen et al. 2019). DESeq2 and Bonferroni correction of p values were used to test for significant differences between counts of distinct OTUs after fertilizer treatment and controls (Love et al. 2014). OTUs were functionally annotated as symbiotroph, pathotroph, and saprotroph using the FUNGuild database (Nguyen et al. 2016).

Statistical procedures and calculations

The statistical analyses were performed with R version 3.6.0 (R Core Development Team 2012). Normal distribution and homogeneity of variances were tested by analyzing the residuals of the models and performing a Shapiro–Wilk test for chemical soil and root parameters and EMF relative abundance. Data were logarithmically or square root-transformed to meet the criteria of normal distribution and homogeneity of variances, where necessary.

To determine the effects of forest type, soil layer, season, habitat, and treatment linear mixed effect models ("lmer," R package lme4) were used. The factor plot was used as random effect and the factor season (spring and fall) was defined as repeated measurement in the model. Pairwise comparisons of the sample means were conducted using Tukey's HSD (package: "multcomp"). Means were considered to be significantly different from each other when $p \leq 0.05$. Data are shown as means and standard error (± SE) of the three plots per treatment, if not indicated otherwise. If not indicated otherwise, count data were not transformed. Visual inspection of their residuals showed homogenous distribution and therefore, these data also were analyzed by linear mixed effect models using a quasi-Poisson distribution. P values of repeated tests were adjusted with Bonferroni, as indicated in figure and table legends. Shannon diversity and fungal richness were determined with the package "vegan" (Oksanen et al. 2019) and analyzed by generalized linear models. For comparisons of OL and ML a paired rank test was used.

We used the following fungal communities for our analyses: EMF, SAF, and RAF. RAF and SAF were distinguished in the Illumina dataset and further discerned as SYM, SAP, and PAT corresponding to the groups of ectomycorrhizal (SYM), saprotrophic (SAP), and pathogenic fungi (PAT). To obtain ectomycorrhizal fungi, the guild of symbiotrophic fungi was manually screened and other symbiotrophic fungi (arbuscular mycorrhizal, orchid mycorrhizal, endophyte, lichenized, combinations of saprotrophic, or pathotrophic with EMF) were eliminated and then used as SYM for further analyses.

Detrended correspondence analysis (DCA) was used to explore and visualize fungal community composition. The function ADONIS (multivariate analysis of variance using distance matrices) (package: "vegan") (Oksanen et al. 2019) was used to analyze the dissimilarities among the fungal

communities for the factors: forest type, habitat, layer, season, and treatment.

To determine the influence of fertilization in different soil layers and seasons, we grouped fungal taxa according to fungal orders. We used the most abundant fungal orders, which encompassed > 1% of the fungal sequences to calculate Generalized Adaptive Models for Location, Scale and Shape with a zero-inflated beta family (GAMLSS-BEZI) with the R package "metamicrobiomeR" (Ho et al. 2019). By using a zero-inflated beta (BEZI) family, GAMLSS regression model is applicable for any distribution type exhibited by a response variable (Rigby and Stasinopoulos, 2005). GAMLSS can be used for analyses of relative abundance data and utilizes the log of odds ratio to compute meta-analysis (Ho et al., 2019).

Results

Influence of P and N addition on soil and root chemistry

Soil and root chemistry varied with forest site and season, while treatment effects due to P, N, or N + P addition were mainly found for P (Table 1). P addition resulted in increased soil P_{lab} concentrations across the three study forests but did not affect the soil P_{tot} concentrations (Fig. 1a-c, Table 1). The effects were also present when the forests were fertilized with P + N (Fig. 1a–c) and were more pronounced during fall than spring (Table 1, Supplement Table S4). Furthermore, the P fertilization effects were stronger in the organic layer than in the mineral soil (Fig. 1a–c, Table 1, HP: $F = 12.6$, $p = 0.001$; LP: $F = 111.1$, $p < 0.001$, lmer), with the exception of the MP forest soils ($F = 0.02$, $p = 0.902$, lmer, Fig. 1b).

In P-fertilized forest soils with higher availabilities of P_{lab}, the fine root P_{tot} concentrations were only higher in the LP forest, whereas P_{tot} in fine roots in the HP and MP forests were unaffected compared with the controls (Fig. 2a–c). In all three forests, the P_{lab} concentrations of the roots were higher after P fertilization (Fig. 2d–f). Across the three forests, fine root P_{lab} was higher during spring (556 ± 29 µg g^{-1} dw) than fall (320 ± 19 µg g^{-1} dw, $F = 25.3$, $p < 0.001$) and higher in the organic layer (526 ± 31 µg g^{-1} dw) than in the mineral layer (351 ± 20 µg g^{-1} dw, $F = 22.0$, $p < 0.001$, lmer) (Supplement Table S4).

N fertilization slightly lowered the C/N ratio in the MP forest and raised it in the LP forest (Table 1, Supplement Table S4). The N addition did not increase soluble, inorganic N concentrations (NO_3^-, exchangeable NH_4^+) in the mineral soil (Fig. 1d–f). In the organic layer, the N-fertilized MP forest contained lower exchangeable NH_4^+ concentrations than the controls (Fig. 1e), while no effect was found in the HP and an increase in the LP forest (Fig. 1d,f). No significant seasonal effects of the treatments on the NO_3^- or exchangeable NH_4^+ concentrations were observed (Table 1).

N fertilization significantly decreased root P_{tot} concentrations in the organic layer (Fig. 2a–c) but did not change root N concentrations (Table 1, Supplement Table S4). Therefore, the treatments increased the N:P ratios of the roots but not of the soil (Table 1, Supplement Table S4).

Influence of P and N addition on EMF and root- or soil-associated fungal diversity indices and fungal community composition

We analyzed fungal species richness and Shannon diversity at three scales: EMF, RAF, and SAF (Fig. 3, Table 2). EMF richness did not vary in response to the treatments (Fig. 3a, Table 2). We detected a mean of 8.0 ± 0.4 EMF species per treatment and forest. The dominant EMF species belonged to the genera Russula, Lactarius, Xerocomus, Laccaria, Hydnotrya, Elaphomyces, Clavulina, and Cenococcum and various members of Helotiales (Supplement Fig. S1). The Shannon diversity of EMF was unaffected by the fertilizer treatments (Fig. 3d, Table 2). Fertilization did not affect the fraction of mycorrhizal root tips (99.9 ± 0.4%) nor the fraction of dead root tips (29.7 ± 1.6%) (Supplement Table S5). However, in the mineral layer in the MP and LP forests, root mortality was higher than that in the HP forest (Supplement Table S5).

Using Illumina sequencing (288 samples in total), we obtained 3169 million fungal sequence reads, which clustered into 4134 different OTUs. Across all conditions, the RAF communities contained approximately three times fewer OTUs (219 ± 12) than the SAF communities (764 ± 10, $F = 16{,}155$, $p < 0.001$). P fertilization caused a reduction in the OTU richness of RAF in the organic layer (Fig. 3b) but did not affect Shannon diversity (Fig. 3e). The N and N + P treatments did not have any effects on RAF richness or Shannon diversity (Fig. 3b, e, Table 2). SAF richness and Shannon diversity were unaffected by any fertilizer treatment (Fig. 3c, f, Table 2).

Variations in RAF and SAF OTU richness or Shannon diversity were often observed during different seasons and among the forests (Table 2). OTU richness in the organic layer of RAF and SAF was higher in the organic layer than in the mineral soil (Fig. b,c).

Differential abundance measurements aimed at identifying the distinct fungal OTUs that responded to treatments were unsuccessful because the majority of the OTUs were represented by low and variable numbers of sequence reads spread across 92 fungal orders (Supplement Table S6). We tested whether fertilizer treatments influenced fungal community composition, but we did not detect any treatment effects, whereas forest, soil layer and season caused

Table.1 Statistical information on the effects of forest site, season and fertilization treatments on soil and root chemistry in the organic layer and in the mineral topsoil. Soils and roots were collected in a P-rich (HP), P-medium (MP), and P-poor (LP) forest in spring and fall 2018 and separated into organic layer and the mineral topsoil for analyses. Means can be found in the supplementary information (Supplement Table S4). Differences among means per forest type, season, treatment, and the two-way interactions (forest × season, forest × treatment, season × treatment, P × N) were tested by a linear mixed effect model with plot number as random effect. Calculations were performed separately for the organic layer and mineral topsoil. Tukey HSD was used as posthoc test. Bold letters indicate significant differences at $p \leq 0.05$. RWC = relative water content. $p = p$-value

	Forest		Season		Treatment		Forest × season		Forest × treatment		Season × treatment		N		P		P × N	
	F	*P*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*P*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Soil: Organic layer																		
RWC	77.5	**< 0.001**	221.8	**< 0.001**	0.9	0.451	37.4	**< 0.001**	0.9	0.502	2.4	0.078	0.0	0.989	0.3	0.608	0.1	0.705
P_{tot}	315.5	**< 0.001**	73.8	**< 0.001**	1.6	0.200	4.0	**0.025**	4.0	**0.003**	2.3	0.085	0.8	0.379	0.5	0.475	0.1	0.722
P_{lab}	120.8	**< 0.001**	65.8	**< 0.001**	7.7	**< 0.001**	4.2	**0.020**	0.7	0.651	2.3	0.089	0.0	0.980	10.4	**0.002**	0.0	0.879
N	57.7	**< 0.001**	21.5	**< 0.001**	1.6	0.212	1.9	0.154	0.2	0.976	1.8	0.163	0.0	0.879	2.2	0.142	0.6	0.448
C	52.6	**< 0.001**	42.6	**< 0.001**	1.1	0.346	3.2	**0.048**	0.1	0.993	2.0	0.134	0.0	0.879	2.2	0.142	0.6	0.431
C:N	53.1	**< 0.001**	51.9	**< 0.001**	1.3	0.283	20.3	**< 0.001**	1.2	0.208	0.4	0.717	0.4	0.493	0.5	0.470	0.0	0.886
N:P	123.9	**< 0.001**	11.9	**0.001**	0.5	0.674	3.4	**0.042**	0.4	0.888	0.5	0.665	0.0	0.974	0.0	0.923	0.4	0.531
pH	148.2	**< 0.001**	151.2	**< 0.001**	2.6	0.066	15.7	**< 0.001**	0.8	0.546	0.6	0.588	1.8	0.180	0.1	0.723	0.0	0.947
NH_4^+	6.9	**0.002**	2.6	0.112	0.9	0.459	1.8	0.172	2.9	**0.018**	0.6	0.590	2.0	0.166	0.6	0.449	0.1	0.785
NO_{3-}^-	11.0	**< 0.001**	3.9	0.054	0.2	0.910	1.3	0.272	0.3	0.952	1.1	0.340	0.4	0.508	0.0	0.997	0.1	0.824
Soil: Mineral layer																		
RWC	324.6	**< 0.001**	206.5	**< 0.001**	3.5	**0.022**	0.7	0.482	2.0	0.088	4.1	**0.012**	0.8	0.388	1.2	0.280	0.1	0.744
P_{tot}	1363.0	**< 0.001**	270.1	**< 0.001**	3.2	**0.031**	107.4	**< 0.001**	2.1	0.065	0.1	0.951	0.8	0.365	0.2	0.644	0.0	0.948
P_{lab}	105.3	**< 0.001**	15.5	**< 0.001**	1.5	0.218	7.4	**0.002**	2.4	**0.044**	1.3	0.301	0.0	0.917	3.2	0.081	0.0	0.915
N	291.1	**< 0.001**	0.1	0.815	2.6	0.067	0.2	0.815	2.0	0.078	1.9	0.136	4.7	**0.034**	4.6	**0.037**	0.2	0.627
C	135.5	**< 0.001**	0.2	0.665	1.4	0.268	0.5	0.630	2.1	0.070	1.7	0.188	1.9	0.176	4.2	**0.045**	0.4	0.516
C:N	815.7	**< 0.001**	41.1	**< 0.001**	5.5	**0.002**	73.5	**< 0.001**	8.1	**< 0.001**	7.5	**< 0.001**	0.0	0.875	0.5	0.467	0.0	0.924
N:P	59.3	**< 0.001**	1.4	0.248	0.3	0.849	0.6	0.569	0.4	0.864	0.2	0.871	0.2	0.665	0.1	0.821	0.1	0.838
pH	58.3	**< 0.001**	8.1	**0.007**	0.5	0.692	4.0	0.024	1.2	0.312	0.3	0.810	0.4	0.554	0.4	0.546	0.1	0.718
NH_4^+	1.7	0.195	1.6	0.216	0.5	0.664	0.1	0.951	1.3	0.259	0.6	0.606	0.0	0.872	1.0	0.332	1.2	0.282
NO_3^-	1.9	0.168	2.8	0.102	0.3	0.790	4.8	0.012	1.0	0.414	1.3	0.280	0.4	0.511	0.5	0.503	0.0	0.902

Table.1 (continued)

	Forest		Season		Treatment		Forest × season		Forest × treatment		Season × treatment		N		P		P × N	
	F	*P*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*P*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Fine roots: Organic layer																		
RWC	9.7	**< 0.001**	101.4	**< 0.001**	0.5	0.678	6.8	0.002	0.4	0.904	0.4	0.783	0.0	0.891	0.4	0.538	0.1	0.707
P_{tot}	86.2	**< 0.001**	188.7	**< 0.001**	8.8	**< 0.001**	12.6	**< 0.001**	1.8	0.111	0.4	0.776	3.8	0.057	0.8	0.386	0.8	0.373
P_{lab}	3.0	0.057	69.1	**< 0.001**	6.6	**0.001**	10.1	**< 0.001**	1.0	0.441	1.6	0.209	0.1	0.755	7.8	**0.007**	0.0	0.902
N	11.4	**< 0.001**	3.0	0.089	0.5	0.679	29.3	**< 0.001**	0.4	0.878	0.3	0.856	0.1	0.771	0.3	0.579	0.4	0.535
C	119.5	**< 0.001**	0.1	0.733	0.5	0.714	17.9	**< 0.001**	0.7	0.660	0.1	0.944	0.0	0.866	0.8	0.361	0.0	0.925
C:N	19.4	**< 0.001**	4.1	**0.048**	0.6	0.572	21.3	**< 0.001**	0.2	0.965	0.5	0.642	0.0	0.826	0.3	0.587	0.6	0.423
N:P	30.1	**< 0.001**	98.3	**< 0.001**	3.9	**0.013**	19.3	**< 0.001**	0.5	0.808	0.2	0.886	1.5	0.227	2.3	0.134	0.1	0.675
Fine roots: Mineral layer																		
RWC	71.1	**< 0.001**	1.5	0.231	2.1	0.111	34.7	**< 0.001**	0.5	0.803	1.1	0.376	0.9	0.352	0.5	0.471	0.1	0.706
P_{tot}	110.4	**< 0.001**	10.2	**0.003**	4.5	**0.007**	0.2	0.802	2.7	**0.025**	2.3	0.093	5.9	**0.018**	4.4	**0.040**	0.8	0.373
P_{lab}	94.4	**< 0.001**	69.1	**< 0.001**	12.2	**< 0.001**	3.8	**0.028**	1.7	0.149	2.3	0.093	1.1	0.291	14.2	**< 0.001**	0.0	0.899
N	58.2	**< 0.001**	3.4	0.073	2.2	0.105	15.1	**< 0.001**	0.4	0.880	1.2	0.338	3.6	0.064	0.2	0.620	0.4	0.545
C	9.2	**< 0.001**	6.4	**0.015**	0.6	0.587	4.1	**0.022**	0.6	0.708	0.1	0.952	1.4	0.241	0.2	0.692	0.0	0.925
C:N	65.1	**< 0.001**	2.0	0.164	3.3	**0.028**	9.9	**< 0.001**	1.1	0.401	0.8	0.467	2.5	0.116	0.2	0.691	0.6	0.423
N:P	47.9	**< 0.001**	13.6	**< 0.001**	7.0	**< 0.001**	7.3	**0.002**	3.1	**0.011**	5.3	**0.003**	1.7	0.195	4.1	**0.045**	0.2	0.675

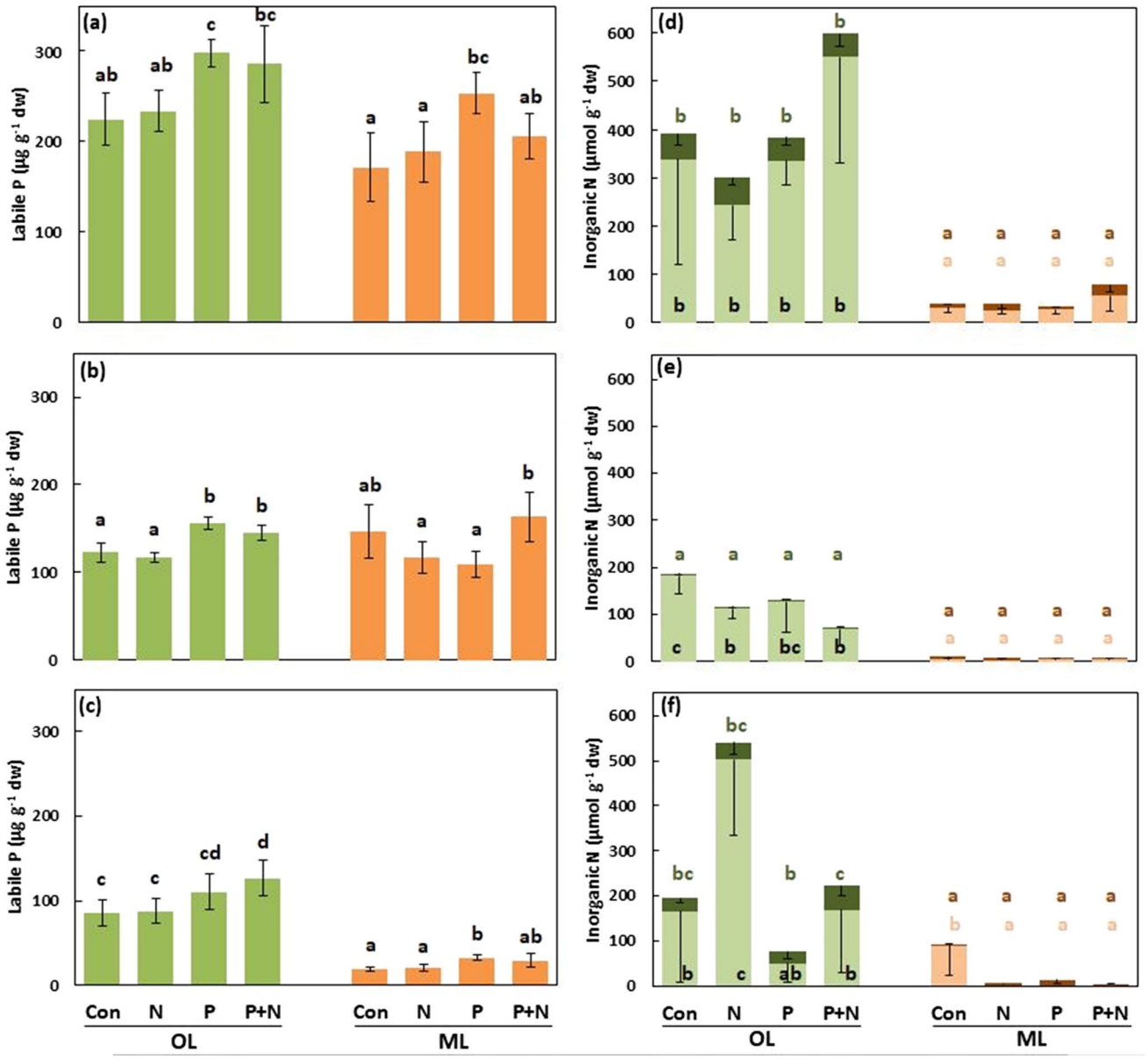


Fig. 1 Labile phosphorus (**a**, **b**, **c**) and inorganic nitrogen (**d, e**, **f**) concentrations the soil of beech forests (*Fagus sylvatica* L.) Soils were collected in a P-rich (**a**, **d**), P-medium (**b**, **e**), and P-poor (**c**, **f**) forest and separated into organic layer (green) and the mineral topsoil (orange) for analyses. Stacked bars show ammonium (bright colors) and nitrate (dark colors). Data for spring and fall were pooled. Data indicate means ($n = 6 \pm$ SE). Differences among means were tested by a linear mixed effect model with plot number as random effect and Tukey HSD post hoc test. Different letters indicate significant differences at $p \leq 0.05$

significant differentiation within the EMF, RAF, and SAF communities (Supplement Fig. S2a, b, c).

Phylogenetic and functional groups of fungi respond to N and P treatments

Since important fungal traits for nutrient use and turnover are conserved at higher classification levels (Treseder and Lennon, 2015), we assigned the OTUs to fungal orders (Supplement Table S6). Fifteen of the 92 detected orders were abundant, each accounting for more than 1% of the fungal sequences (Supplement Fig. S3, Supplement Table S6). Seven orders were unaffected by the fertilizer treatments (Agaricales, Atheliales, Cantharellales, Thelephorales, Eurotiales, Hypocreales, and Mortierellales, Supplement Figs. S4 and S5).

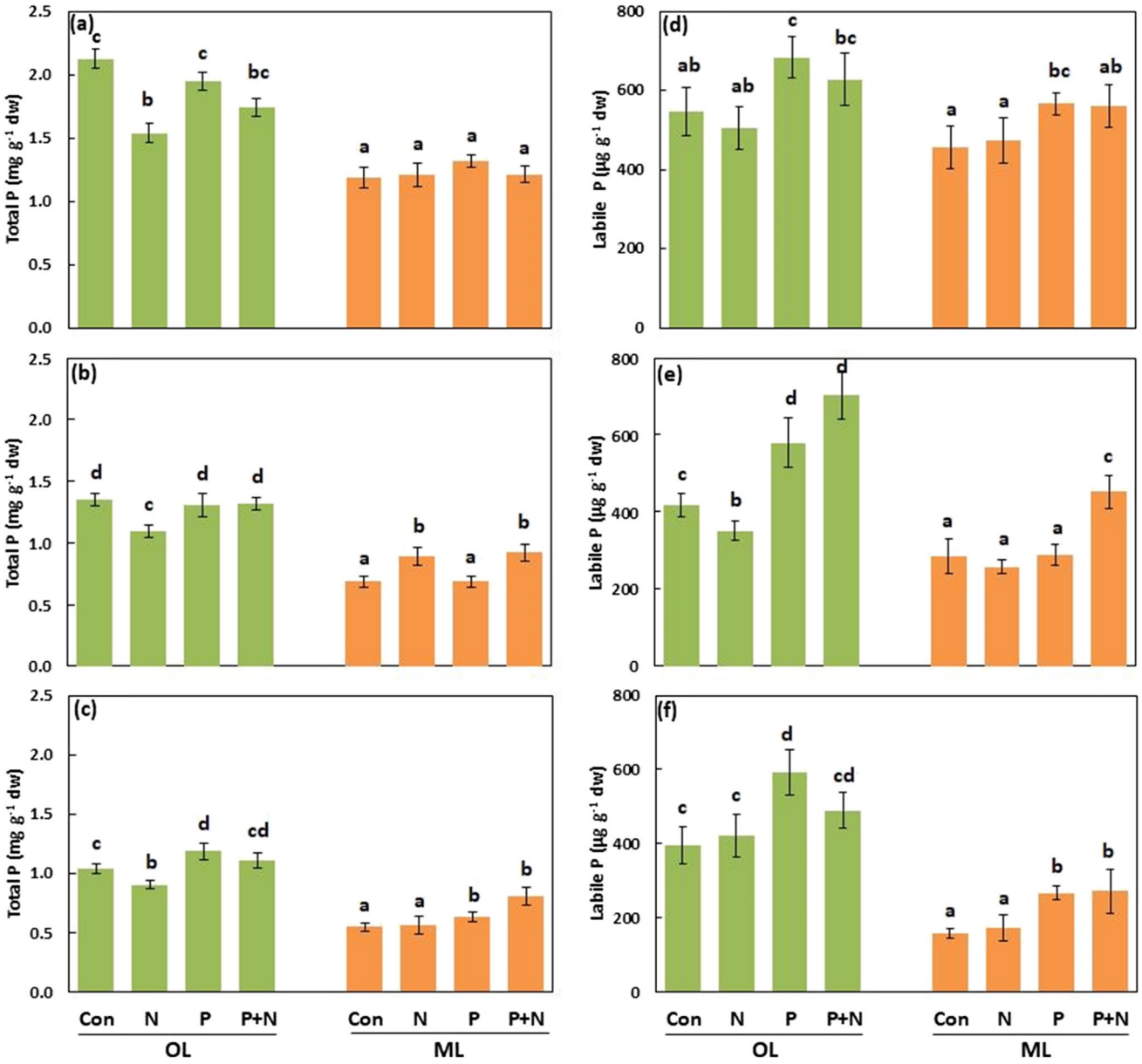


Fig. 2 Total phosphorus (**a**, **b**, **c**) and labile phosphorus (**d**, **e**, **f**) in roots of beech forests (*Fagus sylvatica* L.) Fine roots were collected in a P-rich (**a**, **d**), P-medium (**b**, **e**), and P-poor (**c**, **f**) forest and separated into organic layer (green) and the mineral topsoil (orange) for analyses. Season data were pooled. Data indicate means ($n = 6 \pm$ SE). Differences among means were tested by a linear mixed effect model with plot number as random effect and Tukey HSD post hoc test. Different letters indicate significant differences at $p \leq 0.05$

A heatmap differentiating the organic and mineral soil fungal responses during spring and fall showed that four RAF orders (Russulales, Boletales, Trechisporales, and Pezizales) and seven SAF orders (Boletales, Trechisporales, Helotiales, Hypocreales, Sodariales, Pleosporales, and Mytilinidales) were significantly affected by the fertilization treatments (Fig. 4). Only SAF responded to N, with negative responses for Helotiales in spring in the mineral soil and positive responses for the Hypocreales in the organic layer when both seasons were considered together (Fig. 4). Further positive effects on the SAF were observed after P or N + P treatment, but the responses of different orders were scattered among different soil layers and seasons (Fig. 4). When the SAF data for seasons and soil layers were analyzed together, the treatment effects were masked (Fig. 5a).

Among the RAF, Russulales and Boletales showed the most consistent and strongest responses to the P and N + P treatments (Fig. 4). The P treatment caused relative enrichment of Russulales during spring in both soil layers and a decrease during fall compared with that in the controls

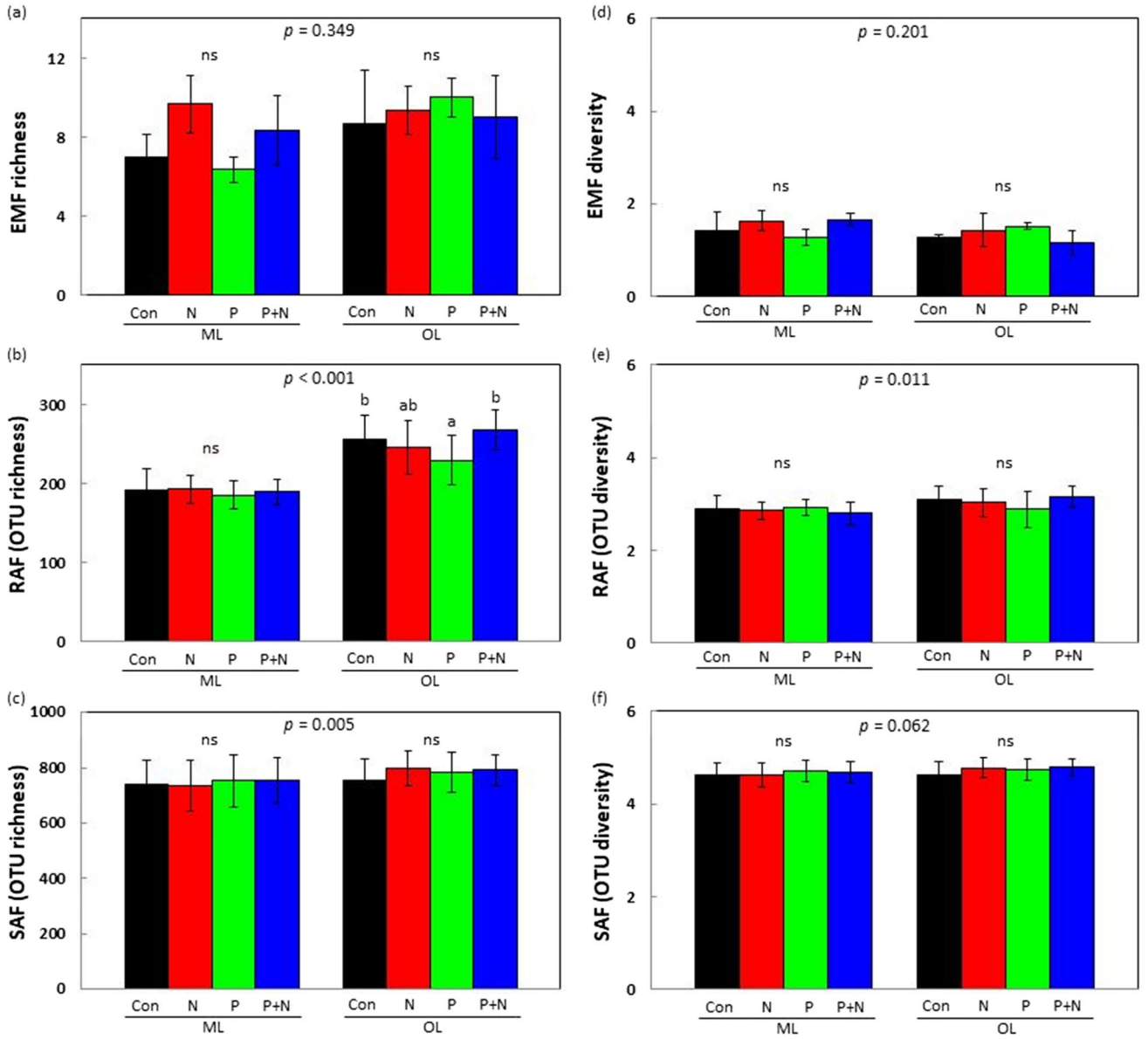


Fig. 3 Richness (**a**, **b**, **c**) and Shannon diversity (**d**, **e**, **f**) of ectomycorrhizal (EMF), root (RAF), and soil (SAF) associated fungi in the organic layer (OL) and in the mineral topsoil (ML) in response to fertilization (Con, N, P, P + N). Soils and fine root samples were collected in a P-rich, P-medium, and P-poor beech forest (*Fagus sylvatica* L.) in spring and fall 2018 and separated into organic layer and the mineral topsoil for analyses. Data indicate means ($n = 36 \pm$ SE). Differences among treatments were tested by generalized linear models followed by a post hoc test (Tukey). Calculations were performed separately for the organic layer and mineral topsoil. Data for richness were log transformed prior analyses. Different letters indicate significant differences at $p \leq 0.05$ for each soil layer seperatley. Controls (Con) = black, N = red, P = green, P + N = blue. ns., not significant. ***P*** values in each subpanel refer to the comparisons between ML and OL conducted by a paired rank test. Further statistical information is shown in Table 2

(Fig. 4). The Boletales in the RAF showed higher relative abundances in response to P and N + P fertilization during fall compared with those in the controls (Fig. 4). When the data for RAF in different soil layers and seasons were pooled, the P treatment still resulted in approximately twice as high relative abundances of Boletales than those in the controls (Fig. 5b), whereas the positive effects of P on Russulales during spring were masked by negative effects during fall (Fig. 5b). N + P treatment significantly reduced Russulales in the RAF (Fig. 5b). Other fungal orders in the RAF that responded to fertilization were Trechisporales, which showed positive effects under the N + P treatment in the mineral layer, and Pezizales, which showed negative effects under the P treatments in the organic layer during spring (Fig. 4).

Table.2 Statistical information on the effects of forest site, season and fertilization treatments on richness and Shannon diversity of soil- and root-associated fungi and on ectomycorrhizal fungi colonizing the root tips in the organic layer and in the mineral topsoil. Soil and fine root samples were collected in a P-rich, P-medium, and P-poor forest in spring and fall 2018 and separated into organic layer and the mineral topsoil for analyses. Differences among means per forest type, season, and treatment were tested by a generalized linear model (GLM) with lg-transformed data. Calculations were performed separately for the organic layer and mineral topsoil. Interactions were tested but not found. Bold letters indicate significant differences at $p \leq 0.05$. SoS = sum of squares, MS = mean square, R^2 (ad) indicates the explained variance adjusted for degrees of freedom

		Organic layer				Mineral soil			
Richness	Soil-associated fungi								
Source	Df	SoS	MS	*F*-ratio	*P* value	SoS	MS	*F*-ratio	*P* value
Model	6	0.1905	0.0318	16.97	**< 0.0001**	0.4927	0.0821	17.81	**< 0.0001**
Treatment	3	0.0067	0.0022	1.19	0.3220	0.0021	0.0007	0.15	0.9276
Forest	2	0.0129	0.0065	3.45	**0.0377**	0.2656	0.1328	28.79	**< 0.0001**
Season	1	0.1710	0.1710	91.39	**< 0.0001**	0.2250	0.2250	48.80	**< 0.0001**
Residual	65	0.1216	0.0019			0.2997	0.0046		
Total (corrected)	71	0.3121			R^2 (adj) = 57.4%	0.7925			R^2 (adj) = 58.7%
Shannon diversity									
Source	Df	SoS	MS	*F*-ratio	*P* value	SoS	MS	*F*-ratio	*P* value
Model	6	4.8024	0.8004	9.03	**< 0.0001**	6.0542	1.0090	10.06	**< 0.0001**
Treatment	3	0.2751	0.0917	1.04	0.3830	0.0692	0.0231	0.23	0.8751
Forest	2	0.1464	0.0732	0.83	0.4423	4.6718	2.3359	23.29	**< 0.0001**
Season	1	4.3809	4.3809	49.45	**< 0.0001**	1.3132	1.3132	13.10	**0.0006**
Residual	65	5.7585	0.0886			6.5184	0.1003		
Total (corr)	71	10.5609			R^2 (adj) = 40.4%	12.5726			R^2 (adj) = 43.4%
Richness	Root-associated fungi								
Source	Df	SoS	MS	*F*-ratio	*P* value	SoS	MS	*F*-ratio	*P* value
Model	6	0.3024	0.0504	10.03	**< 0.0001**	0.0254	0.0042	0.70	0.6506
Treatment	3	0.0537	0.0179	3.56	**0.0188**	0.0022	0.0007	0.12	0.9475
Forest	2	0.1138	0.0569	11.33	**0.0001**	0.0075	0.0037	0.62	0.5431
Season	1	0.1349	0.1349	26.85	**< 0.0001**	0.0158	0.0158	2.60	0.1114
Residual	65	0.3265	0.0050			0.3937	0.0061		
Total (corr)	71	0.6289			R^2 (adj) = 43.3%	0.4192			R^2 (adj) = 0.0%
Shannon diversity									
Source	Df	SoS	MS	*F*-ratio	*P* value	SoS	MS	*F*-ratio	*P* value
Model	6	5.9332	0.9889	4.87	**0.0004**	0.9767	0.1628	1.14	0.3516
Treatment	3	0.7756	0.2585	1.27	0.2911	0.1481	0.0494	0.34	0.7932
Forest	2	3.1337	1.5669	7.71	**0.0010**	0.4777	0.2388	1.67	0.1968
Season	1	2.0239	2.0239	9.96	**0.0024**	0.3509	0.3509	2.45	0.1224
Residual	65	13.2041	0.2031			9.3125	0.1433		
Total (corr)	71	19.1373			R^2 (adj) = 24.6%	10.2891			R^2 (adj) = 1.1%
Richness	Ectomycorrhizal fungi on root tips								
Source	Df	SoS	MS	*F*-ratio	*P* value	SoS	MS	*F*-ratio	*P* value
Model	5	34.4167	6.8833	1.69	0.2707	92.1667	18.4333	6.70	0.0192
Treatment	3	14.2500	4.7500	1.16	0.3984	2.0000	0.6667	0.24	0.8639
Forest	2	20.1667	10.0833	2.47	0.1650	90.1667	45.0833	16.39	**0.0037**
Residual	6	24.5000	4.0833			16.5	2.75		
Total (corr)	11	58.9167			R^2 (adj) = 23.8%	108.667			R^2 (adj) = 72.2%
Shannon diversity									
Source	Df	SoS	MS	*F*-ratio	*P* value	SoS	MS	*F*-ratio	*P* value
Model	5	0.4782	0.0956	0.58	0.7199	0.6851	0.1370	0.71	0.6386
Treatment	3	0.2354	0.0785	0.47	0.7131	0.2875	0.0958	0.50	0.6985
Forest	2	0.2428	0.1214	0.73	0.5203	0.3976	0.1988	1.03	0.4130
Residual	6	0.9980	0.1663			1.1597	0.1933		

Table.2 (continued)

		Organic layer		Mineral soil	
Total (corr)	11	1.4763	R^2 (adj) = 0.0%	1.8445	R^2 (adj) = 0.0%

Since the treatment effects on the RAF were confined to orders that consisted of EMF and in the SAF mainly to saprotrophic orders (except Boletales and Trechisporales), we tested whether N, P or P + N treatment (Fig. 5b) influenced the relative abundances of symbiotrophs or saprotrophs. The relative abundance of symbiotrophic fungi in the SAF and RAF increased, whereas that of saprotrophic fungi decreased in these compartments under the P and N treatments (Fig. 5c, d). We excluded pathotropic fungi from these analyses because their mean relative abundance was below 1%.

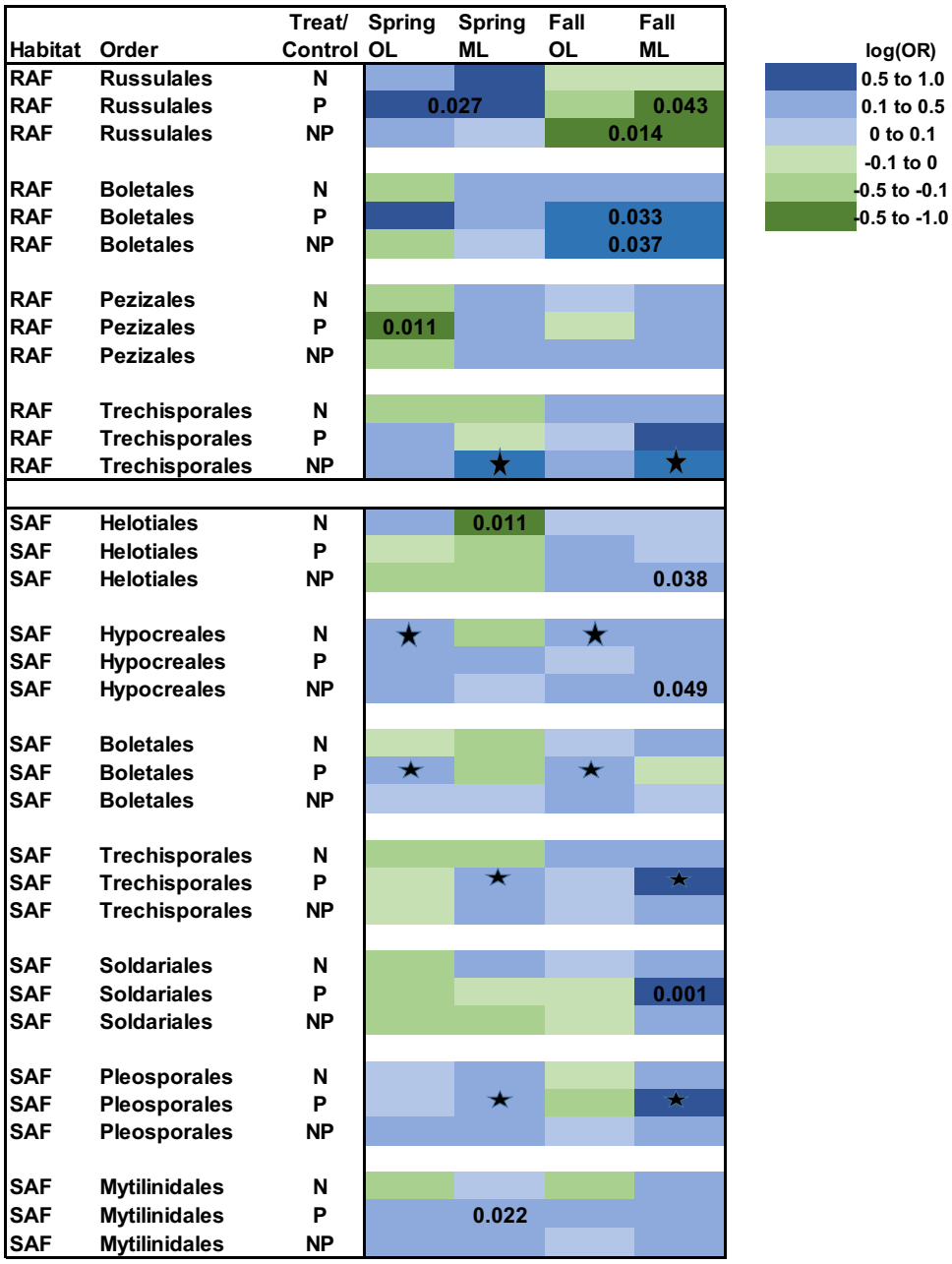


Fig. 4 Heatmap for the effect sizes of N, P, or N + P treatments on selected fungal orders relative to controls. RAF, root-associated fungi; SAF, soil-associated fungi; OL, organic layer; ML, mineral soil. Blue show positive and green color code negative responses. The responses are indicated as log of odds ratio (log(OR)) as the result of GAMLSS analyses. Numbers in cells indicate *p* values for significant changes. *P* values for shared effects of ML + OL are centered. Shared effects of spring + fall are indicated by * when $p \leq 0.05$

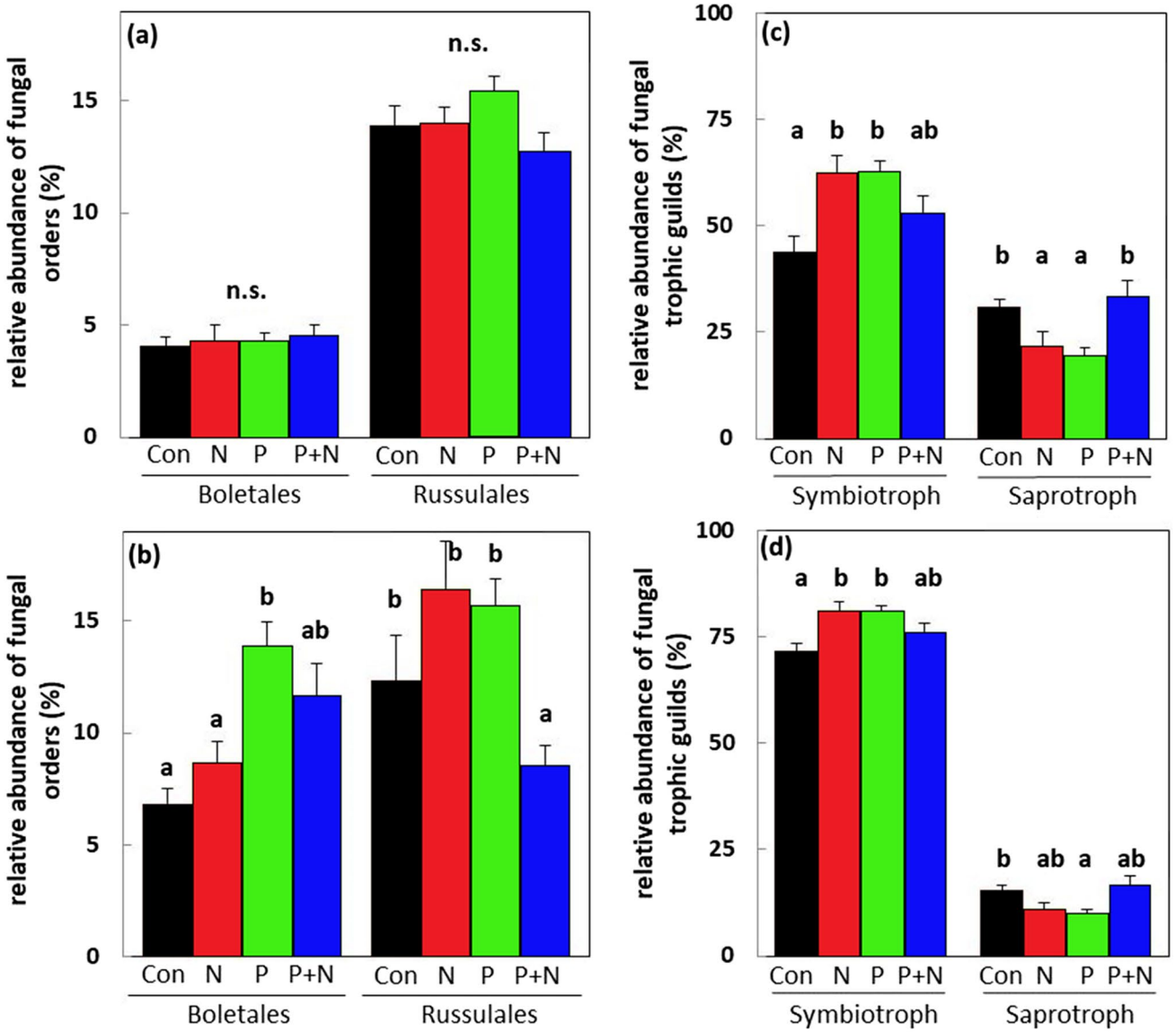


Fig. 5 Relative abundance of Boletales and Russulales in soil (**a**) and roots (**b**) and of trophic guilds in soil (**c**) and roots (**d**) in response to fertilization (Con, N, P, P + N). Soil and fine root samples of the organic layer and mineral topsoil were collected in a P-rich, P-medium, and P-poor beech forest (*Fagus sylvatica* L.) in spring and fall 2018. Data of the forest types and season were merged to evaluate effects of the treatments on the soil-residing fungi (**a**, **c**) and root-associated fungi (**b**, **d**). Data indicate means ($n = 36 \pm$ SE). Significant differences between the treatments were calculated by a linear mixed effect model using Poisson distribution and Tukey HSD post hoc test for the treatments with site as random effect and season as repeated measure. Different letters indicate significant differences for each fungal order and trophic group separately. Controls (Con) = black, N = red, P = green, P + N = blue. n.s., not significant

Discussion

P and N inputs affect P nutrition of beech

In agreement with our expectations, we found that under low P soil availabilities, root P concentrations decreased with N addition and increased with P addition. Unexpectedly, we found that N addition also decreased the P concentrations in roots in soils with higher P availabilities, i.e., in the HP and MP forests. These observations suggest that the applied amounts of N (here, 60 N kg ha^{-1} a^{-1}, other studies: 15 to 5 kg N $ha^{-1}a^{-1}$, De Vries et al. 2014; Gonzales and Yanei, 2019; Wardle et al. 2016), which exceed ambient deposition in unpolluted areas (approximately 6 kg N ha^{-1} a^{-1}, Schwede et al. 2018), might have caused N:P imbalances. Etzold et al. (2020) reported tipping points at 24 to 34 kg N ha^{-1} a^{-1} for positive

growth responses of forest trees in Europe, with potential negative effects at higher deposition values. In a meta-analysis, Deng et al. (2016) reported decreases in tissue P concentrations upon N fertilization, although the labile P pools in soils were unaffected. Our results are consistent with these findings.

Several previous studies in LP and HP forests clearly demonstrated that soil microbes and young beech trees in LP soil are limited by low P availabilities (Bergkemper et al. 2016; Lang et al. 2017; Pastore et al. 2020; Zavišić et al. 2018). Experimental studies with young trees in HP and LP soil showed that negative effects of P limitation, such as a reduction in photosynthesis were mitigated by P fertilization, while the photosynthesis of beech trees in HP soil was unaffected by P addition (Zavišić et al. 2018). In the present study, the NH_4^+ enrichment after N addition in LP, but not in HP soil suggests that N utilization was impaired due to P shortage. The observation that the accumulation of NH_4^+ in soil was relieved by P fertilization further supports this suggestion. N turnover is rapid, and great variations in NO_3^- and exchangeable NH_4^+ in soil solutions are known (Cheng et al. 2019; Ollivier et al. 2011). Although our analyses of NO_3^- and NH_4^+ represent only snapshots during our sampling campaigns, our results agree with those of other studies (Li et al. 2015; Liu et al. 2013; Xia et al. 2020), by showing that P addition counteracted the negative effects of high N input on root P contents.

P addition increased potential plant-available P in all three forest soils. This result might have been expected since the annual P uptake of forest trees is much lower (ca. 9 kg ha^{-1} a^{-1}, Rosling et al. 2016) than the amount of added P. In our study, the increase in P_{lab} was small compared to the content of P_{tot} in soil; therefore, we did not observe significant increases in P_{tot}. Consequently, the N:P ratios of the soils remained stable after N and P additions. Soil N:P ratios of approximately 15 have been suggested to indicate a nutritional balance in beech forests (Mellert and Göttlein 2012). Here, we found extremely large differences in these ratios among the forests and seasons (organic layer: 7 to 31, mineral layer: 3 to 17). Plant-available fractions of N and P are critical to tree nutrition. Here, the N_{min}:P_{lab} ratios decreased slightly in response to P or N addition in the HP forest but increased more than threefold after N addition in the organic layer of the LP forest compared to that in the HP forest (estimated with data from the organic layer, Supplement Table S4). This dynamic was also partly detected in roots, where N fertilization decreased P_{tot}, while P fertilization increased P_{lab}. These results support the metabolic flexibility of beech to cope with differences in nutrient availabilities (Meller et al. 2019; Zavišić et al., 2016).

Fungal taxonomic composition is driven by long-term habitat conditions

A main goal of this study was to disentangle the soil fungal community composition response to the addition of N, P, and P + N under conditions of P shortage or P sufficiency. In general, the assembly of soil fungi is predominately driven by deterministic processes, such as abiotic habitat conditions and soil properties, while stochastic effects play a minor role (Chase, 2007; Glassman et al. 2017; Mykrä et al. 2016; Peay et al. 2016; Štursová et al. 2014). In agreement with the expectation that abiotic habitat filters are important drivers of fungal community composition, our results showed that environmental variables such as humidity, P, Ca and N in soil and roots explained the variation in fungal community composition in different forests.

In line with the findings of other studies (Goldmann et al. 2016; Zhang et al. 2017), RAF were less diverse than SAF. Furthermore, the EMF assemblage involved in active symbiosis was far less diverse than the EMF detected by Illumina or other deep sequencing methods (Pena et al. 2017; Schröter et al. 2019). For example, Pena et al. (2017) found approximately 10 to 15 EMF species colonizing root tips per plot, while Schröter et al. (2019), through pyrosequencing, detected approximately 50 EMF species in the same plots. Our EMF results were in a similar range. The high number of OTUs is partly due to methodological bias (Castaño et al. 2018; Nilsson et al. 2019) resulting in species overestimation. Furthermore, the choice of the barcoding sequence, such as ITS or LSU, affects estimates of diversity (Xue et al. 2019). However, it should be noted that consistent response patterns of fungal diversity to environmental factors were detected, irrespective of the method used for fungal community analysis (Xue et al. 2019). Here, the enhanced EMF species richness discovered in the SAF and RAF compared to EMF colonizing root tips likely reflects the ability of EMF, which are not engaged in active symbiosis, to live as saprotrophs in soil or on root surfaces (Iwański and Rudawaska, 2007; Kohler et al. 2015; Lindahl and Tunlid, 2015; Phillips et al. 2014).

According to ecological theory, stress reduces diversity by filtering out species that can tolerate harsh environments (Chase, 2007). This is supported for soil fungi, including ectomycorrhizal communities (Glassman et al. 2017; Schröter et al. 2019; Štursová et al. 2014). For example, along a biogeographic gradient in temperate forests, fungal assemblages were generally less diverse in dry and more acidic soil than in more humid and nutrient-rich soil (Goldmann et al. 2016; Pena et al. 2017; Schröter et al. 2019). Therefore, we anticipated here that P fertilization would result in stress relief and lead to more species-rich, diverse assemblages. While no effect on EMF or SAF richness or diversity was found, P fertilization caused a moderate

decrease in RAF richness in the organic layer, in contrast to our initial hypothesis. Analyses of taxonomic fungal community composition did not reveal any significant response to the fertilization treatments, indicating that the taxonomic dissimilarities and species turnover among the forests dominated small effects at the OTU level.

As outlined in the introduction, high N inputs often cause reductions in fungal diversity and shifts in the community toward nitrophilic assemblages (Bahr et al. 2013; Cox et al. 2010; Lilleskov et al. 2002, 2008; Suz et al. 2014; de Witte et al. 2017). Field studies in temperate forests also identified soil N as an important driver of RAF composition (Lilleskov et al. 2019; Nguyen et al. 2020; Schröter et al. 2019). In other studies, N deposition did not influence fungal community composition (Lilleskov et al. 2019; Purahong et al. 2018). Similarly, we did not observe effects of N addition on the fungal assemblages, irrespective of whether the fungi in soil or those in direct contact with the roots were inspected. Upon N fertilization, less C is allocated belowground to ectomycorrhizal fungi associated with roots (Högberg et al. 2017, 2020). Reduced C availability to the EMF reduces root P uptake (Clausing et al. 2021). These physiological feedback controls might have caused decreased root P concentrations after N addition without requiring strong reshaping of the fungal assemblage. In conclusion, our hypothesis that P fertilization increases fungal richness in P-poor soil and shifts fungal communities to those found in P-rich soil was not supported by our results.

N and P inputs affect the phylogenetic and functional compositions of fungal assemblages

Phylogenetic composition carries information on ecological assembly processes because high relatedness between members of a community suggests similar ecological requirements or functions (Cavender-Bares et al. 2009; Pausas and Verdú, 2010). Information on functions is important to better understand the assembly processes of soil microbes (Nannipieri et al. 2019). Treseder and Lennon (2015) analyzed the fungal traits required for nutrient cycling (e.g., phosphatases and ammonium transporters) in fungal genomes. They found that these potential traits were more conserved, in terms of gene counts, in phylogenetically more closely related taxa (up to the subphylum level) than in the more distant ones (Treseder and Lennon, 2015). Therefore, we reasoned that adaptation of fungal community composition to enhanced N or P inputs might be traceable after aggregating OTUs at a higher classification level, i.e., at the order level. However, in contrast to our hypothesis that N fertilization shifts fungal taxa toward more nitrophilic EMF communities, we observed increases in only Hypocreales (mainly saprotrophic and pathotrophic members) in soil. A novel result of our study was that P fertilization affected mycorrhizal orders (Russulales, Boletales) of both SAF and RAF.

Members of the Russula lineage (*Russula* sp., *Lactarius* sp.) were dominant in our study forests (this study; Clausing et al. 2021; Zavišić et al. 2016). All known members of the Russulales are ectomycorrhizal fungi and are very common in temperate beech forests (Buée et al. 2005; Lang et al. 2011; Taylor et al. 2000). *Russula* and *Lactarius* spp. lack extensive extramatrical hyphae and thus absorb nutrients from their immediate surroundings (Agerer, 2001). Therefore, we assumed that elevated inorganic nutrient availability might favor this fungal genus. However, Russulales showed a significant decrease in response to the combined P + N treatment and increased during spring in soils fertilized only with P. Similar to our study, Mason et al. (2020) found subtle increases in Russulales after P fertilization (5 years) in an LP forest (Ohio, USA). Nicolás et al. (2017) found no significant effects of N fertilization on *Russula* sp. in a boreal forest. However, root colonization and sporocarp formation of *Russula* sp. increased significantly after strong long-term disturbance by high N input (16 years, 170 kg N ha^{-1} a^{-1}) (Avis et al. 2003). Therefore, various *Russula* spp. were classified as nitrophilic species (Avis, 2012). Our results indicate that N availability alone was not decisive. Rather, the N:P ratio regulated the relative abundance of this important fungal order, as Russulales declined significantly when high N addition was accompanied by high P availability.

P fertilization had the most pronounced effects on Boletales, especially in the RAF. Long-distance rhizomorphs and a thick hyphal mantle characterize members of the Boletales (Agerer, 2001). The relative abundance of Boletales increased almost two-fold upon P addition. This result was surprising because investment in high-biomass fungi is considered profitable in nutrient-limited ecosystems in terms of accessing distant resources (Hobbie and Agerer, 2010). For example, Almeida et al. (2019) found increases in *Imleria badia* (formerly known as *Boletus badius*) hyphae accessing apatite (a recalcitrant P source) in N-fertilized soil but not if the N fertilized soil was amended with easily available P sources. Therefore, they argued that *Imleria* is a P-efficient species that responds to an enhanced P demand of the tree (Almeida et al. 2019). However, our results do not support this suggestion because the roots of N-fertilized plots showed decreases in P, which would support an enhanced tree demand under these conditions, whereas the increases in Boletales occurred only in P- or P + N-fertilized plots. Boletales accumulate P in the hyphal mantle and store P in polyphosphate granules in the mycelium (Kottke et al. 1998). One possibility is that the increases in Boletales were responsible for the observed P accumulation in roots in the P-fertilized plots, but it is also feasible that Boletales used P for their own requirements.

In addition to fertilizer treatments, the soil layers also showed differences in the fungal orders present, especially for saprotrophic fungi. Other studies also reported shifts in fungal composition with increasing soil depth (Peršoh et al. 2018; Toju et al. 2016). For example, Toju et al. (2016) found a lower relative abundance of Russulales in the organic layer than in deeper horizons. However, this observation deviates from our results. We found that EMF did not vary between the layers (this study; Clausing and Polle 2020), whereas fungal orders showed significant differences between the organic layer and the mineral soil. In general, the relative abundance of saprotrophic taxa was lower in the RAF than in the SAF. This pattern reflects different nutritional strategies of saprotrophs and mycorrhizal fungi. Saprotrophs prefer environments where bound nutrients can be unlocked from organic compounds, while mycorrhizal fungi mine the mineral layer for inorganic compounds and rely on plant-derived carbohydrates.

An important result of our study was that the relative abundance of saprotrophic fungi was reduced in response to both N and P fertilization. This observation might imply that enhanced availability of mineral nutrients occurs with a trade-off in saprotrophic potential. Future studies should address this proposition by analyzing enzymatic activities. The shift toward symbiotrophs suggests that inorganic nutrient addition might have led to a competitive advantage for the growth of ectomycorrhizal fungi because they obtain carbohydrates from their host, while free-living saprotrophic fungi need to degrade organic compounds to access C. Similarly, field experiments showed that enhanced ectomycorrhizal fungal growth also results in enhanced phosphodiesterase activities and thus higher P_{lab} availability (Müller et al. 2020). Pot experiments under controlled conditions revealed that ectomycorrhizal diversity fostered P uptake efficiency (Köhler et al. 2018), and in forest soils, ectomycorrhizal P uptake efficiency was further related to P_{lab} availability (Clausing and Polle, 2020). These results suggest that P_{lab} availability drives a positive feedback mechanism for plant nutrition. The increase in the relative abundances of symbiotrophic to saprotrophic fungi upon N or P addition might indicate an advantage in capturing mineral nutrients under those conditions. In contrast to enhanced P_{lab} availability, enhanced N_{min} availability resulted in a decrease in root P contents. The shift away from saprotrophic toward symbiotrophic activities may have resulted in lower P mineralization, thereby decreasing P availability and contributing to the reduced root P content in the N-fertilized plot. These considerations underline the important role of saprotrophic fungi in the mineralization of organic P.

Conclusion

Revisiting our hypotheses, we reject our first postulate that P fertilization of P-poor soil leads to increased EMF or RAF richness and diversity and shifts the community composition toward those present in P-rich beech forests. Instead, we found that P fertilization caused a decrease in RAF richness and an increase in the relative abundance of Boletales. At the level of distinct taxa (OTU-based), these shifts were not detectable, indicating that individual responses were diminutive but spread across a group of related species, thus aggregating to measurable effects at higher classification levels. Since Boletales are known for their ability to sequester P (Kottke et al. 1998), our results support the concept of phylogenetic trait conservatism (Powell et al. 2009). Thus, our results may shed light on the apparent ectomycorrhizal community stability in response to nutrient inputs as species-rich fungal assemblages may distribute the response among related members. This suggestion needs to be tested in future studies and may provide an ecological explanation for a frequently observed phenomenon (Lilleskov et al. 2019). We also reject the hypothesis that N fertilization, at least when applied at moderate doses for a relatively short period of time (2.5 years), affects the fungal communities, thus supporting the notion that EMF in temperate beech forests are relatively resistant to N inputs (Lilleskov et al. 2019). Since the relative abundance of SAF and RAF symbiotrophic fungi increased under N fertilization while root P contents declined irrespective of the soil P content, N inputs may lock P in fungal biomass, with negative consequences for tree P nutrition. We speculate that Russulales play a prominent role in this regard because the negative feedback of N fertilization on root P was compensated for by additional P application and was accompanied by decreases in Russulales. Overall, our results emphasize the importance of distinguishing different habitats and including the major nutrients N and P to better understand the drivers of fungal communities in relation to nutrient cycling.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-021-01593-x.

Acknowledgements We are grateful to Nadine Kubsch and PhD Karolin Müller for help with the harvests. We appreciate the skilled technical assistance by M. Franke-Klein (Forest Botany and Tree Physiology, Göttingen), G. Lehmannn, and T. Klein (Laboratory for Radio-Isotopes, LARI, Göttingen).

Author contributions SC and AP conceived the study. FL and JK realized the fertilization experiment, maintained the plots and contributed field data. SC conducted field and laboratory measurements. LEL conducted EMF sampling and analyses. HYF determined CN. SC, LEL, DJ, DS, RD, and AP analyzed data. SC compiled the data and wrote the first manuscript draft. AP revised the draft. All authors contributed to, commented, and agreed on the final version.

Funding This research was conducted in the Collaborative Research Program "Ecosystem Nutrition, SPP1685" funded by the Deutsche Forschungsgemeinschaft (DFG) with financial support to AP to project Po362/22–2 and FL to project LA1398/13–2. HYF was funded by the Institutional foundation of Chinese Academy of Forestry, CAFYBB2018GC010 and LEL was funded by a Special Research Fellowship (PhD scholarship) of the University of Zambia and as an associated student by the RTG2300 project SP4.

Data availability The datasets generated for this study can be found in the Dryad digital repository (https://doi.org/10.5061/dryad.rv15dv473). The sequences for identified mycorrhizal fungi are available in NCBI GenBank under accession numbers MT859114 to MT859131. The Illumina MiSeq sequences of fungal ITS2 can be found in the Sequence Read Archive (SRA) of NCBI under Bioproject PRJNA680926.

Declarations

Conflict of interest The authors declare no competing interests.

References

Agerer R (Ed.) (1987) Colour atlas of ectomycorrhizae: with glossary. Delivery 2: 1st-5th ed., Einhorn-Verlag Dietenberger, Schwäbisch Gmünd, Germany.

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

Allison SD, Hanson CA, Treseder KK (2007) Nitrogen fertilization reduces diversity and alters community structure of active fungi in boreal ecosystems. Soil Biol Biochem 39:1878–1887. https://doi.org/10.1016/j.soilbio.2007.02.001

Almeida JP, Rosenstock NP, Forsmark B, Bergh J, Wallander H (2019) Ectomycorrhizal community composition and function in a spruce forest transitioning between nitrogen and phosphorus limitation. Fungal Ecol 40:20–31. https://doi.org/10.1016/j.funeco.2018.05.008

Amend AS, Martiny AC, Allison SD, Berlemont R, Goulden ML, Lu Y, Treseder KK, Weihe C, Martiny JBH (2016) Microbial response to simulated global change is phylogenetically conserved and linked with functional potential. ISME J 10:109–118. https://doi.org/10.1038/ISMEJj.2015.96

Augusto L, Achat DL, Jonard M, Vidal D, Ringeval B (2017) Soil parent material—a major driver of plant nutrient limitations in terrestrial ecosystems. Glob Chang Biol 23:3808–3824. https://doi.org/10.1111/gcb.13691

Avis PG, McLaughlin DJ, Dentinger BC, Reich PB (2003) Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. New Phytol 160:239–253. https://doi.org/10.1046/j.1469-8137.2003.00865.x

Avis PG (2012) Ectomycorrhizal iconoclasts: the ITS rDNA diversity and nitrophilic tendencies of fetid Russula. Mycologia 104:998–1007. https://doi.org/10.3852/11-399

Awad A, Majcherczyk A, Schall P, Schöning SK, I, Schrumpf M, Ehbrecht M, Boch S, Kahl T, Bauhus J, Seidel D, Ammer C, Fischer M, Kües U, Pena R, (2019) Ectomycorrhizal and saprotrophic soil fungal biomass are driven by different factors and vary among broadleaf and coniferous temperate forests. Soil Biol Biochem 131:9–18. https://doi.org/10.1016/j.soilbio.2018.12.014

Bahnmann B, Mašínová T, Halvorsen R, Davey ML, Sedlák P, Tomšovský M, Baldrian P (2018) Effects of oak, beech and spruce on the distribution and community structure of fungi in litter and soils across a temperate forest. Soil Biol Biochem 119:162–173. https://doi.org/10.1016/j.soilbio.2018.01.021

Bahr A, Ellström M, Akselsson C, Ekblad A, Mikusinska A, Wallander H (2013) Growth of ectomycorrhizal fungal mycelium along a Norway spruce forest nitrogen deposition gradient and its effect on nitrogen leakage. Soil Biol Biochem 59:38–48. https://doi.org/10.1016/j.soilbio.2013.01.004

Bahr A, Ellström M, Bergh J, Wallander H (2015) Nitrogen leaching and ectomycorrhizal nitrogen retention capacity in a Norway spruce forest fertilized with nitrogen and phosphorus. Plant Soil 390:323–335. https://doi.org/10.1007/s11104-015-2408-6

Baldrian P (2017) Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol Rev 41:109–130. https://doi.org/10.1093/femsre/fuw040

Bergkemper F, Welzl G, Lang F, Krüger J, Schloter M, Schulz S (2016) The importance of C, N and P as driver for bacterial community structure in German beech dominated forest soils. J Plant Nutr Soil Sci 179:472–480. https://doi.org/10.1002/jpln.201600077

Bödeker ITM, Clemmensen KE, de Boer W, Martin F, Olson Å, Lindahl BD (2014) Ectomycorrhizal *Cortinarius* species participate in enzymatic oxidation of humus in northern forest ecosystems. New Phytol 203:245–256. https://doi.org/10.1111/nph.12791

Bray RH, Kurtz LT (1945) Determination of total, organic, and available forms of phosphorus in soils. Soil Sci 59:39–46. https://doi.org/10.1097/00010694-194501000-00006

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

Castaño C, Lindahl BD, Alday JG, Hagenbo A, Martínez de Aragón J, Parladé J, Pera J, Bonet JA (2018) Soil microclimate changes affect soil fungal communities in a Mediterranean pine forest. New Phytol 220:1211–1221. https://doi.org/10.1111/nph.15205

Cavender-Bares J, Kozak KH, Fine PVA, Kembel SW (2009) The merging of community ecology and phylogenetic biology. Ecol Lett 12:693–715. https://doi.org/10.1111/j.1461-0248.2009.01314.x

Chase JM (2007) Drought mediates the importance of stochastic community assembly. Proc Natl Acad Sci USA 104:17430–17434. https://doi.org/10.1073/pnas.0704350104

Cheng Y, Wang J, Chang SX, Cai Z, Müller C, Zhang J (2019) Nitrogen deposition affects both net and gross soil nitrogen transformations in forest ecosystems: a review. Environ Pollut 244:608–616. https://doi.org/10.1016/j.envpol.2018.10.054

Clausing S, Polle A (2020) Mycorrhizal phosphorus efficiencies and microbial competition drive root P uptake. Front for Glob Change 3:54. https://doi.org/10.3389/ffgc.2020.00054

Clausing S, Pena R, Song B, Müller K, Mayer-Gruner P, Marhan S, Gräfe M, Schulz S, Krüger J, Lang F, Schloter M, Kandeler E, Polle A (2021) Carbohydrate depletion impedes phosphorus

nutrition of young forest trees. New Phytol 5:2611–2624. https://doi.org/10.1111/nph.17058
Courty P-E, Buée M, Diedhiou AG, Frey-Klett P, Le Tacon F, Rineau F, Turpault M-P, Uroz S, Garbaye J (2010) The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. Soil Biol Biochem 42:679–698. https://doi.org/10.1016/j.soilbio.2009.12.006
Cox F, Barsoum N, Lilleskov EA, Bidartondo MI (2010) Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. Ecol Lett 13:1103–1113. https://doi.org/10.1111/j.1461-0248.2010.01494.x
de Vries W, Du E, Butterbach-Bahl K (2014) Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. Curr Opin Environ Sustain 9–10:90–104. https://doi.org/10.1016/j.cosust.2014.09.001
de Witte LC, Rosenstock NP, van der Linde S, Braun S (2017) Nitrogen deposition changes ectomycorrhizal communities in Swiss beech forests. Sci Total Environ 605:1083–1096. https://doi.org/10.1016/j.scitotenv.2017.06.142
Deng Q, McMahon DE, Xiang Y, Yu C-L, Jackson RB, Hui D (2016) A global meta-analysis of soil phosphorus dynamics after afforestation. New Phytol 213:181–192. https://doi.org/10.1111/nph.14119
Du E, De Vries W (2018) Nitrogen-induced new net primary production and carbon sequestration in global forests. Environ Pollut 242:1476–1487. https://doi.org/10.1016/j.envpol.2018.08.041
Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x
Etzold S, Ferretti M, Reinds GJ, Solberg S, Gessler A, Waldner P, Schaub M, Simpson D, Benham S, Hansen K, Ingerslev M, Jonard M, Karlsson PE, Lindroos A-J, Marchetto A, Manninger M, Meesenburg H, Merilä P, Nöjd P, Rautio P, Sanders TGM, Seidling W, Skudnik M, Thimonier A, Verstraeten A, Vesterdal L, Vejpustkova M, de Vries W (2020) Nitrogen deposition is the most important environmental driver of growth of pure, even-aged and managed European forests. For Ecol Manag 458:117762. https://doi.org/10.1016/j.foreco.2019.117762
Forsmark B, Wallander H, Nordin A, Gundale MJ (2021) Long-term nitrogen enrichment does not increase microbial phosphorus mobilization in a northern coniferous forest. Funct Ecol 35:277–287. https://doi.org/10.1111/1365-2435.13701
Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320:889–892. https://doi.org/10.1126/science.1136674
Geer LY, Marchler-Bauer A, Geer RC, Han L, He J, He S, Liu C, Shi W, Bryant SH (2010) Nucleic Acids Res 38(suppl_1): D492–D496. https://doi.org/10.1093/nar/gkp858
Glassman SI, Wang IJ, Bruns TD (2017) Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales. Mol Ecol 26:6960–6973. https://doi.org/10.1111/mec.14414
Goldmann K, Schröter K, Pena R, Schöning I, Schrumpf M, Buscot F, Polle A (2016) Divergent habitat filtering of root and soil fungal communities in temperate beech forests. Sci Rep 6:31439. https://doi.org/10.1038/srep31439
Gonzales K, Yanai R (2019) Nitrogen–phosphorous interactions in young northern hardwoods indicate P limitation: foliar concentrations and resorption in a factorial N by P addition experiment. Oecologia 189:829–840. https://doi.org/10.1007/s00442-019-04350-y
Heinrichs H, Brumsack H-J, Loftfield N, König N (1986) Verbessertes Druckaufschlußsystem für biologische und anorganische Materialien. Zeitschrift Für Pflanzenernährung Und Bodenkunde 149:350–353. https://doi.org/10.1002/jpln.19861490313
Ho NT, Li F, Wang S, Kuhn L (2019) MetamicrobiomeR: an R package for analysis of microbiome relative abundance data using zero-inflated beta GAMLSS and meta-analysis across studies using random effects models. BMC Bioinform 20:188. https://doi.org/10.1186/s12859-019-2744-2
Hobbie EA, Agerer R (2010) Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. Plant Soil 327:71–83. https://doi.org/10.1007/s11104-009-0032-z
Högberg P, Näsholm T, Franklin O, Högberg MN (2017) Tamm review: on the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. For Ecol Manag 403:161–185. https://doi.org/10.1016/j.foreco.2017.04.045
Högberg MN, Skyllberg U, Högberg P, Knicker H (2020) Does ectomycorrhiza have a universal key role in the formation of soil organic matter in boreal forests? Soil Biol Biochem 140:107635. https://doi.org/10.1016/j.soilbio.2019.107635
ISO 10390 (2005) Soil quality - determination of pH, International Organization for Standardization.
Iwański M, Rudawska M (2007) Ectomycorrhizal colonization of naturally regenerating *Pinus sylvestris* L. seedlings growing in different micro-habitats in boreal forest. Mycorrhiza 17:461–467. https://doi.org/10.1007/s00572-007-0132-7
Jonard M, Fürst A, Verstraeten A, Thimonier A, Timmermann V, Potočić N, Waldner P, Benham S, Hansen K, Merilä P, Ponette Q, de la Cruz AC, Roskams P, Nicolas M, Croisé L, Ingerslev M, Matteucci G, Decinti B, Bascietto M, Rautio P (2015) Tree mineral nutrition is deteriorating in Europe. Glob Chang Biol 21:418–430. https://doi.org/10.1111/gcb.12657
Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N, Clum A, Colpaert J, Copeland A, Costa MD, Doré J, Floudas D, Gay G, Girlanda M, Henrissat B, Herrmann S, Hess J, Högberg N, Johansson T, Khouja H-R, LaButti K, Lahrmann U, Levasseur A, Lindquist EA, Lipzen A, Marmeisse R, Martino E, Murat C, Ngan CY, Nehls U, Plett JM, Pringle A, Ohm RA, Perotto S, Peter M, Riley R, Rineau F, Ruytinx J, Salamov A, Shah F, Sun H, Tarkka M, Tritt A, Veneault-Fourrey C, Zuccaro A, Tunlid A, Grigoriev IV, Hibbett DS, Martin F (2015) Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat Genet 47:410–415. https://doi.org/10.1038/ng.3223
Köhler J, Yang N, Pena R, Raghavan V, Polle A, Meier IC (2018) Ectomycorrhizal fungal diversity increases P uptake efficiency of European beech. New Phytol 220:1200–1210. https://doi.org/10.1111/nph.15208
Kolaříková Z, Kohout P, Krüger C, Janoušková M, Mrnka L, Rydlová J (2017) Root-associated fungal communities along a primary succession on a mine spoil: distinct ecological guilds assemble differently. Soil Biol Biochem 113:143–152. https://doi.org/10.1016/j.soilbio.2017.06.004
Kottke I, Qian XM, Pritsch K, Haug I, Oberwinkler F (1998) *Xerocomus badius – Picea abies*, an ectomycorrhiza of high activity and element storage capacity in acidic soil. Mycorrhiza 7:267–275. https://doi.org/10.1007/s005720050191
Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson K-H (2013) Towards a unified paradigm for sequence-based identification

of fungi. Mol Ecol 22:5271–5277. https://doi.org/10.1111/mec.12481
Lambers H, Raven J, Shaver G, Smith S (2008) Plant nutrient-acquisition strategies change with soil age. Trends Ecol Evol 23:95–103. https://doi.org/10.1016/j.tree.2007.10.008
Lang C, Seven J, Polle A (2011) Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. Mycorrhiza 21:297–308. https://doi.org/10.1007/s00572-010-0338-y
Lang F, Krüger J, Amelung W, Willbold S, Frossard E, Bünemann EK, Bauhus J, Nitschke R, Kandeler E, Marhan S, Schulz S, Bergkemper F, Schloter M, Luster J, Guggisberg F, Kaiser K, Mikutta R, Guggenberger G, Polle A, Pena R, Prietzel J, Rodionov A, Talkner U, Meesenburg H, von Wilpert K, Hölscher A, Dietrich HP, Chmara I (2017) Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. Biogeochemistry 136:5–29. https://doi.org/10.1007/s10533-017-0375-0
Li J, Li Z, Wang F, Zou B, Chen Y, Zhao J, Mo Q, Li Y, Li X, Xia H (2015) Effects of nitrogen and phosphorus addition on soil microbial community in a secondary tropical forest of China. Biol Fertil Soils 51:207–215. https://doi.org/10.1007/s00374-014-0964-1
Lilleskov EA, Fahey TJ, Horton TR, Lovett GM (2002) Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83:104–115. https://doi.org/10.1890/0012-9658(2002)083[0104:BEFCCO]2.0.CO;2
Lilleskov EA, Wargo PM, Vogt KA, Vogt DJ (2008) Mycorrhizal fungal community relationship to root nitrogen concentration over a regional atmospheric nitrogen deposition gradient in the northeastern USA. Can J for Res 38:1260–1266. https://doi.org/10.1139/X07-211
Lilleskov EA, Kuyper TW, Bidartondo MI, Hobbie EA (2019) Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. Environ Pollut 246:148–162. https://doi.org/10.1016/j.envpol.2018.11.074
Lindahl BD, Tunlid A (2015) Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprotrophs. New Phytol 205:1443–1447. https://doi.org/10.1111/nph.13201
Liu L, Zhang T, Gilliam FS, Gundersen P, Zhang W, Chen H, Mo J (2013) Interactive effects of nitrogen and phosphorus on soil microbial communities in a tropical forest. PLoS ONE 8:e61188. https://doi.org/10.1371/journal.pone.0061188
López-Mondéjar R, Brabcová V, Štursová M, Davidová A, Jansa J, Cajthaml T, Baldrian P (2018) Decomposer food web in a deciduous forest shows high share of generalist microorganisms and importance of microbial biomass recycling. ISME J 12:1768–1778. https://doi.org/10.1038/s41396-018-0084-2
Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550. https://doi.org/10.1186/s13059-014-0550-8
Maaroufi NI, Nordin A, Palmqvist K, Hasselquist NJ, Forsmark B, Rosenstock NP, Wallander H, Gundale MJ (2019) Anthropogenic nitrogen enrichment enhances soil carbon accumulation by impacting saprotrophs rather than ectomycorrhizal fungal activity. Glob Chang Biol 25:2900–2914. https://doi.org/10.1111/gcb.14722
Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10. https://doi.org/10.14806/ej.17.1.200
Mason LM, Eagar A, Patel P, Blackwood CB, DeForest JL (2020) Potential microbial bioindicators of phosphorus mining in a temperate deciduous forest. J Appl Microbiol 130:109–122. https://doi.org/10.1111/jam.14761
Meller S, Frossard E, Luster J (2019) Phosphorus allocation to leaves of beech saplings reacts to soil phosphorus availability. Front Plant Sci 10:744. https://doi.org/10.3389/fpls.2019.00744
Mellert KH, Göttlein A (2012) Comparison of new foliar nutrient thresholds derived from van den Burg's literature compilation with established central European references. Eur J for Res 131:1461–1472. https://doi.org/10.1007/s10342-012-0615-8
Müller K, Kubsch N, Marhan S, Mayer-Gruner P, Nassal P, Schneider D, Daniel R, Piepho H-P, Polle A, Kandeler E (2020) Saprotrophic and ectomycorrhizal fungi contribute differentially to organic P mobilization in beech-dominated forest ecosystems. Front for Glob Change 3:47. https://doi.org/10.3389/ffgc.2020.00047
Mykrä H, Tolkkinen M, Markkola AM, Pirttilä AM, Muotka T (2016) Phylogenetic clustering of fungal communities in human-disturbed streams. Ecosphere 7:3. https://doi.org/10.1002/ecs2.1316
Nannipieri P, Penton CR, Purahong W, Schloter M, van Elsas JD (2019) Recommendations for soil microbiome analyses. Biol Fertil Soils 55:765–766. https://doi.org/10.1007/s00374-019-01409-z
Näsholm T, Högberg P, Franklin O, Metcalfe D, Keel SG, Campbell C, Hurry V, Linder S, Högberg MN (2013) Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? New Phytol 198:214–221. https://doi.org/10.1111/nph.12139
Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol 20:241–248. https://doi.org/10.1016/j.funeco.2015.06.006
Nguyen DQ, Schneider D, Brinkmann N, Song B, Janz D, Schöning I, Daniel R, Pena R, Polle A (2020) Soil and root nutrient chemistry structure root-associated fungal assemblages in temperate forests. Environ Microbiol 22:3081–3095. https://doi.org/10.1111/1462-2920.15037
Nicolás C, Almeida JP, Ellström M, Bahr A, Bone SE, Rosenstock NP, Bargar JR, Tunlid A, Persson P, Wallander H (2017) Chemical changes in organic matter after fungal colonization in a nitrogen fertilized and unfertilized Norway spruce forest. Plant Soil 419:113–126. https://doi.org/10.1007/s11104-017-3324-8
Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L (2019) Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol 17:95–109. https://doi.org/10.1038/s41579-018-0116-y
Oksanen J, Blanchet F G, Kindt R, Legendre P, Minchin P R, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2019) Package 'Vegan'. Community Ecology Package, Version 2. Available at: http://CRAN.R-project.org/package=vegan
Ollivier J, Töwe S, Bannert A, Hai B, Kastl E-M, Meyer A, Su MX, Kleineidam K, Schloter M (2011) Nitrogen turnover in soil and global change: key players of soil nitrogen cycle. FEMS Microbiol Ecol 78:3–16. https://doi.org/10.1111/j.1574-6941.2011.01165.x
Op De Beeck M, Troein C, Peterson C, Persson P, Tunlid A (2018) Fenton reaction facilitates organic nitrogen acquisition by an ectomycorrhizal fungus. New Phytol 218:335–343. https://doi.org/10.1111/nph.14971
Pastore G, Kernchen S, Spohn M (2020) Microbial solubilization of silicon and phosphorus from bedrock in relation to abundance of phosphorus-solubilizing bacteria in temperate forest soils. Soil Biol Biochem 151:108050. https://doi.org/10.1016/j.soilbio.2020.108050
Pausas JG, Verdú M (2010) The jungle of methods for evaluating phenotypic and phylogenetic structure of communities. Bioscience 60:614–625. https://doi.org/10.1525/bio.2010.60.8.7
Peay KG, Kennedy PG, Talbot JM (2016) Dimensions of biodiversity in the earth mycobiome. Nat Rev Microbiol 14:434–447. https://doi.org/10.1038/nrmicro.2016.59

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

Peñuelas J, Poulter B, Sardans J, Ciais P, van der Velde M, Bopp L, Boucher O, Godderis Y, Hinsinger P, Llusia J, Nardin E, Vicca S, Obersteiner M, Janssens IA (2013) Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nat Commun 4:2934. https://doi.org/10.1038/ncomms3934

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

Phillips LA, Ward V, Jones MD (2014) Ectomycorrhizal fungi contribute to soil organic matter cycling in sub-boreal forests. ISME J 8:699–713. https://doi.org/10.1038/ISMEJj.2013.195

Powell J, Parrent J, Hart M, Klironomos J, Rillig M, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. Proc Royal Soc b: Biol Sci 76:4237–4245. https://doi.org/10.1098/rspb.2009.1015

Pritsch K, Garbaye J (2011) Enzyme secretion by ECM fungi and exploitation of mineral nutrients from soil organic matter. Ann for Sci 68:25–32. https://doi.org/10.1007/s13595-010-0004-8

Purahong W, Wubet T, Kahl T, Arnstadt T, Hoppe B, Lentendu G, Baber K, Rose T, Kellner H, Hofrichter M, Bauhus J, Krüger D, Buscot F (2018) Increasing N deposition impacts neither diversity nor functions of deadwood-inhabiting fungal communities, but adaptation and functional redundancy ensure ecosystem function: N deposition in highly N-limited habitat. Environ Microbiol 20:1693–1710. https://doi.org/10.1111/1462-2920.14081

R Core Development Team (2012) R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. https://www.r-project.org/

Rigby RA, Stasinopoulos DM (2005) Generalized additive models for location, scale and shape. J R Stat Soc 54:507–554. https://doi.org/10.1111/j.1467-9876.2005.00510.x

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

Rosling A, Midgley MG, Cheeke T, Urbina H, Fransson P, Phillips RP (2016) Phosphorus cycling in deciduous forest soil differs between stands dominated by ecto- and arbuscular mycorrhizal trees. New Phytol 209:1184–1195. https://doi.org/10.1111/nph.13720

Rotter P, Loreau M, de Mazancourt C (2020) Why do forests respond differently to nitrogen deposition? A Modelling Approach Ecol Model 425:109034. https://doi.org/10.1016/j.ecolmodel.2020.109034

Schimel JP, Schaeffer SM (2012) Microbial control over carbon cycling in soil. Front Microbiol 3:348. https://doi.org/10.3389/fmicb.2012.00348

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

Schulte-Uebbing L, de Vries W (2018) Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: a meta-analysis. Glob Chang Biol 24:e416–e431. https://doi.org/10.1111/gcb.13862

Schwede DB, Simpson D, Tan J, Fu JS, Dentener F, Du E, deVries W (2018) Spatial variation of modelled total, dry and wet nitrogen deposition to forests at global scale. Environ Pollut 243:1287–1301. https://doi.org/10.1016/j.envpol.2018.09.084

Štursová M, Žifčáková L, Leigh MB, Burgess R, Baldrian P (2012) Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiol Ecol 80:735–746. https://doi.org/10.1111/j.1574-6941.2012.01343.x

Štursová M, Šnajdr J, Cajthaml T, Bárta J, Šantrůčková H, Baldrian P (2014) When the forest dies: the response of forest soil fungi to a bark beetle-induced tree dieback. ISME J 8:1920–1931. https://doi.org/10.1038/ISMEJj.2014.37

Suz LM, Barsoum N, Benham S, Dietrich H-P, Fetzer KD, Fischer R, García P, Gehrman J, Kristöfel F, Manninger M, Neagu S, Nicolas M, Oldenburger J, Raspe S, Sánchez G, Schröck HW, Schubert A, Verheyen K, Verstraeten A, Bidartondo MI (2014) Environmental drivers of ectomycorrhizal communities in Europe's temperate oak forests. Mol Ecol 23:5628–5644. https://doi.org/10.1111/mec.12947

Taylor A, Martin F, Read, D (2000) Fungal diversity in ectomycorrhizal communities of Norway spruce [*Picea abies* (L.) Karst.] and beech (*Fagus sylvatica* L.) along north-south transects in Europe. In: Schulze ED (ed) Carbon and nitrogen cycling in European Forest Ecosystems. Ecological Studies (Analysis and Synthesis), vol 142. Springer, Berlin, pp. 343–365. https://doi.org/10.1007/978-3-642-57219-7_16

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

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

Toju H, Kishida O, Katayama N, Takagi K (2016) Networks depicting the fine-scale co-occurrences of fungi in soil horizons. PLoS ONE 11:e0165987. https://doi.org/10.1371/journal.pone.0165987

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

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecol Appl 20:5–15. https://doi.org/10.1890/08-0127.1

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

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

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

Wardle DA (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509–513. https://doi.org/10.1126/science.1098778

Wardle DA, Jonsson M, Mayor JR, Metcalfe DB (2016) Above-ground and below-ground responses to long-term nutrient addition across a retrogressive chronosequence. J Ecol 104:545–560. https://doi.org/10.1111/1365-2745.12520

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols. Elsevier, Amsterdam, Netherlands, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

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

Xia Z, Yang J, Sang C, Wang X, Sun L, Jiang P, Wang C, Bai E (2020) Phosphorus reduces negative effects of nitrogen addition on soil microbial communities and functions. Microorganisms 8:1828. https://doi.org/10.3390/microorganisms8111828

Xue C, Hao Y, Pu X, Ryan Penton C, Wang Q, Zhao M, Zhang B, Ran W, Huang Q, Shen Q, Tiedje JM (2019) Effect of LSU and ITS genetic markers and reference databases on analyses of fungal communities. Biol Fertil Soils 55:79–88. https://doi.org/10.1007/s00374-018-1331-4

Zanne AE, Abarenkov K, Afkhami ME, Aguilar-Trigueros CA, Bates S, Bhatnagar JM, Busby PE, Christian N, Cornwell W, Crowther TW, Moreno HF, Floudas D, Gazis R, Hibbett D, Kennedy P, Lindner DL, Maynard DS, Milo AM, Nilsson H, Powell J, Schildhauer M, Schilling J, Treseder KK (2020) Fungal functional ecology: bringing a trait-based approach to plant-associated fungi. Biol Rev 95:409–433. https://doi.org/10.1111/brv.12570

Zavišić A, Nassal P, Yang N, Heuck C, Spohn M, Marhan S, Pena R, Kandeler E, Polle A (2016) Phosphorus availabilities in beech (*Fagus sylvatica* L.) forests impose habitat filtering on ectomycorrhizal communities and impact tree nutrition. Soil Biol Biochem 98:127–137. https://doi.org/10.1016/j.soilbio.2016.04.006

Zavišić A, Yang N, Marhan S, Kandeler E, Polle A (2018) Forest soil phosphorus resources and fertilization affect ectomycorrhizal community composition, beech P uptake efficiency, and photosynthesis. Front Plant Sci 9:463. https://doi.org/10.3389/fpls.2018.00463

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

Zhang K, Adams JM, Shi Y, Yang T, Sun R, He D, Ni Y, Chu H (2017) Environment and geographic distance differ in relative importance for determining fungal community of rhizosphere and bulk soil. Environ Microbiol 19:3649–3659. https://doi.org/10.1111/1462-2920.13865

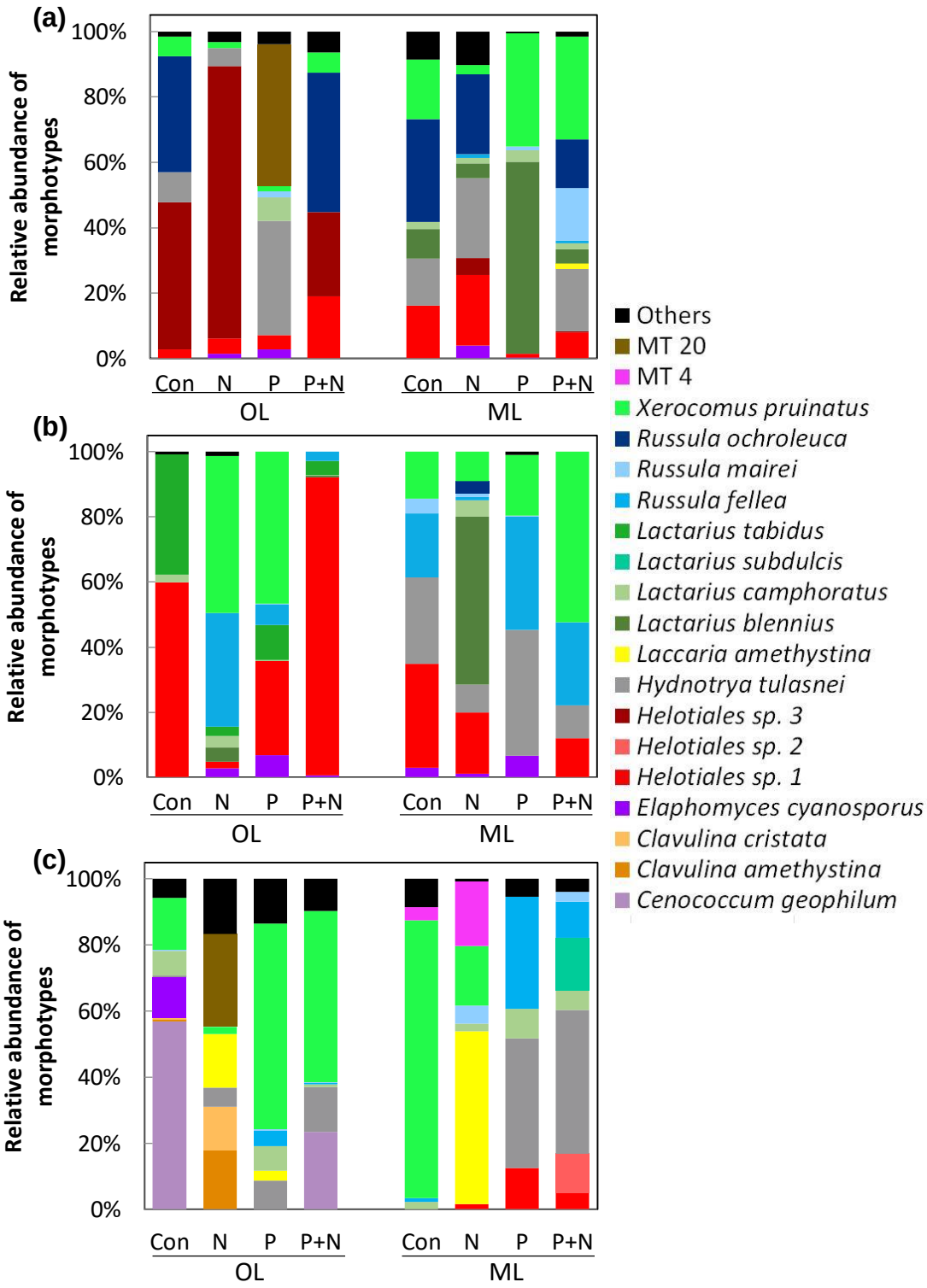
Supplement (Chapter 3)

Impact of nitrogen and phosphorus addition on resident soil and root mycobiomes in beech forests

Simon Clausing, Likulunga Emmanuel Likulunga, Denis Janz, Huang Ying Feng, Dominik Schneider, Rolf Daniel, Jaane Krüger, Friederike Lang, Andrea Polle

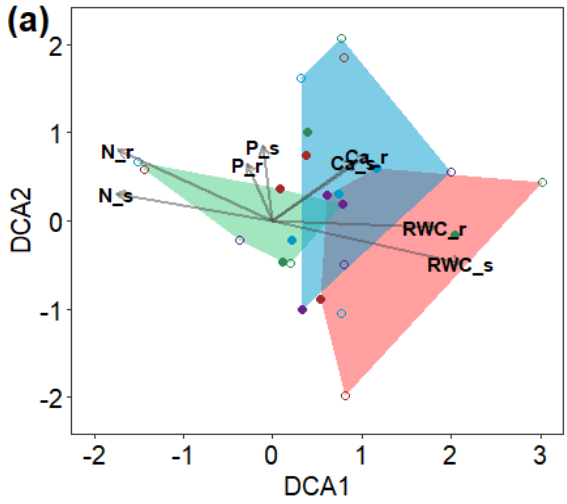
Published in: Biology and Fertility of Soils, 2021 (57), 1031–1052

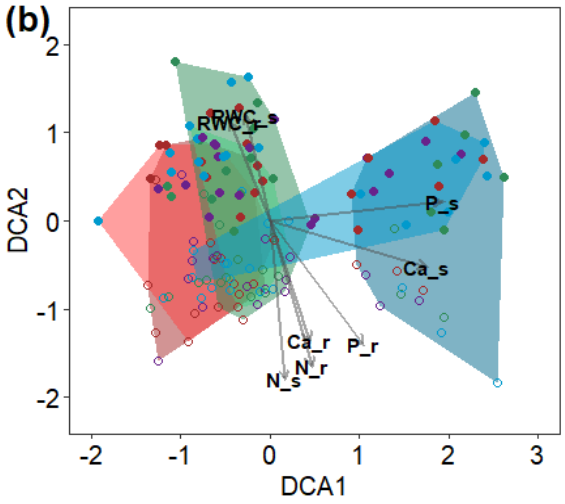
https://link.springer.com/article/10.1007/s00374-021-01593-x

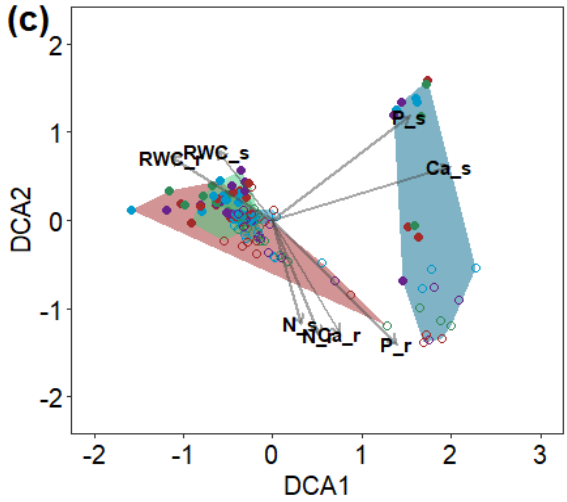


Supplement Figure S1: Ectomycorrhizal fungal community colonizing beech (*Fagus sylvatica* L.) roots under different fertilization treatments (Con, N, P, P+N).

Trees were investigated in P-rich (a), P-medium (b) and P-poor (c) forests. Roots from the organic layer and the mineral topsoil were analyzed separately. Two fungal morphotypes (MT20, MT4) did not yield sequences. “Others” refers to the sum of rare morphotypes, which were not sequenced. Data indicate means (n = 3). ANOSIM revealed significant differences between the following groups: Forest: R = 0.171, p = 0.001; Layer R = 0.018, p = 0.171; Forest type x Layer: R = 0.211, p = 0.001.



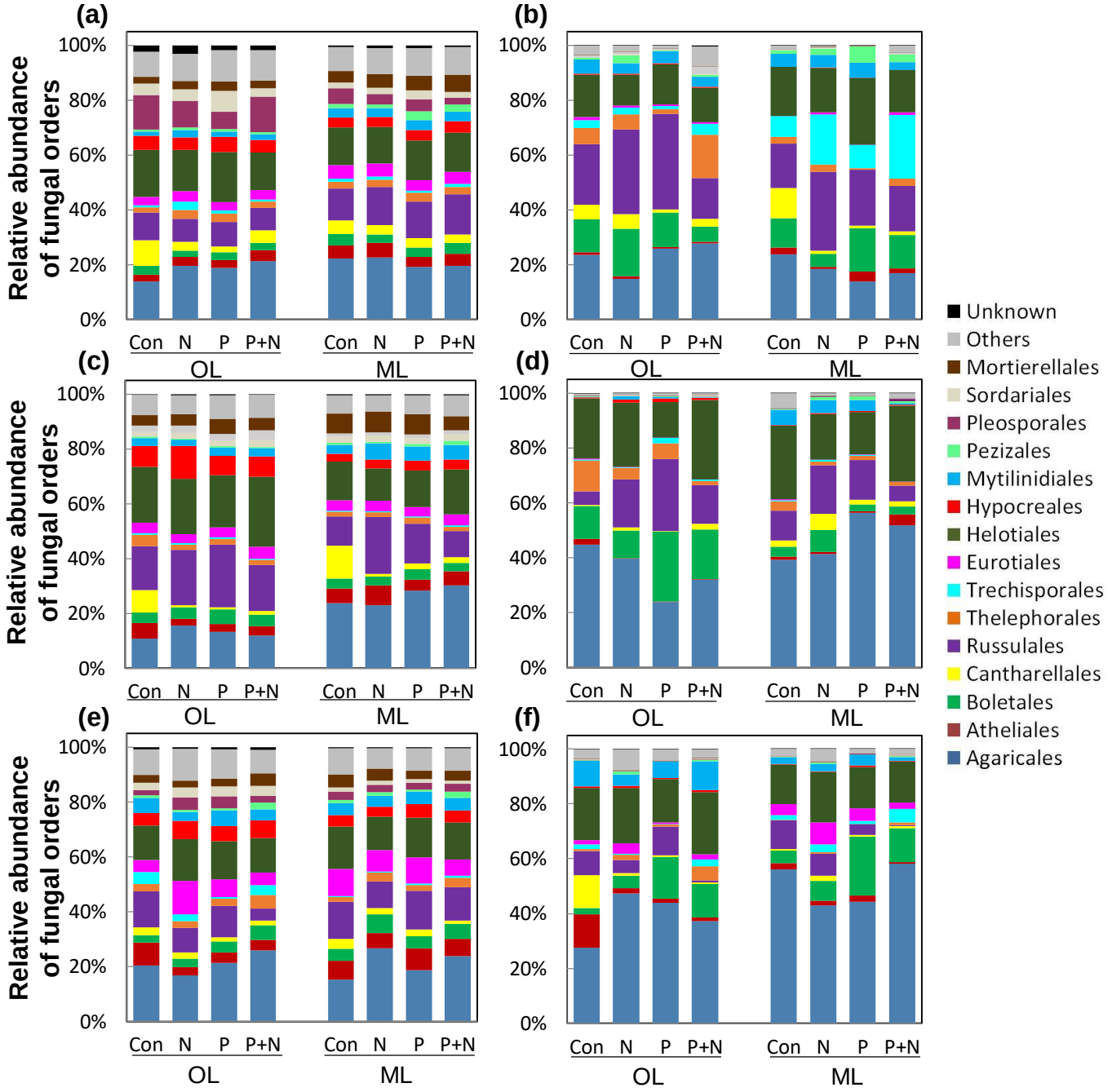




Group	Variable	F	R^2	p-value
EMF	Forest	2.02	0.161	**0.01**
	Soil layer	2.22	0.092	**0.01**
	Treatment	0.65	0.089	0.97
RAF	Forest	12.40	0.150	**0.00**
	Soil layer	10.69	0.070	**0.00**
	Season	2.85	0.020	**0.00**
	Treatment	1.01	0.021	0.42
SAF	Forest	13.08	0.156	**0.00**
	Soil layer	10.49	0.069	**0.00**
	Season	18.30	0.114	**0.00**
	Treatment	0.51	0.011	1.00

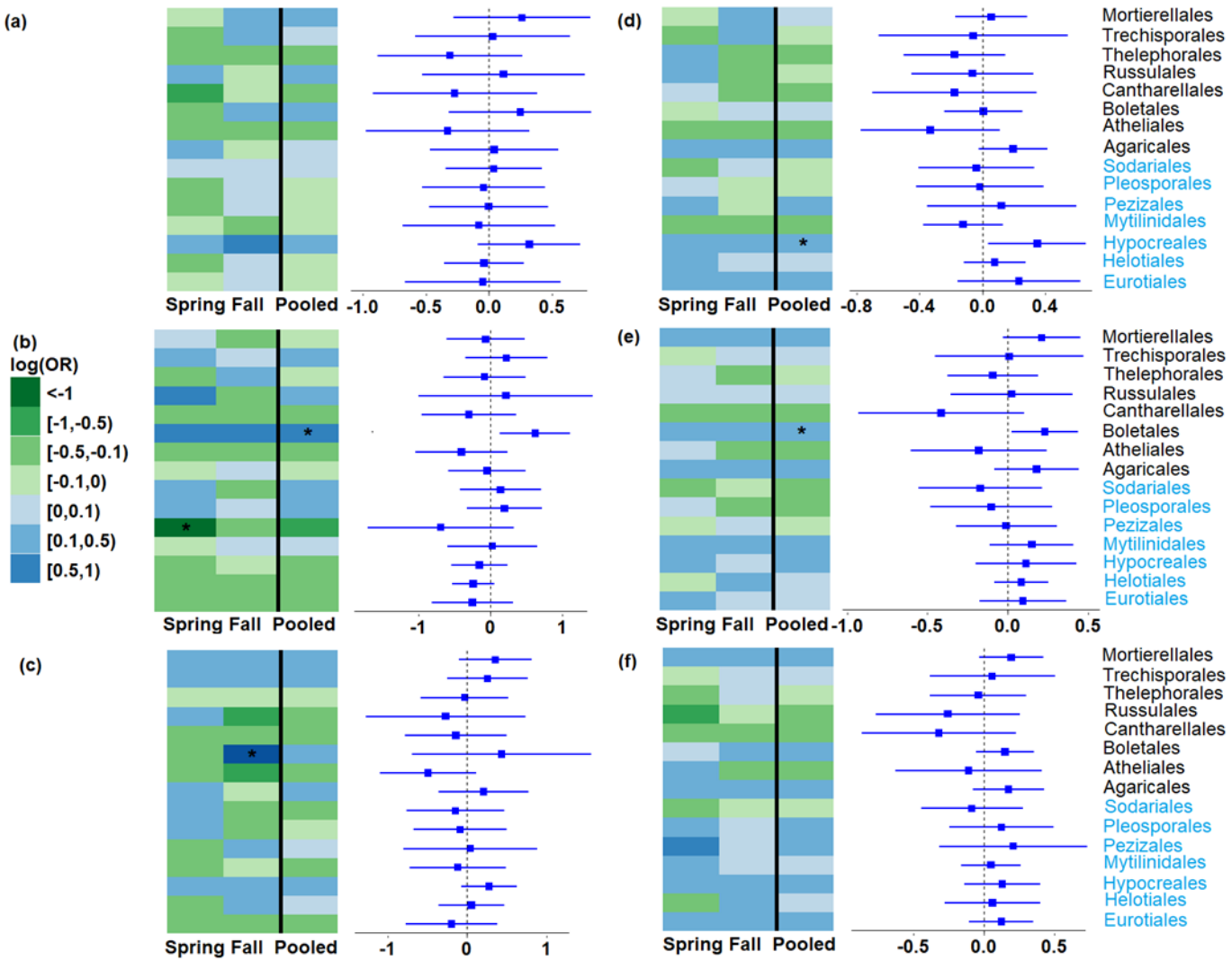
Supplement Figure S2: Detrended Correspondence Analysis (DCA) for ectomycorrhizal (a), root (b) and soil fungi (c) of beech forests (*Fagus sylvatica* L.).

Soil and fine root samples were collected in a P-rich (blue), P-medium (green) and P-poor (red) forest in 2018. The figure shows samples from the organic layer (open circles), the mineral topsoil (filled dots) collected in spring (dark colors) and fall (bright colors). Different fertilization treatments are labeled with colored symbols (red = Con = unfertilized, green = N = nitrogen, purple= P = phosphorus, blue = P+N = phosphorus and nitrogen). We measured elements in soil and roots as indicated in Materials and methods. We tested multicollinearity by Pearson's pairwise correlation for nutrient elements (P_{tot}, P_{lab}, N, C, Na, K, Ca, Mg, Mn, Fe, Al, S) and RWC (relative water content) We used variables that were unrelated as explanatory variables for the DCA and showed significant vectors ($p < 0.05$). Abbreviations: RWC = relative water content, Ca = calcium, P = total phosphorous, N = nitrogen; lower case letters s and r at the end of the variables indicate soil and fine roots respectively. Differences among groups for the factors forest type, soil layer, season and treatment were tested by ADONIS. R^2 correspond to the percentage of variation.

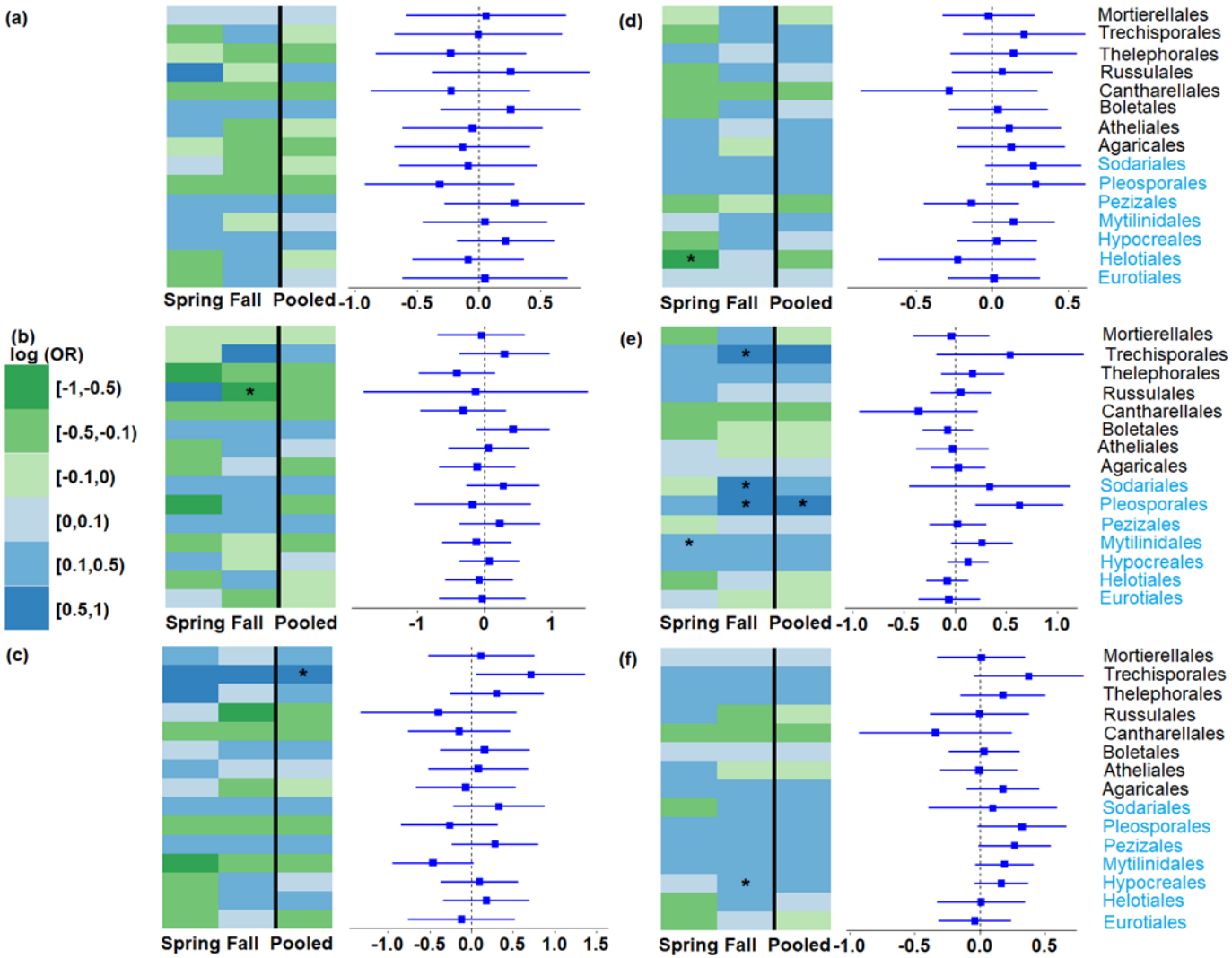


Supplement Figure S3: Relative abundance of fungal orders residing in soil (a, c, e) and associated with roots (b, d, f) of beech forests (*Fagus sylvatica* L.) without fertilization (Con) or with addition of N, P or N+P.

Soil and fine root samples were collected in a P-rich (a, b), P-medium (c, d) and P-poor (e, f) forest in 2018. Fungi in all forests, soil layers, seasons and compartments were analyzed separately by Illumina sequencing. Samples from the spring and fall season were pooled for the analyses. Data indicate means (n = 6). Fungal order with > 1% of the sequences are shown: Basidiomycota: Agaricales – dark blue, Atheliales – dark red, Boletales - green, Cantharellales – yellow, Russulales – purple, Thelephorales – orange, Trechisporales - turqouise ;Ascomycota: Eurotiales – pink, Helotiales – dark green, Hypocreales – red, Mytinidiales – blue, Pezizales – mint green, Pleosporales – sand, Sordariales – dark purple ; Zygomycota: Mortierellales – brown; Others (= sum of fungal orders < 1% of the sequences) – grey, Unknown (= sum of fungal sequences without an annotation for a fungal order) – black. Significant differences between the forest, season and treatment were calculated by a linear mixed effect model using Poisson distribution with plot number as random effect.



Supplement Figure S4: Effect sizes of N, P or N+P treatments on root (a, b and c) and soil (d, e and f) fungal orders in the organic soil layer for the treatments N/Con (a and d), P/Con (b and e) and (P+N)/Con (c and f). Blue show positive and green color code negative responses. The responses are indicated as log of odds ratio (log(OR)) as the result of GAMLSS analyses. Significant effects are indicated by * when $p \leq 0.05$. Fungal orders in blue are Ascomycetes while in black are Basidiomycetes.



Supplement Figure S5: Effect sizes of N, P or N+P treatments on root (a, b and c) and soil (d, e and f) fungal orders in the mineral top soil for the treatments N/Con (a and d), P/Con (b and e) and (P+N)/Con (c and f). Blue show positive and green color code negative responses. The responses are indicated as log of odds ratio (log(OR)) as the result of GAMLSS analyses. Significant effects are indicated by * when p ≤ 0.05. Fungal orders in blue are Ascomycetes while in black are Basidiomycetes.

Supplement Table S1: Characteristics of the research sites in the P-rich (HP, Bad Brückenau) the P-medium (MP, Mitterfels) and the P-poor forest (LP Luess). Data were compiled from publications (Haußmann and Lux, 1997; Lang et al., 2017). The parameters age, height and diameter refer to beech trees. Soil chemistry was determined before application of fertilizers. Extractable P was determined with the resin method (Lang et al. 2017). Values in parentheses represent standard deviation.

Parameters	HP	MP	LP
Location			
Gauss-Krüger coordinates	50°21'7.2"N 9°55'44.5"E	48°58'34.1"N 12°52'46.7"E	52°50'21.7"N 10°16'2.3"E
Altitude (m a.s.l.)	809	1023	115
Climate			
Mean annual temperature (°C)	5.8	4.9	8.0
Sum of annual precipitation (mm)	1031	1200	779
Stand characteristics			
Potential natural vegetation	Hordelymo-Fagetum	Dryopteris-Fagetum	Luzulo-Fagetum
Tree species composition (%)	*Fagus sylvatica* (99) *Acer pseudoplatanus* (1)	*Fagus sylvatica* (96) *Picea abies* (2) *Abies alba* (2)	*Fagus sylvatica* (91) *Quercus petraea* (9)
Age of beech (a)	137	131	132
Height (mean tree) (m)	26.8	20.8	27.3
Diameter at breast height (cm)	36.8	37.6	27.5
Number of trees (ha^{-1})	335	252	480
Basal area ($m^2\ ha^{-1}$)	35.6	28.1	36.7
Standing volume ($m^3\ ha^{-1}$)	495	274	529
Soil characteristics			
Soil type	Dystric skeletic cambisol	Hyperdystric chromic folic cambisol	Hyperdystric folic Cambisol
Parent material	Basalt	Paragneiss	Sandy till
Humus form	Mull-like Moder	Moder	Mor-like Moder
Texture (topsoil)	Silty clay loam	Loam	Loamy sand
Texture (subsoil)	Loam	Sandy loam	Sand
Soil chemistry (A horizon 0 to 5 cm)			
pH (H_20)	3.8 (0.15)	3.6 (0.1)	3.5 (0.08)
Total P ($mg\ kg^{-1}$)	2966 (45)	1375 (34)	195 (15)
Extractable P ($mg\ kg^{-1}$)	116 (9)	70 (6)	11 (3)
P in leaf litter ($g\ m^{-2}\ a^{-1}$)	0.229 (0.023)	0.213 (0.039)	0.156 (0.018)
P in leaves ($mg\ g^{-1}$ dry mass)	1.41 (0.21)	1.66 (0.16)	1.21 (0.08)

Supplement Table S2: Weather conditions (temperature and precipitation) during the harvest season and deviation from climatic conditions in beech forests (*Fagus sylvatica* L.). Soil samples were collected in a P-rich (HP), P-medium (MP) and P-poor (LP) forest in spring and fall 2018. We show the weather conditions in the month before and in the sampling month, using mean monthly temperatures and the sum of precipitation. The deviation from the long-term climatic conditions (dfc) was calculated as the monthly mean temperature of the sampling month minus the long-term mean in the respective month for the period 1981-2010. Data indicate monthly means of March and April for spring and of August and September for fall. Spring samples collected in April to early May and fall samples were collected in September to early October. The second table shows the weather and climatic data for each month.

	temperature (°C)		precipitation (mm)	
	Spring	fall	Spring	fall
during the harvest + month before				
HP	7.4	13.2	120.4	63.1
MP	6.8	12.1	57.1	110.6
LP	7.7	13.3	53.3	64.0
deviation from climatic conditions				
HP	+1.9	+2.7	-17.9	-96.6
MP	+0.6	+1.5	-37.5	-13.9
LP	+0.6	+1.4	-8.4	-49.8

		temperature (°C)		**precipitation (mm)**	
site	**month**	month	dfc	Month	dfc
	February	-3.1	-2.8	13.6	-59.8
	March	1.8	-1.6	70.3	-10.1
	April	13.0	5.3	50.1	-7.8
	May	16.1	3.8	61.4	-10.1
HP	June	17.6	2.6	46.3	-20.3
	July	21.1	3.9	32.1	-44.2
	August	20.2	3.3	26.3	-35.9
	September	15.2	2.3	36.3	-37.2
	October	11.1	3.0	26.8	-59.7
	February	-3.5	-3.4	20.6	-24.7
	March	1.3	-2.3	43.8	-6.8
	April	12.3	3.4	13.3	-30.7
	May	15.5	3.1	67.9	-28.0
MP	June	16.8	0.6	49.8	-44.1
	July	19.0	1.2	68.2	-39.9
	August	19.7	2.6	33.0	-55.5
	September	14.1	1.1	73.9	7.2
	October	10.1	1.9	36.7	-21.1
	February	1.3	-3.1	3.2	-17.1
	March	2.6	-2.3	3.5	-16.5
	April	12.7	3.5	49.8	8.1
	May	17.5	3.7	38.7	-14.5
LP	June	18.4	2.0	14.1	-47.0
	July	21.1	2.4	39.2	-27.3
	August	20.2	2.1	30.1	-36.7
	September	15.4	1.3	43.8	-13.5
	October	11.1	1.4	20.2	-36.3

Supplement Table S3: Molecular information on ectomycorrhizal species colonizing root tips in beech forests (*Fagus sylvatica* L.) under fertilization treatments. Roots were analyzed in P-rich, P-medium and P-poor forests. Roots from the organic layer and the mineral topsoil were analyzed separately. The table shows the original morphotype number, accession number of the best match (best match in the UNITE Genbank), the sequence length/length in the data base and % similarity of nucleotide sequence, and NCBI accession number (accession number under which the sequences of the present fungi have been deposited in NCBI Genbank). The ITS region of the fungal rDNA was amplified using the PCR primers ITS1F (5′CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′TCCTCCGCTTATTGATATGC-3′) (White et al. 1990).

Ectomycorrhizal fungal species	MT number	Reference accession number UNITE	Length and % similarity of nucleotide sequence	NCBI accession number
Cenococcum geophylum	MT26	LC095162.1	542 (98.9%)	MT859114
Clavulina amethystina	MT19	MK422194.1	736 (96.66%)	MT859115
Clavulina cristata	MT11	MN947349.1	735 (99.03%)	MT859116
Elaphomyces cyanosporus	MT33	KX238826.1	613 (99.83%)	MT859117
Helotiales sp. 1	MT1	HM190117.1	736 (99.73%)	MT859118
Helotiales sp. 2	MT34	JF519582.1	621 (99.67%)	MT859119
Helotiales sp. 3	MT35	LC189022.2	576 (100%)	MT859120
Laccaria amethystina	MT16	MN947342.1	736 (100%)	MT859121
Lactarius blennius	MT39	MN947353.1	782 (99.23%)	MT859122
Lactarius camphoratus	MT8	MN992440.1	763 (99.86%)	MT859123
Lactarius subdulcis	MT9	HM189802.1	765 (99.61%)	MT859124
Russula fellea	MT6	MN959791.1	694 (99.63%)	MT859126
Russula mairei	MT2	MN947352.1	704 (99.15%)	MT859127
Russula ochroleuca	MT38	MT644930.1	741 (99.73%)	MT859128
Xerocomus pruinatus	MT5	MN947367.1	800 (99.38%)	MT859129
Hydnotrya tulasnei	MT15	GQ149454.1	762 (99.87%)	MT859130
Lactarius tabidus	MT42	HM189817.1	752 (99.6%)	MT859131
Unknown - MT 4	MT4			
Unknown - MT 20	MT20			

Supplement Table S4: Soil and root chemistry in the organic layer and mineral top soil in spring and fall in response to fertilization treatments (Con, N, P, P+N) of beech forests (*Fagus sylvatica* L.). Soils and roots were collected in a P-rich (HP), P-medium (MP) and P-poor (LP) forest in spring and fall 2018 and separated into organic layer and the mineral topsoil for analyses. Data indicate means (n = 3 ± SE). RWC = relative water content. Statistical information is provided in Table 1

HP

Spring				
Organic layer	**Con**	**N**	**P**	**P+N**
Bulk soil				
RWC	0.54 ± 0.03	0.42 ± 0.02	0.52 ± 0.07	0.46 ± 0.04
P_{tot} (mg g^{-1} dw)	2.59 ± 0.12	2.29 ± 0.11	2.08 ± 0.12	1.65 ± 0.07
P_{lab} (µg g^{-1} dw)	264.6 ± 25.1	286.6 ± 27.7	313.7 ± 18.4	354.7 ± 30.8
N (mg g^{-1} dw)	17.7 ± 0.8	17.5 ± 0.9	19.2 ± 0.7	17.0 ± 0.9
C (mg g^{-1} dw)	365.8 ± 21.4	349.4 ± 11.7	377.5 ± 10.3	329.5 ± 22.2
C:N	20.7 ± 0.3	20.0 ± 0.8	19.7 ± 0.2	19.4 ± 1.0
N:P	6.8 ± 0.5	7.7 ± 0.7	9.3 ± 0.6	10.4 ± 0.9
pH	4.51 ± 0.05	4.62 ± 0.07	4.53 ± 0.07	4.70 ± 0.07
NH_4^+ (µmol g^{-1} dw)	402.9 ± 232.7	366.5 ± 81.2	562.3 ± 40.2	600.4 ± 214.1
NO_3^- (µmol g^{-1} dw)	39.1 ± 9.5	51.2 ± 6.6	56.0 ± 10.9	28.4 ± 11.9
Fine roots				
RWC	0.71 ± 0.02	0.70 ± 0.02	0.71 ± 0.02	0.72 ± 0.03
P_{tot} (mg g^{-1} dw)	2.78 ± 0.07	1.97 ± 0.06	2.51 ± 0.04	2.16 ± 0.14
P_{lab} (µg g^{-1} dw)	510.1 ± 32.7	637.1 ± 59.3	733.1 ± 13.5	687.8 ± 77.3
N (mg g^{-1} dw)	21.7 ± 0.7	20.7 ± 1.7	22.4 ± 1.2	21.0 ± 1.1
C (mg g^{-1} dw)	471.4 ± 2.5	464.1 ± 1.9	463.6 ± 9.4	467.0 ± 1.7
C:N	21.7 ± 0.6	22.6 ± 1.6	20.8 ± 0.9	22.3 ± 1.3
N:P	7.8 ± 0.2	10.6 ± 1.2	8.9 ± 0.5	9.8 ± 0.6
Mineral topsoil				
Bulk soil				
RWC	0.46 ± 0.01	0.46 ± 0.01	0.45 ± 0.01	0.45 ± 0.01
P_{tot} (mg g^{-1} dw)	3.33 ± 0.14	3.42 ± 0.15	3.60 ± 0.14	3.78 ± 0.16
P_{lab} (µg g^{-1} dw)	233.0 ± 59.4	220.2 ± 34.6	325.3 ± 28.5	230.1 ± 15.7
N (mg g^{-1} dw)	7.40 ± 0.52	7.56 ± 0.32	7.21 ± 0.55	7.70 ± 0.81
C (mg g^{-1} dw)	107.3 ± 9.4	103.5 ± 3.4	104.4 ± 7.4	108.7 ± 14.0
C:N	14.5 ± 0.6	13.7 ± 0.2	14.5 ± 0.1	14.1 ± 0.3
N:P	2.2 ± 0.2	2.2 ± 0.1	2.0 ± 0.2	2.0 ± 0.2
pH	4.54 ± 0.25	4.51 ± 0.10	4.53 ± 0.12	4.54 ± 0.07
NH_4^+ (µmol g^{-1} dw)	7.26 ± 2.91	12.6 ± 1.3	18.1 ± 3.4	64.2 ± 50.6
NO_3^- (µmol g^{-1} dw)	3.37 ± 1.40	1.31 ± 0.35	3.83 ± 2.30	2.34 ± 0.82
Fine roots				
RWC	0.65 ± 0.02	0.65 ± 0.01	0.67 ± 0.01	0.64 ± 0.01
P_{tot} (mg g^{-1} dw)	1.22 ± 0.09	1.21 ± 0.11	1.44 ± 0.07	1.31 ± 0.08
P_{lab} (µg g^{-1} dw)	517.9 ± 11.1	598.1 ± 78.5	713.7 ± 19.8	662.7 ± 67.3
N (mg g^{-1} dw)	14.0 ± 0.5	14.6 ± 0.3	14.6 ± 0.8	14.3 ± 0.5
C (mg g^{-1} dw)	472.7 ± 2.8	463.1 ± 7.1	446.7 ± 10.2	460.8 ± 2.9
C:N	33.8 ± 1.2	31.7 ± 1.1	30.8 ± 1.6	32.3 ± 1.4
N:P	11.6 ± 1.1	12.2 ± 0.9	10.1 ± 0.2	10.9 ± 0.6

Fall				
Organic layer	**Con**	**N**	**P**	**P+N**
Bulk soil				
RWC	0.31 ± 0.05	0.37 ± 0.05	0.30 ± 0.01	0.36 ± 0.07
P_{tot} (mg g^{-1} dw)	1.51 ± 0.05	1.80 ± 0.19	1.66 ± 0.09	1.53 ± 0.29
P_{lab} (µg g^{-1} dw)	183.9 ± 32.2	180.3 ± 18.2	281.8 ± 12.5	216.2 ± 54.7
N (mg g^{-1} dw)	12.9 ± 0.9	12.3 ± 1.9	13.8 ± 0.5	16.0 ± 4.3
C (mg g^{-1} dw)	222.7 ± 18.8	178.6 ± 26.6	216.8 ± 3.2	265.4 ± 93.3
C:N	17.3 ± 0.8	14.6 ± 0.1	15.7 ± 0.3	15.9 ± 1.3
N:P	8.6 ± 0.6	7.1 ± 1.7	8.4 ± 0.4	9.6 ± 0.9
pH	4.00 ± 0.04	4.11 ± 0.07	4.10 ± 0.04	4.07 ± 0.18
NH_4^+ (µmol g^{-1} dw)	272.4 ± 204.4	121.3 ± 61.8	108.3 ± 60.8	500.0 ± 225.0
NO_3^- (µmol g^{-1} dw)	68.3 ± 41.1	62.3 ± 27.2	42.2 ± 22.2	66.9 ± 38.5
Fine roots				
RWC	0.61 ± 0.06	0.60 ± 0.02	0.61 ± 0.05	0.61 ± 0.01
P_{tot} (mg g^{-1} dw)	1.47 ± 0.19	1.10 ± 0.13	1.39 ± 0.10	1.32 ± 0.14
P_{lab} (µg g^{-1} dw)	581.5 ± 117.0	371.9 ± 48.1	633.2 ± 120.2	565.9 ± 113.6
N (mg g^{-1} dw)	20.6 ± 0.9	17.0 ± 1.3	18.2 ± 0.6	19.3 ± 0.2
C (mg g^{-1} dw)	448.2 ± 3.6	453.9 ± 7.8	438.9 ± 6.7	429.3 ± 24.9
C:N	21.9 ± 1.0	27.1 ± 2.4	24.2 ± 0.8	22.3 ± 1.1
N:P	14.4 ± 1.7	15.5 ± 0.6	13.2 ± 1.3	15.0 ± 1.9
Mineral topsoil				
Bulk soil				
RWC	0.27 ± 0.03	0.31 ± 0.01	0.32 ± 0.03	0.32 ± 0.01
P_{tot} (mg g^{-1} dw)	1.84 ± 0.21	2.14 ± 0.12	2.06 ± 0.20	2.09 ± 0.13
P_{lab} (µg g^{-1} dw)	109.0 ± 17.2	156.6 ± 32.4	180.9 ± 18.0	180.9 ± 34.4
N (mg g^{-1} dw)	6.97 ± 0.55	7.95 ± 0.33	6.66 ± 0.43	8.81 ± 0.78
C (mg g^{-1} dw)	93.9 ± 6.4	101.2 ± 3.1	91.5 ± 4.5	123.9 ± 19.3
C:N	13.5 ± 0.3	12.7 ± 0.2	13.8 ± 0.4	13.9 ± 0.9
N:P	3.9 ± 0.4	3.7 ± 0.1	3.3 ± 0.1	4.2 ± 0.3
pH	4.30 ± 0.02	4.32 ± 0.06	4.32 ± 0.02	4.27 ± 0.09
NH_4^+ (µmol g^{-1} dw)	56.6 ± 18.4	38.8 ± 14.3	37.0 ± 13.0	48.4 ± 12.2
NO_3^- (µmol g^{-1} dw)	10.4 ± 1.7	24.0 ± 17.9	7.0 ± 1.4	42.0 ± 29.6
Fine roots				
RWC	0.51 ± 0.06	0.60 ± 0.01	0.57 ± 0.01	0.59 ± 0.03
P_{tot} (mg g^{-1} dw)	1.15 ± 0.18	1.20 ± 0.10	1.19 ± 0.02	1.11 ± 0.05
P_{lab} (µg g^{-1} dw)	393.1 ± 94.4	348.9 ± 36.1	417.5 ± 37.9	459.6 ± 39.7
N (mg g^{-1} dw)	18.0 ± 0.7	17.4 ± 0.3	17.0 ± 0.6	18.7 ± 1.4
C (mg g^{-1} dw)	437.6 ± 12.7	434.7 ± 9.5	433.4 ± 0.4	436.4 ± 9.5
C:N	24.4 ± 1.5	25.1 ± 0.8	25.6 ± 0.9	23.5 ± 1.5
N:P	16.3 ± 1.9	14.6 ± 0.9	14.3 ± 0.6	16.8 ± 0.7

Supplement Table S4 continued

MP

Spring								
Organic layer	**Con**	**N**	**P**	**P+N**
Bulk soil				
RWC	0.71 ± 0.02	0.71 ± 0.02	0.69 ± 0.01	0.72 ± 0.00
P_{tot} (mg g^{-1} dw)	1.29 ± 0.05	1.35 ± 0.09	1.41 ± 0.10	1.28 ± 0.09
P_{lab} (µg g^{-1} dw)	147.1 ± 13.5	127.4 ± 8.6	163.0 ± 7.6	173.5 ± 6.8
N (mg g^{-1} dw)	23.1 ± 0.3	23.4 ± 0.4	22.8 ± 0.1	23.0 ± 0.2
C (mg g^{-1} dw)	465.9 ± 2.9	471.3 ± 1.5	465.3 ± 4.3	470.7 ± 3.3
C:N	20.2 ± 0.2	20.1 ± 0.4	20.4 ± 0.2	20.5 ± 0.1
N:P	18.1 ± 0.9	17.5 ± 1.1	16.3 ± 1.1	18.1 ± 1.6
pH	3.72 ± 0.02	3.80 ± 0.02	3.76 ± 0.03	3.77 ± 0.03
NH_4^+ (µmol g^{-1} dw)	144.7 ± 53.4	118.8 ± 21.7	191.0 ± 109.5	64.8 ± 22.1
NO_3^- (µmol g^{-1} dw)	4.87 ± 0.68	3.59 ± 1.54	4.55 ± 0.55	3.78 ± 0.58
Fine roots				
RWC	0.70 ± 0.02	0.70 ± 0.01	0.71 ± 0.03	0.74 ± 0.01
P_{tot} (mg g^{-1} dw)	1.74 ± 0.04	1.37 ± 0.08	1.50 ± 0.06	1.65 ± 0.04
P_{lab} (µg g^{-1} dw)	555.1 ± 36.8	451.1 ± 28.4	696.6 ± 134.8	1020.2 ± 180.8
N (mg g^{-1} dw)	17.7 ± 0.9	17.5 ± 1.3	17.9 ± 1.0	18.5 ± 1.6
C (mg g^{-1} dw)	499.6 ± 2.0	495.0 ± 2.7	498.3 ± 4.3	496.3 ± 5.7
C:N	28.4 ± 1.5	28.7 ± 2.4	28.1 ± 2.0	27.4 ± 3.0
N:P	10.1 ± 0.4	12.8 ± 0.8	11.9 ± 0.3	11.2 ± 0.8
Mineral topsoil				
Bulk soil				
RWC	0.43 ± 0.02	0.42 ± 0.01	0.42 ± 0.01	0.42 ± 0.02
P_{tot} (mg g^{-1} dw)	1.58 ± 0.11	1.76 ± 0.07	1.47 ± 0.06	1.72 ± 0.05
P_{lab} (µg g^{-1} dw)	146.0 ± 27.3	136.6 ± 19.0	131.2 ± 23.4	152.2 ± 3.8
N (mg g^{-1} dw)	6.86 ± 0.64	7.11 ± 0.19	6.17 ± 0.56	6.55 ± 0.38
C (mg g^{-1} dw)	120.1 ± 11.4	121.2 ± 2.8	106.9 ± 11.1	112.5 ± 7.0
C:N	17.5 ± 0.2	17.0 ± 0.2	17.3 ± 0.2	17.2 ± 0.3
N:P	4.3 ± 0.3	4.0 ± 0.1	4.2 ± 0.2	3.8 ± 0.2
pH	3.92 ± 0.01	3.96 ± 0.05	3.97 ± 0.04	3.92 ± 0.02
NH_4^+ (µmol g^{-1} dw)	13.3 ± 4.0	7.0 ± 1.7	41.9 ± 33.3	6.8 ± 2.6
NO_3^- (µmol g^{-1} dw)	1.64 ± 0.27	1.51 ± 0.10	1.71 ± 0.36	2.43 ± 0.21
Fine roots				
RWC	0.39 ± 0.02	0.30 ± 0.05	0.32 ± 0.07	0.38 ± 0.02
P_{tot} (mg g^{-1} dw)	0.59 ± 0.02	1.09 ± 0.13	0.71 ± 0.08	1.00 ± 0.08
P_{lab} (µg g^{-1} dw)	297.1 ± 41.4	314.4 ± 9.5	359.9 ± 48.1	542.9 ± 45.9
N (mg g^{-1} dw)	11.7 ± 0.1	14.4 ± 1.3	11.2 ± 0.4	12.7 ± 1.1
C (mg g^{-1} dw)	485.9 ± 4.0	464.7 ± 15.4	484.9 ± 3.5	477.9 ± 10.0
C:N	41.4 ± 0.4	33.1 ± 4.3	43.4 ± 1.5	38.3 ± 3.7
N:P	19.9 ± 0.5	13.4 ± 1.7	15.9 ± 1.4	12.7 ± 0.4

Fall				
Organic layer	**Con**	**N**	**P**	**P+N**
Bulk soil				
RWC	0.51 ± 0.04	0.57 ± 0.06	0.56 ± 0.04	0.67 ± 0.06
P_{tot} (mg g^{-1} dw)	0.92 ± 0.01	0.89 ± 0.07	1.07 ± 0.06	0.97 ± 0.14
P_{lab} (µg g^{-1} dw)	98.3 ± 8.5	106.4 ± 2.3	149.4 ± 6.0	117.3 ± 10.7
N (mg g^{-1} dw)	21.5 ± 1.4	20.4 ± 1.3	22.0 ± 1.7	23.6 ± 0.4
C (mg g^{-1} dw)	428.0 ± 36.9	395.8 ± 36.0	408.5 ± 39.5	444.4 ± 24.4
C:N	19.9 ± 0.5	19.4 ± 0.5	18.6 ± 0.4	18.8 ± 0.7
N:P	23.3 ± 1.8	23.2 ± 2.6	20.9 ± 2.8	25.3 ± 3.6
pH	3.54 ± 0.03	3.69 ± 0.08	3.68 ± 0.03	3.61 ± 0.03
NH_4^+ (µmol g^{-1} dw)	220.1 ± 25.1	107.8 ± 20.8	63.3 ± 21.8	73.4 ± 49.9
NO_3^- (µmol g^{-1} dw)	3.54 ± 1.07	4.04 ± 1.09	4.36 ± 1.04	4.83 ± 0.43
Fine roots				
RWC	0.64 ± 0.01	0.64 ± 0.01	0.68 ± 0.01	0.67 ± 0.01
P_{tot} (mg g^{-1} dw)	0.96 ± 0.06	0.81 ± 0.02	1.11 ± 0.12	0.99 ± 0.12
P_{lab} (µg g^{-1} dw)	281.8 ± 23.3	251.3 ± 22.1	461.9 ± 39.2	385.2 ± 31.9
N (mg g^{-1} dw)	22.5 ± 0.3	22.5 ± 0.4	23.9 ± 1.1	23.0 ± 0.5
C (mg g^{-1} dw)	503.0 ± 2.3	504.9 ± 0.9	495.1 ± 3.4	506.0 ± 2.4
C:N	22.4 ± 0.4	22.4 ± 0.4	20.8 ± 1.1	22.0 ± 0.6
N:P	23.6 ± 1.1	27.9 ± 0.6	21.8 ± 1.4	23.9 ± 2.8
Mineral topsoil				
Bulk soil				
RWC	0.19 ± 0.08	0.34 ± 0.02	0.30 ± 0.01	0.38 ± 0.02
P_{tot} (mg g^{-1} dw)	1.07 ± 0.08	1.07 ± 0.10	0.87 ± 0.10	1.25 ± 0.02
P_{lab} (µg g^{-1} dw)	147.7 ± 34.5	97.1 ± 15.6	86.9 ± 5.8	174.3 ± 53.2
N (mg g^{-1} dw)	6.80 ± 1.24	6.08 ± 0.49	4.99 ± 0.11	7.96 ± 0.62
C (mg g^{-1} dw)	122.7 ± 23.2	99.7 ± 7.8	83.3 ± 3.5	131.4 ± 11.6
C:N	18.0 ± 0.3	16.4 ± 0.1	16.7 ± 0.4	16.5 ± 0.3
N:P	6.3 ± 0.7	5.7 ± 0.2	5.8 ± 0.5	6.3 ± 0.4
pH	3.90 ± 0.08	4.01 ± 0.01	4.07 ± 0.04	3.88 ± 0.04
NH_4^+ (µmol g^{-1} dw)	24.9 ± 4.9	25.4 ± 10.6	39.2 ± 15.7	20.6 ± 4.4
NO_3^- (µmol g^{-1} dw)	10.07 ± 5.47	5.92 ± 1.57	5.50 ± 1.91	5.34 ± 0.50
Fine roots				
RWC	0.42 ± 0.00	0.42 ± 0.01	0.41 ± 0.02	0.41 ± 0.02
P_{tot} (mg g^{-1} dw)	0.78 ± 0.06	0.69 ± 0.07	0.66 ± 0.01	0.84 ± 0.11
P_{lab} (µg g^{-1} dw)	272.1 ± 46.0	199.3 ± 26.4	216.7 ± 6.2	361.3 ± 39.4
N (mg g^{-1} dw)	11.5 ± 0.6	11.1 ± 0.6	10.5 ± 0.9	11.3 ± 0.3
C (mg g^{-1} dw)	462.5 ± 7.7	454.8 ± 13.2	451.0 ± 21.7	459.1 ± 12.6
C:N	40.4 ± 1.5	41.3 ± 3.3	43.3 ± 3.0	40.6 ± 0.4
N:P	14.8 ± 0.6	16.3 ± 1.1	15.9 ± 1.3	13.9 ± 2.1

Supplement Table S4 continued

LP

Spring organic layer	Con	N	P	P+N
Bulk soil				
RWC	0.70 ± 0.02	0.65 ± 0.02	0.69 ± 0.03	0.66 ± 0.03
P_{tot} (mg g^{-1} dw)	0.62 ± 0.07	0.57 ± 0.06	0.72 ± 0.06	0.75 ± 0.05
P_{lab} (µg g^{-1} dw)	124.5 ± 24.7	119.4 ± 19.1	153.4 ± 25.3	190.2 ± 33.7
N (mg g^{-1} dw)	16.9 ± 2.3	14.7 ± 1.6	17.0 ± 2.1	16.4 ± 1.6
C (mg g^{-1} dw)	358.1 ± 32.3	315.7 ± 26.6	368.5 ± 44.2	331.3 ± 33.1
C:N	21.4 ± 0.9	21.6 ± 0.7	21.7 ± 0.9	20.2 ± 0.3
N:P	27.2 ± 1.2	25.8 ± 0.7	23.5 ± 1.1	21.8 ± 0.8
pH	4.38 ± 0.10	4.39 ± 0.08	4.34 ± 0.15	4.55 ± 0.03
NH_4^+ (µmol g^{-1} dw)	278.1 ± 265.4	539.2 ± 122.3	23.5 ± 16.8	77.5 ± 66.7
NO_3^- (µmol g^{-1} dw)	42.5 ± 18.1	11.0 ± 2.4	12.7 ± 2.4	11.9 ± 2.4
Fine roots				
RWC	0.69 ± 0.01	0.72 ± 0.00	0.70 ± 0.02	0.71 ± 0.02
P_{tot} (mg g^{-1} dw)	1.08 ± 0.14	1.15 ± 0.11	1.56 ± 0.12	1.31 ± 0.13
P_{lab} (µg g^{-1} dw)	596.9 ± 167.7	646.4 ± 92.5	953.8 ± 136.5	764.4 ± 224.4
N (mg g^{-1} dw)	19.2 ± 2.7	20.3 ± 1.3	19.8 ± 1.7	20.9 ± 2.9
C (mg g^{-1} dw)	491.2 ± 3.4	498.9 ± 3.8	495.1 ± 1.6	489.3 ± 7.1
C:N	26.6 ± 3.8	24.8 ± 1.7	25.4 ± 2.2	24.5 ± 4.0
N:P	17.7 ± 0.8	17.8 ± 0.9	12.7 ± 1.1	15.8 ± 0.9
Mineral topsoil				
Bulk soil				
RWC	0.18 ± 0.02	0.17 ± 0.01	0.21 ± 0.01	0.18 ± 0.01
P_{tot} (mg g^{-1} dw)	0.10 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.01
P_{lab} (µg g^{-1} dw)	23.5 ± 3.8	27.0 ± 5.3	38.0 ± 3.3	32.3 ± 4.7
N (mg g^{-1} dw)	1.19 ± 0.14	1.40 ± 0.10	1.80 ± 0.27	1.38 ± 0.18
C (mg g^{-1} dw)	26.2 ± 2.8	32.8 ± 2.2	41.8 ± 5.0	30.3 ± 3.0
C:N	22.1 ± 0.4	23.5 ± 1.0	23.5 ± 0.9	22.1 ± 0.7
N:P	12.2 ± 0.7	15.5 ± 3.1	17.0 ± 0.7	14.2 ± 2.3
pH	4.25 ± 0.06	4.41 ± 0.13	4.16 ± 0.05	4.35 ± 0.14
NH_4^+ (µmol g^{-1} dw)	126.7 ± 111.8	15.4 ± 19.5	1.8 ± 1.1	27.3 ± 25.0
NO_3^- (µmol g^{-1} dw)	4.73 ± 0.62	7.20 ± 1.91	22.7 ± 16.6	5.08 ± 0.25
Fine roots				
RWC	0.42 ± 0.01	0.43 ± 0.02	0.42 ± 0.01	0.42 ± 0.02
P_{tot} (mg g^{-1} dw)	0.55 ± 0.04	0.55 ± 0.07	0.73 ± 0.04	0.89 ± 0.10
P_{lab} (µg g^{-1} dw)	202.1 ± 8.9	218.5 ± 35.1	363.2 ± 19.8	310.1 ± 48.7
N (mg g^{-1} dw)	12.4 ± 1.6	11.9 ± 1.2	12.0 ± 1.4	11.6 ± 1.0
C (mg g^{-1} dw)	467.1 ± 20.8	466.5 ± 15.5	487.6 ± 12.0	459.9 ± 31.5
C:N	38.7 ± 4.0	39.8 ± 3.1	41.7 ± 4.4	39.6 ± 0.8
N:P	22.4 ± 1.6	21.9 ± 0.6	16.4 ± 1.2	13.3 ± 1.8

Fall organic layer	Con	N	P	P+N
Bulk soil				
RWC	0.25 ± 0.02	0.20 ± 0.06	0.27 ± 0.05	0.27 ± 0.01
P_{tot} (mg g^{-1} dw)	0.42 ± 0.03	0.40 ± 0.08	0.43 ± 0.13	0.49 ± 0.03
P_{lab} (µg g^{-1} dw)	47.2 ± 5.5	55.4 ± 10.4	67.9 ± 16.0	63.5 ± 8.0
N (mg g^{-1} dw)	11.8 ± 1.4	11.2 ± 3.1	10.9 ± 1.1	15.4 ± 1.6
C (mg g^{-1} dw)	242.5 ± 31.9	239.2 ± 61.9	228.6 ± 19.4	321.4 ± 37.4
C:N	20.4 ± 0.5	21.6 ± 0.8	21.1 ± 0.3	20.8 ± 0.8
N:P	28.3 ± 1.4	27.3 ± 2.4	29.1 ± 7.7	31.4 ± 3.3
pH	3.90 ± 0.05	3.98 ± 0.11	3.82 ± 0.11	3.99 ± 0.05
NH_4^+ (µmol g^{-1} dw)	53.8 ± 47.4	467.7 ± 212.7	75.9 ± 67.9	261.5 ± 216.0
NO_3^- (µmol g^{-1} dw)	15.6 ± 3.8	63.8 ± 50.7	41.1 ± 28.1	92.8 ± 41.9
Fine roots				
RWC	0.60 ± 0.02	0.54 ± 0.00	0.56 ± 0.01	0.57 ± 0.03
P_{tot} (mg g^{-1} dw)	0.99 ± 0.32	0.66 ± 0.04	0.81 ± 0.11	0.91 ± 0.13
P_{lab} (µg g^{-1} dw)	192.6 ± 10.9	198.5 ± 24.6	229.4 ± 34.3	214.6 ± 5.5
N (mg g^{-1} dw)	15.0 ± 0.2	14.1 ± 2.9	14.1 ± 1.9	15.3 ± 0.1
C (mg g^{-1} dw)	508.2 ± 0.9	504.7 ± 7.1	515.0 ± 8.3	511.0 ± 4.5
C:N	34.0 ± 0.5	38.6 ± 7.3	38.0 ± 5.6	33.5 ± 0.5
N:P	18.2 ± 4.7	21.2 ± 3.6	17.6 ± 1.4	17.5 ± 2.3
Mineral topsoil				
Bulk soil				
RWC	0.04 ± 0.00	0.04 ± 0.01	0.07 ± 0.02	0.05 ± 0.01
P_{tot} (mg g^{-1} dw)	0.08 ± 0.01	0.08 ± 0.02	0.12 ± 0.02	0.09 ± 0.02
P_{lab} (µg g^{-1} dw)	14.6 ± 2.0	14.5 ± 4.0	27.1 ± 3.1	26.0 ± 11.3
N (mg g^{-1} dw)	0.94 ± 0.13	1.07 ± 0.48	2.00 ± 0.84	1.46 ± 0.67
C (mg g^{-1} dw)	26.2 ± 2.7	30.3 ± 11.3	51.1 ± 17.7	38.5 ± 15.5
C:N	24.6 ± 0.7	27.1 ± 0.6	29.4 ± 0.5	33.6 ± 1.8
N:P	12.8 ± 3.1	12.8 ± 4.9	15.9 ± 4.3	16.2 ± 7.5
pH	4.26 ± 0.06	4.24 ± 0.08	4.10 ± 0.07	4.20 ± 0.04
NH_4^+ (µmol g^{-1} dw)	51.9 ± 20.1	94.6 ± 26.5	21.8 ± 6.5	62.1 ± 40.1
NO_3^- (µmol g^{-1} dw)	2.52 ± 0.72	3.64 ± 1.96	3.15 ± 0.37	3.58 ± 1.04
Fine roots				
RWC	0.51 ± 0.02	0.53 ± 0.01	0.53 ± 0.02	0.57 ± 0.01
P_{tot} (mg g^{-1} dw)	0.55 ± 0.03	0.59 ± 0.08	0.53 ± 0.04	0.72 ± 0.11
P_{lab} (µg g^{-1} dw)	114.0 ± 17.2	129.6 ± 35.9	171.4 ± 18.0	235.7 ± 69.9
N (mg g^{-1} dw)	11.0 ± 0.3	12.8 ± 0.9	10.4 ± 0.3	13.8 ± 1.5
C (mg g^{-1} dw)	487.2 ± 3.9	475.9 ± 7.8	481.4 ± 11.0	467.8 ± 7.6
C:N	44.3 ± 1.4	37.7 ± 3.0	46.3 ± 2.1	34.6 ± 3.1
N:P	19.9 ± 0.9	20.9 ± 1.2	19.9 ± 1.2	19.5 ± 1.8

Supplement Table S5: Mortality (%) of beech fine roots (*Fagus sylvatica* L.) in different forests, soil layers and in response to fertilization treatments (Con, N, P, P+N).Fine root samples were collected in a P-rich (HP), P-medium (MP) and P-poor (LP) forest in spring and fall 2018. The organic layer (OL) and mineral topsoil (ML) were analyzed separately; data for season were analyzed together. Data indicate means (n = 6 ± SE). Differences of means were tested by a linear mixed model and Tukey HSD posthoc test for the factors layer and treatment with plot number as random effect. We tested the effects of layer and treatment for each site. Bold letters indicate significant differences at $p \leq 0.05$.

Forest	Layer	Con	N	P	P+N	Treatment		Layer	
						F	*p*	F	*p*
HP	OL	30.5 ±3.2	30.6 ±2.0	35.3 ±6.6	42.0 ±10.4	0.7	0.573	0.5	0.478
	ML	32.8 ±6.4	37.7 ±5.5	23.6 ±4.0	21.4 ±7.9	0.9	0.481		
MP	OL	17.4 ±3.8	30.4 ±5.9	19.2 ±1.1	18.4 ±2.2	2.7	0.116	4.7	**0.047**
	ML	35.1 ±7.7	29.0 ±3.9	25.0 ±4.6	23.7 ±3.2	0.9	0.448		
LP	OL	29.3 ±10.0	10.9 ±3.9	21.0 ±4.1	23.5 ±3.4	1.6	0.256	13.7	**0.003**
	ML	52.6 ±28.3	33.9 ±11.4	51.8 ±1.8	49.6 ±9.6	0.3	0.809		

Supplement Table S6: Relative abundance of all fungal orders obtained by Ilumina MiSeq in soil and associated with roots in beech forests (*Fagus sylvatica* L.). Soil and fine root samples of the organic and mineral layer were collected in a P-rich, P-medium and P-poor forest in 2018. All data were pooled the abundance of an order was expressed relative to the total number of sequences.

> 1%		**0.1% - 1%**			
abundance (%)	**Order**	**abundance (%)**	**Order**		
27.34	Agaricales	0.75	Sebacinales		
16.99	Helotiales	0.70	Rhytismatales		
13.60	Russulales	0.69	Tremellales		
7.36	Boletales	0.50	Chaetosphaeriales		
3.91	Mytilinidiales	0.47	Chaetothyriales		
3.36	Atheliales	0.43	Incertae		
3.21	Eurotiales	0.36	Auriculariales		
3.00	Cantharellales	0.28	Archaeorhizomycetales		
2.89	Thelephorales	0.24	Polyporales		
2.87	Hypocreales	0.22	Trichosporonales		
2.40	Mortierellales	0.17	Hymenochaetales		
2.19	Trechisporales	0.16	Capnodiales		
2.07	Pleosporales	0.15	Xylariales		
1.47	Sordariales	0.15	Thelebolales		
1.06	Pezizales	0.13	Agaricomycetes		
		0.11	Venturiales		
< 0.1 %		**< 0.01 %**		**< 0.003 %**	
abundance (%)	**Order**	**abundance (%)**	**Order**	**abundance (%)**	**Order**
0.082	Togniniales	0.0099	Diaporthales	0.0029	Magnaporthales
0.051	Umbelopsidales	0.0096	Atractiellales	0.0027	Verrucariales
0.050	Phacidiales	0.0094	Geastrales	0.0025	Jaapiales
0.049	Leucosporidiales	0.0092	Microascales	0.0024	Lecanoromycetes
0.045	Zoopagales	0.0091	Tubeufiales	0.0022	Myrmecridiales
0.045	Coniochaetales	0.0089	Microbotryomycetes	0.0019	Onygenales
0.037	Mucorales	0.0085	Spizellomycetales	0.0017	Diversisporales
0.036	Phallales	0.0083	Erythrobasidiales	0.0015	Kriegeriales
0.036	Saccharomycetales	0.0081	Rhizophydiales	0.0015	Botryosphaeriales
0.031	Sporidiobolales	0.0070	Filobasidiales	0.0014	Georgefischeriales
0.030	Orbiliales	0.0062	Ustilaginales	0.0013	Cystofilobasidiales
0.029	Glomerales	0.0062	Lecanorales	0.0012	Exobasidiales
0.029	Glomerellales	0.0059	Diaporthales	0.0010	Myriangiales
0.024	Basidiobolales	0.0055	Malasseziales	0.0009	Umbilicariales
0.022	Pyxidiophorales	0.0047	Taphrinales	0.0007	Calosphaeriales
0.018	Dothideales	0.0045	Ophiostomatales	0.0007	Lobulomycetales
0.013	Hysterangiales	0.0042	Dothideomycetes	0.0007	Urocystidales
0.011	Corticiales	0.0037	Geoglossales	0.0005	Agaricostilbales
0.010	Ostropales	0.0035	Acarosporales	0.0004	Phomatosporales
				0.0003	Cystobasidiomycetes
				0.0003	Phaeomoniellales
				0.0003	Dacrymycetales
				0.0003	Gloeophyllales
				0.0001	Erysiphales

CHAPTER 4: TREE SPECIES COMPOSITION AND SOIL PROPERTIES IN PURE AND MIXED BEECH-CONIFER STANDS DRIVE SOIL FUNGAL COMMUNITIES

Likulunga Emmanuel Likulunga, Carmen Alicia Rivera Pérez, Dominik Schneider, Rolf Daniel, Andrea Polle

Published in: Forest Ecology and Management, 2021 (502), 119709

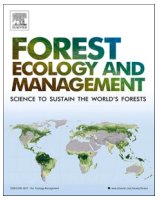
https://doi.org/10.1016/j.foreco.2021.119709

Forest Ecology and Management 502 (2021) 119709

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco



Tree species composition and soil properties in pure and mixed beech-conifer stands drive soil fungal communities

Likulunga Emmanuel Likulunga [a,b,*], Carmen Alicia Rivera Pérez [a], Dominik Schneider [c], Rolf Daniel [c], Andrea Polle [a]

[a] Forest Botany and Tree Physiology, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
[b] Biological Sciences Department, University of Zambia, Great East Road Campus, 32379 Lusaka, Zambia
[c] Genomic and Applied Microbiology and Göttingen Genomics Laboratory, University of Göttingen, 37077 Göttingen, Germany

ARTICLE INFO

Keywords:
Mycobiome
Forest stands
Ectomycorrhiza
Saprotrophic
Soil properties
Fungal ITS

ABSTRACT

Soil fungi, especially the functional guilds of saprotrophs and symbiotrophs, play a central role in ecosystem processes such as decomposition and plant nutrient acquisition. Fungal communities are influenced by soil properties and management strategies such as tree species selection. Yet, the implications of the enrichment of temperate forests consisting of tree species in their native range with non-native tree species on soil fungal diversity and their functional groups are unknown. Here, we studied fungal communities in 40 plots (2500 m^2 size per plot) located in two regions differing in site conditions (nutrient content, soil moisture and climate) in forests composed of European beech, spruce and Douglas-fir (non-native) and mixtures of beech with either spruce or Douglas-fir. We hypothesized that fungal community structures are driven by soil properties and tree species composition, generally resulting in higher fungal diversity in mixed than in mono-specific forests. We further hypothesized that Douglas-fir has a negative effect on ectomycorrhizal fungal species richness compared to native species, whereas saprotrophic fungal richness is unaffected. Fungal DNA barcoding and dissimilarity analyses showed significant separation of fungal communities between nutrient-rich and nutrient-poor regions and among forest types. Mycorrhizal species richness did not vary with forest type, but the relative abundance of mycorrhizal species was lower in Douglas-fir and mixed beech-Douglas-fir forests than in spruce or beech-spruce mixture forests. Conifer forests contained higher relative abundances of saprotrophic fungi than mono-specific beech forests. Among 16 abundant fungal orders in the soil, two containing saprotrophic fungi (Tremellales and Hymenochaetales) were enriched in conifer forests, regardless of site conditions and tree species mixture. The other fungal orders, including those dominated by mycorrhizal fungi (Russulales, Boletales, Atheliales and Cantharellales) showed variable patterns depending on site conditions and tree species. Our results show the impact of tree species composition and soil properties on soil fungi. The response of fungal guilds and taxa to forest types and soil properties can potentially influence nutrient fluxes.

1. Introduction

In temperate and boreal forests, ecosystem functions such as nutrient cycling, decomposition, nutrient acquisition, and protection against plant diseases are mediated by soil microbes (Bardgett and Wardle, 2010). Soil fungi contribute substantially in this regard (Baldrian, 2017; Brundrett and Tedersoo, 2018). Based on carbon acquisition strategies, soil fungi can be divided into saprotrophic, symbiotrophic and pathotrophic fungal categories (Schmit and Mueller, 2007; Nguyen et al., 2016). Saprotrophic fungi are the main decomposers of litter and wood (Rayner and Boddy, 1988), while symbiotrophic fungi form mutualistic associations with roots facilitating plant nutrient uptake in exchange for carbohydrates (Smith and Read, 1997). Since fungi play a fundamental role in ecosystem processes, it is imperative to understand how forest management strategies affect the composition of fungal guilds.

In large parts of Europe, European beech (*Fagus sylvatica* L.) and spruce (*Picea abies* [L.] Karst) are ecologically and economically important tree species (Leuschner et al., 2006; Leuschner and Ellenberg, 2017). Due to climate change and calamities jeopardizing beech and spruce forests (Schlyter et al., 2006; Gessler et al., 2007; Bolte et al.,

* Corresponding author: Forest Botany and Tree Physiology, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany.
E-mail address: llikulu@gwdg.de (L.E. Likulunga).

https://doi.org/10.1016/j.foreco.2021.119709
Received 27 July 2021; Received in revised form 13 September 2021; Accepted 15 September 2021
Available online 24 September 2021

2010), there is the need to introduce tree species that resist these changes and show compatibility with beneficial soil microbes. Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), which is native to North America (Hermann, 1987), is one of the long-term introduced species in Europe (Essl, 2005). Douglas-fir exhibits desirable growth characteristics (Sicard et al., 2006; von Lüpke, 2009; Isaac-Renton et al., 2014) and the ability to thrive well with other broadleaved and coniferous tree species (Rothe and Binkley, 2001). These characteristics make Douglas-fir a favorable species for European forestry. However, little is known about the influence of Douglas-fir on belowground fungal communities. Previous studies (Moeller et al., 2015; Parlade et al., 1995; Dučić et al., 2009) conducted with Douglas-fir growing outside its natural range focused on the ability to form mycorrhizal associations with resident fungal species. Information on the impact of Douglas-fir on soil fungal diversity and composition under varying site conditions and soil properties in temperate European forests is lacking.

In general, the taxonomic and functional composition of soil fungi are driven by abiotic and biotic environmental factors such as soil pH, moisture, C/N ratio, and vegetation composition (Kivlin et al., 2014; Wubet et al., 2012; Bahnmann et al., 2018). The latter has strong influence on litter input, shading, rain interception, transpiration and root exudation (Stoutjesdijk and Barkman, 1992; Augusto et al., 2002). Consequently, traits of forest species influence habitat properties (Bahnmann et al., 2018; Põlme et al., 2018; Urbanová et al., 2015). For example, litter of conifers (spruce and Douglas-fir) contains lower nitrogen (N) and cation contents than that of beech trees (Kubartová et al., 2009). In conifer stands of Douglas-fir or spruce, soil organic carbon (C) and N stocks are higher than in beech stands (Cremer et al., 2016; Cremer and Prietzel, 2017; Dawud et al., 2017). Douglas-fir and beech forest soils exhibit higher exchangeable calcium and magnesium concentrations than soil in spruce or mixed beech-spruce stands (Foltran et al., 2020). Changes in soil properties impact saprotrophic and symbiotrophic fungal guilds (Lindahl et al., 2007). Recent studies (Schröter et al., 2019; Nguyen et al., 2020; Ballauff et al., 2021) suggest that the phylogenetic composition of both guilds is responding to different ecological factors, leading to divergent communities in response to environmental changes. Therefore, we anticipated that the introduction of Douglas-fir into European forests has profound effects on the functional and taxonomic composition of soil fungal communities.

Here, we investigated whether pure Douglas-fir cultivation or in mixture with beech affect soil fungal richness, diversity and community composition in comparison with spruce or beech. We conducted our study in forests composed of either pure stands of beech, spruce, and Douglas-fir or of beech-conifer mixtures (beech-spruce, beech-Douglas-fir) stocking on nutrient-rich silt/clay containing soils as well as on nutrient-poor, sandy soils (Foltran et al., 2020). We used this experimental design to test the following hypotheses: i) There is reduction of soil fungal richness in Douglas-fir compared to beech stands. The reduction is attributed to less adaptability of fungal communities to an introduced than native tree species. ii) Each stand type is characterized by a distinct soil fungal community composition because of distinct effects of tree species and soil properties on fungal assemblages. Therefore, we expect that mixed stands contain highest fungal richness and diversity. Alternatively, we assume that the soil mycobiome is adapted to a wide range of conditions and therefore, no differences in fungal species richness occur. iii) Soils under conifers exhibit higher saprotrophic fungal richness and diversity than those under beech because lower litter quality of conifers favors decomposer communities (Cornelissen et al., 2001; Kubartová et al., 2009). iv) We reasoned that phylogenetically more closely related fungi will show similar responses to changes in habitat conditions than phylogenetically distant fungi because functional traits are conserved among related fungal groups (Treseder and Lennon, 2015). This expectation is based on the results of large-scale sequencing projects, showing higher similarities of functional genes (Kohler et al., 2015; Treseder and Lennon, 2015). Therefore, we hypothesized that the specific impact of Douglas-fir is reflected by shifts in fungal orders compared with beech or spruce and occurs irrespective of abiotic site conditions.

2. Material and methods

2.1. Study sites

The study was conducted in eight study sites (Harz, Dassel, Winnefeld, Nienover, Nienburg, Unterlüss, Göhrde II and Göhrde I) located in Germany. In each of the study sites, five plots with an area of 2500 m^2 were established in the different forest types, which were composed of either European beech (B) (*Fagus sylvatica* L.), spruce (S) (*Picea abies* [L.] Karst), Douglas-fir (D) (*Pseudotsuga menziesii* [Mirb.] Franco), the mixture of European beech with spruce (BS) or the mixture of European beech with Douglas-fir (BD). The stand age varied from 41 to 129 years. All plots were limed with approximately 1.9 t ha^{-1} $CaCO_3$ and 0.8 t ha^{-1} $MgCO_3$ applied in two doses (one at end of the 1980s and in the first decade of the 2000s) and seven plots (Unterlüss: D, BD, B and BS; Göhrde I: D, BD and S) were fertilized with P_2O_5 (0.24 t ha^{-1} approximately 30 years ago). The stand characteristics and climatic conditions are available in PANGEA (Ammer et al., 2020). Further site descriptions are provided in Table 1.

Each plot was divided into quarters (North East, South East, South West and North West). The North West quarter of the plot was not used for destructive sampling. Five soil cores (8 cm diameter × 10 cm depth) were collected in each of the three quarters in late fall (November/December 2017), after removal of coarse litter in non-decomposing state (L layer). The five soil cores per quarter were pooled to yield one sample. Thereby, we collected three replicates per plot in each study site. Soil samples were immediately transported in cooling boxes with ice to the laboratory. The soil was sieved (4 mm mesh size) and divided in three aliquots: fresh aliquots frozen at −20 °C, aliquots dried at 40 °C and aliquots dried at 105 °C.

2.2. Soil properties

Relative soil moisture was determined by weighing soil (fresh and dried at 105 °C for 72 h) and calculated as:

$$\text{Moisture}\ (\%) = \frac{\text{Fresh weight - Dry weight}}{\text{Dry weight}} \times 100$$

To determine soil pH, 25 ml of water was added to 10 g of oven-dried soil (40 °C), followed by the addition of a spatula tip of KCl. The suspension was shaken for 2 h. After sedimentation of particles, the pH was measured (WTW pH meter 538, Wissenschaftlich-Technische-Werkstätten, Weilheim, Germany). To calculate means, the pH values were converted to proton (H^+) concentrations, from which means (from three measurements) were calculated and back-transformed to pH.

To measure soil C and N contents, oven-dried soil samples (40 °C) were milled in a ball mill (MN400, Retsch GmbH, Haan, Germany). About 20 mg of milled soil samples were weighed into 4 × 4 × 11 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) on a microbalance (Model: Cubis MSA 2.7S-000-DM, Sartorius, Göttingen, Germany) and analyzed in a CN analyzer (vario MICRO cube CN analyzer, Elementar Analysensysteme, GmbH, Langenselbold Germany). We used acetanilide (10.36 % N, 71.09% C) as the standard.

Nutrient elements were extracted from dry (40 °C), milled soil samples by microwave digestion and determined by inductively coupled plasma–optical emission spectroscopy (ICP-OES) as follows: ultra-pure water (arium® pro, Sartorius Lab Instruments GmbH & Co. KG, Duderstadt, Germany) was added to 2 ml of 65 % HNO_3 up to the final volume of 25 ml followed by addition of a defined weight (about 50 mg) of the milled soil sample. The sample was extracted by digestion in a microwave (Etho.start, Mikrowellen-Labor-Systeme GmbH, Leutkirch im Allgäu, Germany) at increasing temperatures of 90 °C (2:30 min), 150 °C (5 min) and 210 °C (22:30 min). Phosphate-free filter paper (MN

Table 1
Description of the climate (MAT: mean annual temperature; MAP: mean annual precipitation sum), altitude (ALT), soil characteristics and location of the study sites (Ammer et al., 2020; Foltran et al., 2020).

Region	Site	Coordinates	MAT (°C)	MAP (mm)	ALT (m.a.s.l)	Clay (%)	Sand (%)	Silt (%)
South	Harz	51°46'12" N; 10°23'49" E	7.6	1029	511	68	16	16
	Dassel	51°42'33" N; 9°43'14" E	8.6	823	426	21	26	53
	Winnefeld	51°39'52" N; 9°34'19" E	8.9	818	349	23	20	57
	Nienover	51°41'54" N; 9°31'47" E	9.1	870	323	23	20	57
North	Nienburg	52°37'14" N; 9°16'52" E	9.7	733	92	7	80	13
	Unterlüss	52°50'12" N; 10°19'51" E	9.0	747	161	6	79	15
	Göhrde II	53°7'35" N; 10°48'48" E	9.2	682	130	3	73	24
	Göhrde I	53°12'4" N; 10°48'3" E	9.2	673	120	6	79	15

280 1/4, Macherey-Nagel, Düren, Germany) was used to filter the extracts. The filtered extracts were used to measure the elements by ICP-OES (iCAP 7400 Series ICP–OES, Thermo Fisher Scientific, Dreieich, Germany) at the following wavelengths (nm): 589.592 (radial) for Na, 766.490 (radial) for K, 317.933 (axial) for Ca, 285.213 (axial) for Mg, 260.569 (axial) for Mn, 238.204 (axial) for Fe, 308.215 (axial) for Al, 182.034 (axial) for S and 185.942 (axial) for P. The calibration was performed using element concentrations of 1 g l^{-1} (Einzelstandards, Bernd Kraft, Duisburg, Germany) and an internal mixed standard.

2.3. DNA extraction and Illumina sequencing

Frozen soil samples (−20 °C) were milled in liquid nitrogen using a ball mill (MN400, Retsch GmbH). About 250 mg of frozen, milled soil was used for the extraction of DNA with the DNeasy® PowerSoil® Pro kit (Qiagen, Hilden, Germany) and purified with the DNeasy® PowerClean® kit (Qiagen) according to the manufacturer's recommendations. The concentration of extracted DNA was quantified using the NanoDrop 2000 spectrophotometer (PEQLAB Biotechnologie GmbH, Erlangen, Germany). The internal transcribed spacer (ITS) region was amplified by polymerase chain reaction (PCR) using the forward primer ITS3KYO2 (Toju et al., 2012) and reverse primer ITS4 (White et al., 1990). The PCR reactions, purification, pooling of triplicate technical replicates, and quantification of amplicons were conducted as described previously (Clausing et al., 2021). The amplicons were sequenced with MiSeq Reagent Kit v3 (Illumina Inc., San Diego, USA) using the MiSeq platform at the Göttingen Genomics Laboratory (G2L).

2.4. Bioinformatics data processing

We applied the bioinformatic pipeline described by Clausing et al. (2021) to generate amplicon sequence variants, which were clustered at 97% sequence identity resulting in operational taxonomic units (OTUs). We kept only OTUs with fungal classification. The count numbers of fungal OTUs per soil sample ranged from 13,668 to 108,070. The OTUs were assigned to fungal trophic modes (symbiotrophs, saprotrophs and pathotrophs) based on the FUNGuild database (Nguyen et al., 2016). The resulting OTU table was rarefied to 13,668 reads per sample (i.e. minimum number of reads present in one of our 120 samples) using the rrarefy() function in R vegan package v2.5.7 (Oksanen et al., 2020). The saturation curves for each sample, which show the variation among the three replicate samples, are displayed in Supplement Fig. S1. For further analyses, the three replicate samples per plot were pooled to cover the ploťs species richness. Thereby, we obtained an OTU data matrix with 40 samples, available in PANGEA (Likulunga et al., 2021). The raw sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (Leinonen et al., 2011) with Bioproject accession number PRJNA704813.

2.5. Statistical analysis

The statistical analyses were performed with R software version 4.0.2 (R Core Team, 2020). Normal distribution and homogeneity of variances for soil properties were tested using model residuals by conducting the Shapiro Wilk test (n = 4 per region and forest type). If the normality assumption was not met, data were subjected to square root or log transformation. If data sets were not normal-distributed after transformation, non-parametric tests (Kruskal-Wallis test) were used. Under normality assumptions, analysis of variance (ANOVA) was performed followed by a post hoc Tukey HSD test for comparison of means (package: "multcomp"). Differences of the means were considered to be significant at $p \leq 0.05$.

Non-metric multidimensional scaling (NMDS) with Bray Curtis as metric distance was used to visualize the dissimilarities of soil fungal communities. Analysis of similarity (ANOSIM) (package: "vegan") (Oksanen et al., 2020) with 9999 iterations was performed to determine significant differences of soil fungal communities among tree stand types. Pearson's pairwise correlation matrix was used to determine collinearity among variables using the "Hmisc" package (Harrell et al., 2020) and variables with high correlation coefficients ($r > 0.8$) were not used for analysis with exception of N, P and C/N ratio. We used the "envfit function" in the "vegan" package (Oksanen et al., 2020) to correlate the soil properties (moisture, concentration of total phosphorus, total calcium, and nitrogen) and proportion of conifers to soil fungal communities. The "vegan" package was also used to determine fungal richness and Shannon diversity.

We used general linear and linear mixed effect models with site as random factor and applied the post hoc Tukey HSD test to test for differences at $p \leq 0.05$ of species richness and diversity of soil fungal communities and fungal modes (symbiotrophs (SYM) and saprotrophs (SAP) among the tree stands.

The effect size for a distinct stand type relative to beech for SYM fungi was determined using log response ratios (Hedges et al., 1999) as:

$$\text{Effect size} = \log\left\{\frac{\sum \text{OTU abundance (SYM) in tree stand } i}{\sum \text{OTU abundance (SYM) in beech stand}}\right\}$$

where tree stand i refers to a specific stand type (such as Douglas-fir, spruce, mixture of beech with Douglas-fir or mixture of beech with spruce) and "OTU abundance" refers to the number counts. The effect size of saprotrophic fungi was determined accordingly. The effect sizes were analyzed for differences among tree stands using linear mixed effect models with site as random factor and applying the post hoc Tukey HSD test. We used One-sample Wilcoxon signed-rank test (tested against median = 0) to test for differences of effect size from zero in each tree stand.

We used Linear Discriminant Analysis (LDA) analysis to test for differences among sites by soil properties and forest types using the "MASS" R package (Venables and Ripley, 2002). Generalized Adaptive Models for Location, Scale and Shape with a zero-inflated beta family (GAMLSS-BEZI) from the "metamicrobiomeR" package (Ho et al., 2019) were used to test for differences of the relative abundances of a distinct group of fungi between tree stands.

3. Results

3.1. Soil fungal diversity in stand types and sites

We sequenced 120 soil samples and obtained a total number of 4,941 million sequence reads, which clustered into 2,198 OTUs (potential species). Fungal OTU richness showed significant differences among the stand types ($p < 0.001$, $Chi^2 = 85.39$), with Douglas-fir stands exhibiting the highest (609 ± 42) and beech stands the lowest OTU richness (514 ± 26) (Fig. 1a). Mixed stands of beech and conifers had higher species richness (574 ± 23) than pure beech stands and the fungal OTU richness of spruce stands was intermediate compared to beech and Douglas-fir (Fig. 1a).

In order to investigate whether stand type affected fungal richness in distinct trophic groups, we analyzed OTU richness of symbiotrophs and saprotrophs. Symbiotrophic fungi, a group which was formed here mainly by ectomycorrhizal fungi (83%, read abundance table: Likulunga et al., 2021), did not show any differences in species richness (74 ± 3) across the five stand types studied here (Fig. 1b). However, in contrast to species richness, the relative abundances (based on sequence reads) of the symbiotrophic fungi varied among forest types and were lowest in the Douglas-fir and beech-Douglas-fir stands, intermediate in beech and beech-spruce mixture and highest in spruce stands (Fig. 1c).

The richness of saprotrophic fungi varied significantly among the stand types, with pure Douglas-fir stands having the highest (166 ± 15) and beech stands the lowest OTU richness of saprotrophic fungi (137 ± 9) (Fig. 1d). The relative sequence abundance of the saprotrophic fungi was lowest in beech and highest in Douglas-fir stands (Fig. 1e). Overall, the relative sequence abundance of the saprotrophic fungi was much lower than that of the symbiotrophic fungi ($p < 0.001$, $Chi^2 = 26.14$) (Fig. 1c, e; Supplement Fig. S2).

Fungal OTU richness was not only affected by stand type but also by

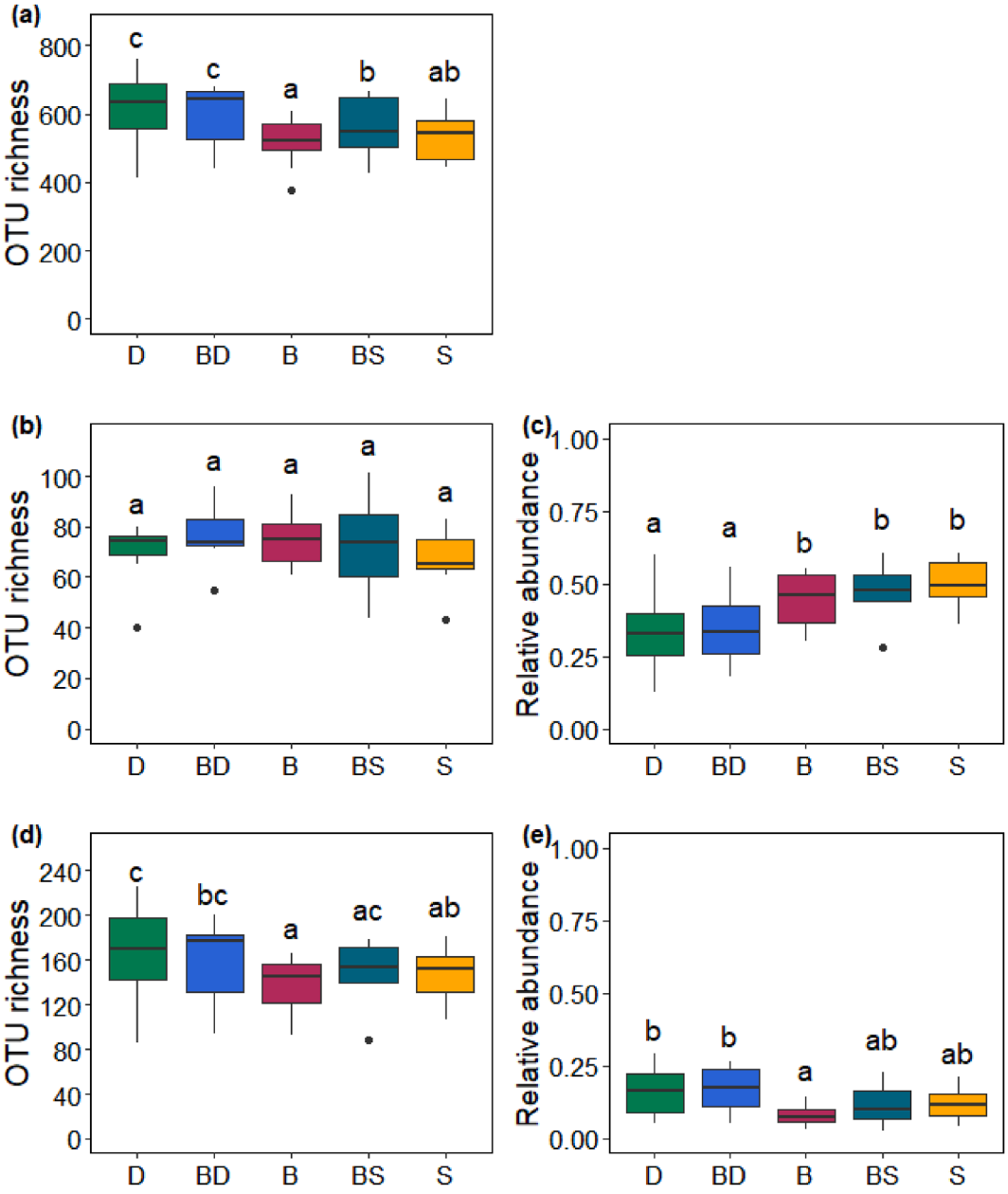


Fig. 1. Soil fungal species richness and relative abundance of all (a), symbiotrophic (b and c) and saprotrophic (d and e) fungi in beech (B), Douglas-fir (D), spruce (S), mixture of beech with Douglas-fir (BD) and mixture of beech with spruce (BS). Lower case letters indicate significant differences at $p \leq 0.05$ ($n = 8$) using linear mixed effect and Kruskal-Wallis test.

site ($p < 0.001$, Deviance = 375.53) and location of the study sites ($p < 0.001$, Deviance = 12.06).

Similarly, Shannon diversity showed differences among the sites, an effect that was due to significantly lower Shannon diversity of symbiotrophic fungi in beech-spruce mixture than in beech or Douglas-fir stands (Table 2). The Shannon diversity of the saprotrophic fungi was unaffected by stand type and site (Table 2).

We also evaluated the effect sizes of the changes in fungal abundances relative to pure beech stands (Fig. 2). A significant, negative effect of Douglas-fir and beech-Douglas-fir stands on symbiotrophic fungal abundances compared to the native spruce and beech-spruce stands ($p < 0.001$, $Chi^2 = 24.40$) was recorded, (Fig. 2a). In spruce or beech-spruce stands, no significant effects were observed (Fig. 2a). The effect sizes of saprotrophic fungi in conifer or mixed beech-conifer stands did not differ significantly among each other ($p = 0.145$, $Chi^2 = 5.40$) (Fig. 2b). However, we observed significantly higher effect sizes of saprotrophic fungi in Douglas-fir ($p = 0.030$), spruce ($p = 0.042$) and beech-Douglas-fir ($p = 0.014$) stands compared with pure beech, which resulted in positive effects (Fig. 2b).

3.2. Soil properties in stand types and sites

Soil properties (relative soil moisture, pH, C, N, C/N ratio, Ca, Mg, K, Na, Mn, Fe, S, P and Al) varied significantly among the different stand types and sites (Table 3). We tested whether these properties could be used to discriminate between stand types and sites by using Linear Discriminant Analysis (LDA). The LDA of all measured soil properties did not reveal a clear differentiation among different forest types (Fig. 3a, confusion matrix in Supplement Table S1). The separation of sites was more distinct (Fig. 3b) but the confusion matrix showed that only four out of eight sites were correctly assigned (Supplement Table S1). The best separation was obtained when the sites were grouped according to location in the southern (Harz, Dassel, Winnefeld, Nienover) and northern region (Nienburg, Unterlüss, Göhrde I, Göhrde II, Fig. 3c) of our study area. This separation also reflected different soil types with clay-silt soils in the south and sandy soils in the north (Foltran et al., 2020).

3.3. Stand type and soil properties shape soil fungal communities

To determine whether the composition of soil fungi varied with stand type and soil properties, we visualized the dissimilarities of fungal assemblages with an NMDS (Fig. 4 a). The first ordination axis separated the fungal communities according northern and southern locations (ANOSIM for location: $R = 0.346$, $p < 0.0001$). The separation along the first axis was accompanied by changes in soil chemistry (pH, N, C/N ratio, P, Ca and soil moisture, Fig. 4b, Supplement Table S2).

The second axis separated the fungal community composition according to stand type (Fig. 4a, ANOSIM for the stand types: $R = 0.291$ and $p < 0.0001$) and was related to the proportion of conifers (Fig. 4b, Supplement Table S2). Soil fungal communities in beech stands clustered separately from those in mixed and conifers stands (Fig. 4a). The fungal community compositions of spruce and Douglas-fir stands showed strong overlap while those in mixed beech-conifer stands were in-between pure stands of spruce, Douglas-fir and beech (Fig. 4a).

3.4. Tree stand type and site show shifts in fungal orders

Since we found that the separation of the fungal species (OTUs) was mainly driven by stand type and location (north and south), the question arose whether this pattern was caused by loss or appearance of distinct phylogenetic groups of the fungi. To address this question, OTUs were grouped at the level of fungal orders. We used all fungal orders ($n = 16$) with relative abundances above 1% of the sequences for our analysis (Supplement Table S3, Supplement Fig. S3). These orders encompassed together more than 80% of the sequences (Supplement Fig. S3, Supplement Table S4).

Russulales, an ectomycorrhizal fungal order (Rinaldi et al., 2008; Tedersoo et al., 2010), was enriched in beech stands in the south but not in the north locations compared to Douglas-fir (Fig. 5a). Therefore, no difference for the Russulales between beech and Douglas-fir forests was observed when the south and north locations were combined (Fig. 5a). None of the other fungal orders showed a significant shift between Douglas-fir and beech in the north or south locations (Fig. 5a). However, the combined data for both locations revealed that Tremellales (mainly mycoparasitic yeasts, Sterkenburg et al., 2015), Hymenochaetales (containing important forest pathogens and wood degrading fungi, Tedersoo et al., 2014) and Helotiales (saprotrophic and ectomycorrhizal fungi, Cannon and Kirk, 2007) increased in Douglas-fir stands, while Boletales (mainly ectomycorrhizal fungi, Cannon and Kirk, 2007) and Agaricales (ectomycorrhizal and saprotrophic fungi, Kirk et al., 2008) were enhanced in beech stands (Fig. 5a).

In the mixed beech-Douglas-fir stands, Tremellales, Hymenochaetales and Helotiales were also enriched compared with beech stands (Fig. 5b). Furthermore, Sebacinales (many ectomycorrhizal and saprotrophic species, Cannon and Kirk, 2007) increased in the mixed beech-Douglas-fir stands, while Hypocreales (saprotrophic and pathogenic species, Sterkenburg et al., 2015) were enriched in beech stands (Fig. 5b).

In spruce and spruce-beech mixtures Tremellales (under most conditions) and Hymenochaetales were enriched compared to beech, suggesting that these orders prefer conifer forests (Fig. 5c,d). In the south locations, Russulales were enriched in beech forests compared to spruce and Atheliales (saprotrophic and mycorrhizal species, Jülich, 1981; Kirk et al., 2008) in spruce and mixed forests compared to beech (Fig. 5c,d). Pezizales (many saprotrophic and ectomycorrhizal species, Cannon and Kirk, 2007) were strongly enriched across all sites in beech forests compared with spruce-beech mixtures (Fig. 5d). Overall, a higher number of fungal orders showed significant variation in spruce or beech-spruce mixture compared with beech than Douglas-fir and its mixture (Fig. 5).

We also compared the changes in fungal relative abundances between spruce and Douglas-fir stands and between spruce-beech and Douglas-fir beech stands (Supplements Fig. S4a,b). The spruce-Douglas-fir comparison showed a number of significantly affected fungal orders similar to that found in the beech comparisons (i.e. 5 or 6 out of 16 orders) (Supplement Fig. S4a). The comparison of the mixtures showed the highest number of significant variations (11 out 16) in our study (Supplement Fig. 4b).

Table 2
Shannon diversity of soil fungi, symbiotrophic (SYM) and saprotrophic (SAP) functional guilds in stand types (Douglas-fir: D; beech: B; spruce: S; mixture of beech with Douglas-fir: BD; mixture of beech with spruce: BS), study sites and locations of the study sites. Data are indicated as means (±SE) analyzed by linear models and post hoc Tukey HSD test. Different letters in rows indicate significant differences at $p \leq 0.05$.

	Mean values					Statistical information		
Group	D	BD	B	BS	S	Stand type p (n = 5)	Site p (n = 8)	Location p (n = 20)
All fungi	3.89 (0.07) a	4.07 (0.14) a	3.96 (0.10) a	3.94 (0.20) a	3.97 (0.10) a	0.081	**<0.001**	**0.014**
SYM	2.68 (0.09) b	2.51 (0.12) b	2.54 (0.13) b	2.04 (0.23) a	2.43 (0.09) ab	**<0.001**	**0.001**	**0.05**
SAP	3.22 (0.13) a	3.02 (0.33) a	3.24 (0.18) a	3.56 (0.22) a	3.49(0.12) a	0.848	0.175	0.132

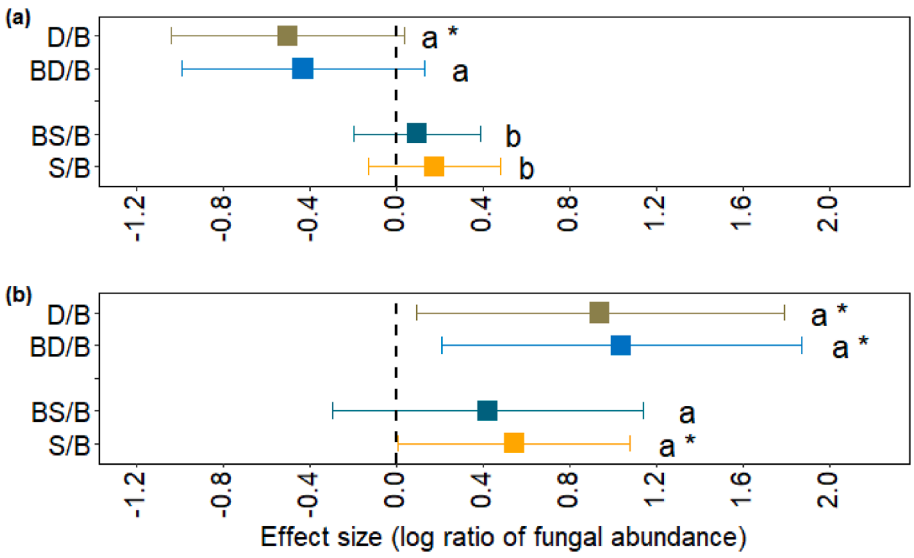


Fig. 2. Effect size for symbiotrophic (a) and saprotrophic (b) fungal abundance in conifer and mixed tree stands relative to pure beech stands (D/B: Douglas-fir and beech; BD/B: Mixture of beech with Douglas-fir and beech; BS/B: Mixture of beech with spruce and beech; S/B: spruce with beech). Lower case letters indicate significant differences at $p \leq 0.05$ (ANOVA for linear mixed effect model with site as random factor, n = 8). Significant differences ($p < 0.05$) of effect sizes from zero are indicated by * (One-sample Wilcoxon signed-rank test).

Table 3
Soil properties in forest types (Douglas-fir: D; beech: B; spruce: S; mixture of beech with Douglas-fir: BD; mixture of beech with spruce: BS), study sites and locations of the study sites. Data are indicated as means (±SE) analyzed by linear models and post hoc Tukey HSD test. Different letters in rows indicate significant differences at $p \leq 0.05$.

	Mean values in stand types					Statistical information		
Variable	D	BD	B	BS	S	Stand type p (n = 5)	Site p (n = 8)	Location p (n = 20)
pH	3.47 (0.09) b	3.41 (0.08) b	3.40 (0.06) b	3.18 (0.05) a	3.30 (0.08) ab	**0.001**	**<0.001**	**<0.001**
MC	38.27 (2.56) a	45.26 (3.30) ab	42.07 (2.74) a	54.71 (4.40) c	50.65 (2.69) bc	**<0.001**	**<0.001**	**<0.001**
C/N	23.89 (0.62) b	22.01 (1.00) a	20.53 (0.52) a	23.43 (0.68) b	23.54 (0.76) b	**<0.001**	**<0.001**	**<0.001**
C	63.08 (6.99) ab	75.05 (9.23) bc	52.14 (4.17) a	81.10 (6.10) c	75.45 (4.39) bc	**<0.001**	**<0.001**	0.981
N	2.63 (0.26) a	3.34 (0.33) ab	2.57 (0.19) a	3.58 (0.31) b	3.25 (0.22) b	**<0.001**	**<0.001**	**<0.001**
Na	0.18 (0.03) ab	0.23 (0.05) b	0.16 (0.02) ab	0.13 (0.01) a	0.20 (0.03) b	**0.008**	**<0.001**	**<0.001**
K	0.77 (0.16) b	0.84 (0.16) b	0.77 (0.17) ab	0.45 (0.07) a	0.64 (0.12 (ab)	**0.001**	**<0.001**	**<0.001**
Ca	0.89 (0.06) ab	1.01 (0.12) b	0.79 (1.10) a	0.76 (0.05) ab	0.91 (0.06) ab	**0.022**	**<0.001**	**<0.001**
Mg	1.28 (0.25) bc	1.48 (0.26) c	1.31 (0.31) ab	0.78 (0.12) a	1.14 (0.18) bc	**<0.001**	**<0.001**	**<0.001**
Mn	0.14 (0.02) a	0.26 (0.05) a	0.20 (0.04) a	0.16 (0.04) a	0.15 (0.03) a	0.071	**<0.001**	**<0.001**
Fe	9.88 (1.74) a	10.02 (1.17) a	9.07 (1.78) a	6.51 (0.68) a	8.54 (1.38) a	0.240	**<0.001**	**<0.001**
S	0.34 (0.03) ab	0.36 (0.04) ab	0.28 (0.02) a	0.35 (0.02) ab	0.39 (0.03) b	**0.004**	**<0.001**	0.734
P	0.27 (0.03) a	0.31 (0.03) ab	0.28 (0.03) ab	0.28 (0.03) a	0.34 (0.04) b	0.010	<0.001	<0.001
Al	15.90 (3.25) b	15.29 (3.01) ab	14.47 (3.26) ab	9.27 (1.23) a	13.44 (2.53) ab	0.023	<0.001	<0.001

4. Discussion

4.1. Forest type and soil properties drive soil fungal composition

Our results show that Douglas-fir, spruce, beech and the mixtures of beech with these conifer species shaped the fungal communities in soil. These results are in agreement with other studies demonstrating that tree species or community effects influence the structure of soil fungal assemblages (Nacke et al., 2016; Bahnmann et al., 2018; Prada-Salcedo et al., 2020). Our results further support that main environmental drivers of fungal community composition are soil C/N, pH and soil moisture. These results show that the overall site effects found in our study concur with those reported previously (Clausing et al., 2021; Nguyen et al., 2020; Větrovský et al., 2019) and explain the separation of the fungal communities between the northern and the southern region, which are characterized by strong differences in soil properties, soil resources and climate (Ammer et al., 2020; Foltran et al., 2020; this study).

A major goal was to understand better the influence of an introduced conifer compared with indigenous tree species on soil fungi. In contrast to our first hypothesis, we did not observe a reduction but an increase in fungal species richness under Douglas-fir. Since the Douglas-fir stands in our study had an age of several decades (Ammer et al., 2020) and Douglas-fir has been introduced to Germany about 150 years ago (Essl, 2005), we speculate that adapted fungal species might have evolved the ability to form novel associations. Another explanation is that ecologically compatible fungal species occur in Douglas-fir biomes because of the wide geographic range of fungal species (Tedersoo et al., 2014). Our results support the latter assumption as the fungal community structures in Douglas-fir and spruce forest soils showed a strong overlap. Although we have no evidence for the appearance of new species, we cannot exclude that evolutionary processes are taking place. However, we would expect that new strains would evolve, which are initially similar to the existing ones. Such subtle changes cannot be traced with our marker gene-based approach.

In our study, we report a clear separation of fungal composition between beech and conifers while beech-mixed stands exhibited intermediate soil fungal assemblages. On our study sites, Lu and Scheu (2021) also found separation of microbial community structures between pure beech and conifers (Douglas-fir and spruce) by employing marker lipids. Moreover, they reported intermediate community structures in mixtures of beech with spruce or Douglas-fir (Lu and Scheu, 2021). Previous studies (Nacke et al., 2016; Bahnmann et al., 2018; Asplund et al., 2019) also found differences in soil fungal communities between beech and spruce forests. The shifts observed in soil fungal communities among different forests types have been attributed to

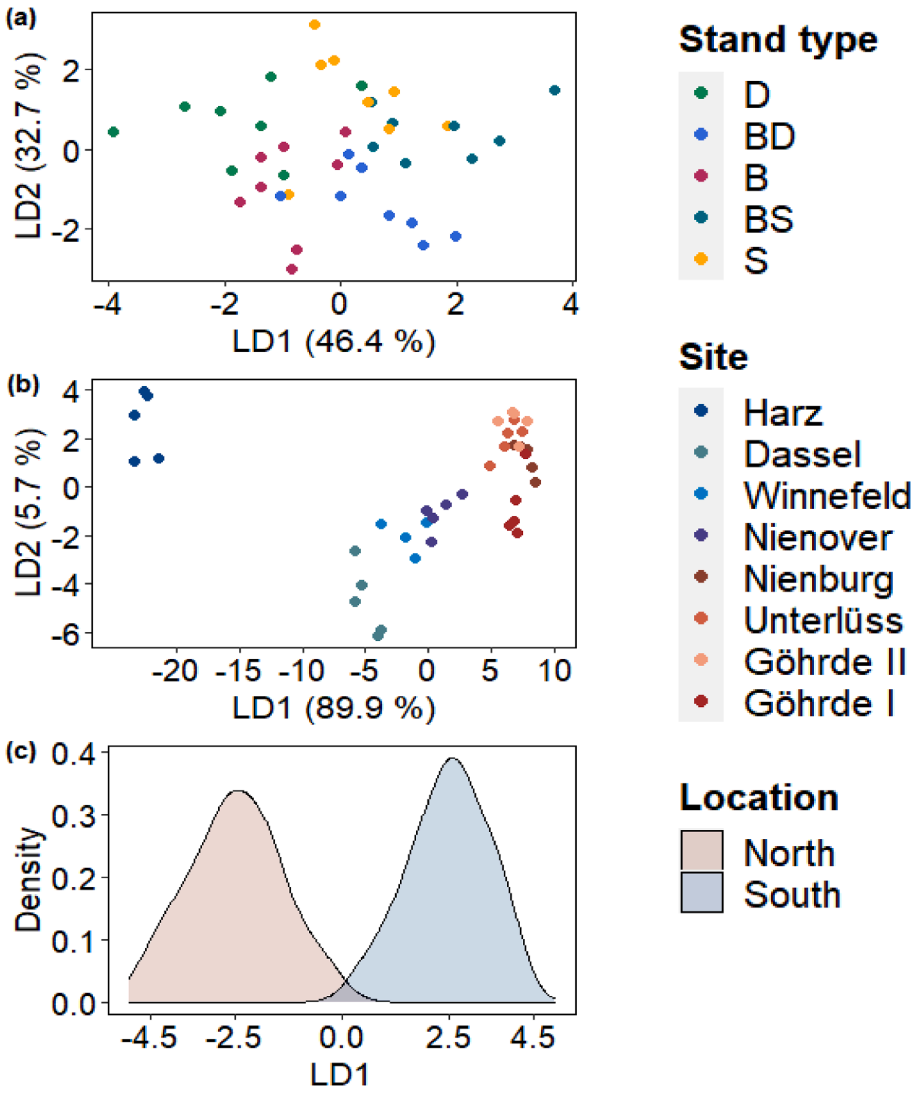


Fig. 3. Linear discriminant analysis (LDA) according to stand types (a), study sites (b) and location of the study sites (c). The analysis was conducted on measured soil properties (relative soil moisture, pH, C, N, C/N ratio, Ca, Mg, K, Na, Mn, Fe, S, P and Al). The corresponding confusion matrix is provided as Supplement Table S3.

indirect effects of dominant tree species through modification of soil resources (Augusto et al., 2002; Augusto et al., 2015). Coniferous ecosystems show lower foliage nutrients (e.g. Ca, K, Mg, P and N) than ecosystems dominated by broadleaf species (Augusto et al., 2002; Augusto et al., 2015), whereby soil nutrients can be influenced through litter production (Stoutjesdijk and Barkman, 1992), consequently leading to tree-driven soil fungal communities (Prescott and Grayston 2013; Urbanová et al., 2015). Such processes are also likely in our study plots and have often been reported for different forest types. For example, Asplund et al. (2019) found that the fungal communities in beech and spruce forests were separated by pH and the C/N ratio. Similarly, Nacke et al. (2016) reported that pH and organic carbon were major drivers for the separation of beech and spruce fungal communities. Further studies demonstrated a strong impact of the litter type on the soil microbiome (Urbanová et al., 2015; Prada-Salcedo et al., 2020; Veen et al., 2021). We found that the separation of fungal microbiomes according to forest type was mainly influenced by the relative abundance of conifers. In general, spruce and Douglas-fir forests contain higher and beech-conifer mixed stands intermediate organic C and N stocks compared to beech stands (Cremer et al., 2016). Therefore, we assume that differences in litter type fostered distinct assemblages observed between beech, conifers and their mixtures.

Bahnmann et al. (2018) showed that soil moisture was a main factor affecting fungal communities at larger scales. Soil moisture was also a significant factor in our study, associated with the separation of the fungal communities between the north and south region. This result was also supported by marker lipid analyses (Lu and Scheu 2021) and highlights the importance of the geographic scale of the studies. Water availability affects plant productivity, resulting in differences in root biomass on our study sites (Lwila et al., 2021). Plant traits such as fine root biomass also influence soil microbes (Prada-Salcedo et al., 2020; Teng et al., 2021; Wambsganss et al., 2021). Therefore, belowground biomass is a further factor, potentially driving differences between the fungal assemblages in the north and south study regions. There is now increasing evidence that root exudates also play profound roles in shaping belowground microbial communities because of differences in quantity and quality among tree species (Brimecombe et al., 2000; Haichar et al., 2014; Kardol and De Long, 2018). Experimental studies on the role of root biomass and root exudates on fungal assemblages are required to clarify the role of Douglas-fir in pure or mixed stands shaping soil fungal communities.

4.2. *Douglas-fir affects the soil fungal functional composition*

We discovered functional differences in the fungal assemblages among distinct forest types with the highest species richness of

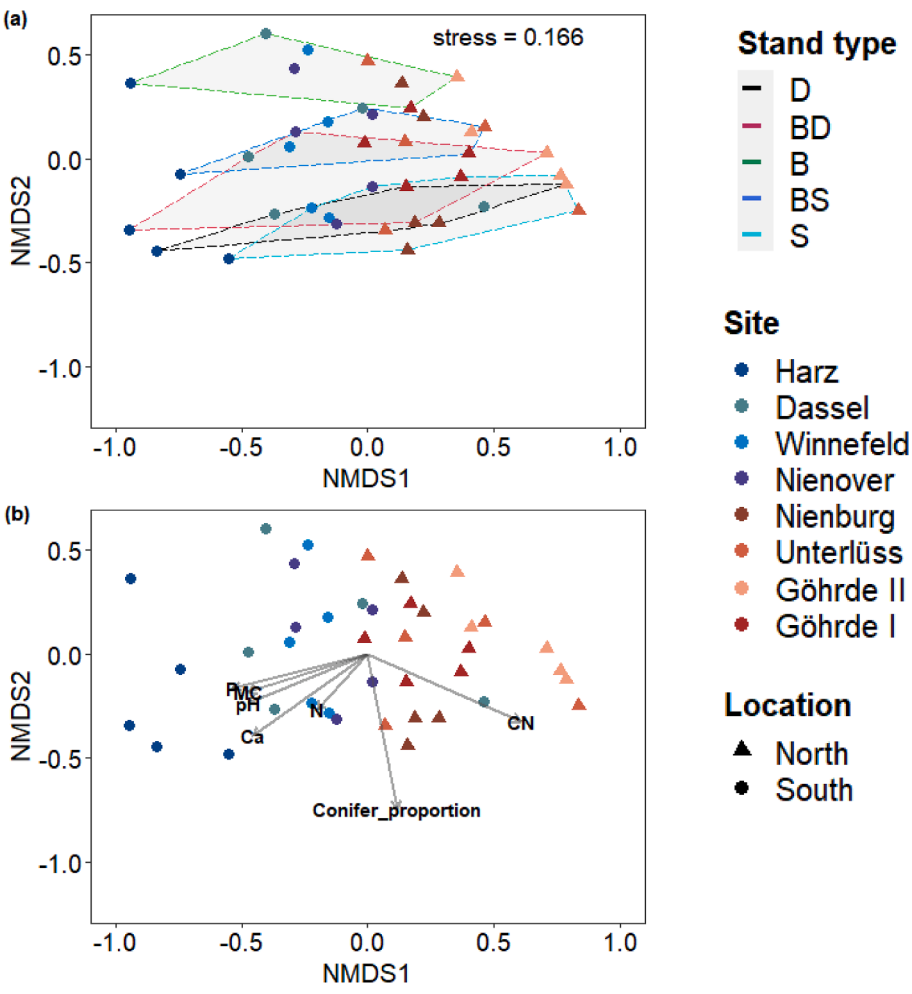


Fig. 4. Non-metric multidimensional scaling (NMDS) of soil fungal OTU communities based on Bray-Curtis dissimilarity measure (a) and main explanatory variables (b). The tree stands in (a) are indicated by hulls: beech (B), spruce (S), Douglas-fir (D), mixture of beech with spruce (BS) and mixture of beech with Douglas-fir (BD). Significant vectors (b) indicate: soil moisture (MC), element concentrations (calcium: Ca; phosphorous: P; nitrogen: N), soil pH, C/N ratio and proportion of conifers (Conifer_proportion). Data points indicate sum of OTUs (n = 3) and means of soil properties (n = 3) per site and forest types).

saprotrophic fungi in Douglas-fir soils. Furthermore, the relative abundance of saprotrophic fungi was higher in Douglas-fir and beech-Douglas-fir mixture than in beech stands. The use of OTU abundance data is critical due to a number of technical issues but comparisons of different methods for fungal community analyses showed consistent response patterns to environmental factors (Xue et al., 2019). In our study, the observed shift in functional guilds might be attributable to litter chemistry, known to shape the identity and composition of saprotrophs (Treseder et al., 2014; Foudyl-Bey et al., 2016; Bahnmann et al., 2018). Kubartová et al. (2009) found that Douglas-fir litter contains high cellulose and low lignin contents, which favor decomposer communities. This may explain the higher proportions of saprotrophs observed in Douglas-fir than beech stands. Further, Awad et al. (2019) found a strong influence of conifers on saprotrophic fungal biomass, also linked to soil resources such as N and C. In spruce forests, the relative abundance of saprotrophic fungi was intermediate between beech and Douglas-fir, while the relative abundance of symbiotrophic fungi was higher in beech and spruce than in Douglas-fir forests. These shifts affected the balance between saprotrophic and symbiotrophic fungi in a complex manner, resulting in positive effect sizes for saprotrophic fungi in the conifer forests but a specific negative effect size for symbiotrophic fungi in Douglas-fir soil. These patterns might reflect inter-guild interactions in which saprotrophs and symbiotrophs compete for soil resources, hence inhibiting each other and slowing down decomposition (Gadgil and Gadgil, 1975; Leake et al., 2002; Fernandez & Kennedy, 2016). Since forest types clearly affected the composition with increases in the fungal decomposer community favored especially by Douglas-fir, we conclude that tree identity effects shape the functional composition of fungal assemblages.

4.3. Forest type and soil properties influence phylogenetically related fungal taxa

We gained insights into the responsiveness of fungal taxa (grouped at the rank of orders) to forest types (relative to beech) and regional factors. Several saprotrophic fungal orders showed variability between north and south locations, indicating susceptibility to microclimate and soil properties. However, two specific fungal orders of Helotiales and Eurotiales were enriched in Douglas-fir and spruce pure stands respectively, which concurs with higher saprotrophic fungal abundances in conifer stands. Furthermore, the orders Hymenochaetales and Tremellales, which are wood degrading fungi and yeasts (Tedersoo et al., 2014; Sterkenburg et al., 2015), were enriched across all site conditions and in conifers stands. This result shows that both spruce and Douglas-fir foster similar saprotrophic fungal groups, thereby contributing to the observed increase of saprotrophic potential. Genome sequencing of members of the Hymenochaetales demonstrated a high number of genes for carbohydrate-active enzymes in the analyzed species (Kohler et al., 2015). Therefore, our results underpin that genomic information can enlighten ecological processes.

In our study, Russulales, which is a ubiquitous fungal group dominated by ectomycorrhiza (Looney et al., 2018), was the most abundant order in our study sites, especially in the beech stands. This result agrees with the widespread occurrence of Russulales in temperate beech stands (Tedersoo et al., 2014; Lang et al., 2011; Pena et al., 2017) and lower abundance in spruce forests (Uroz et al., 2016; Asplund et al., 2019).

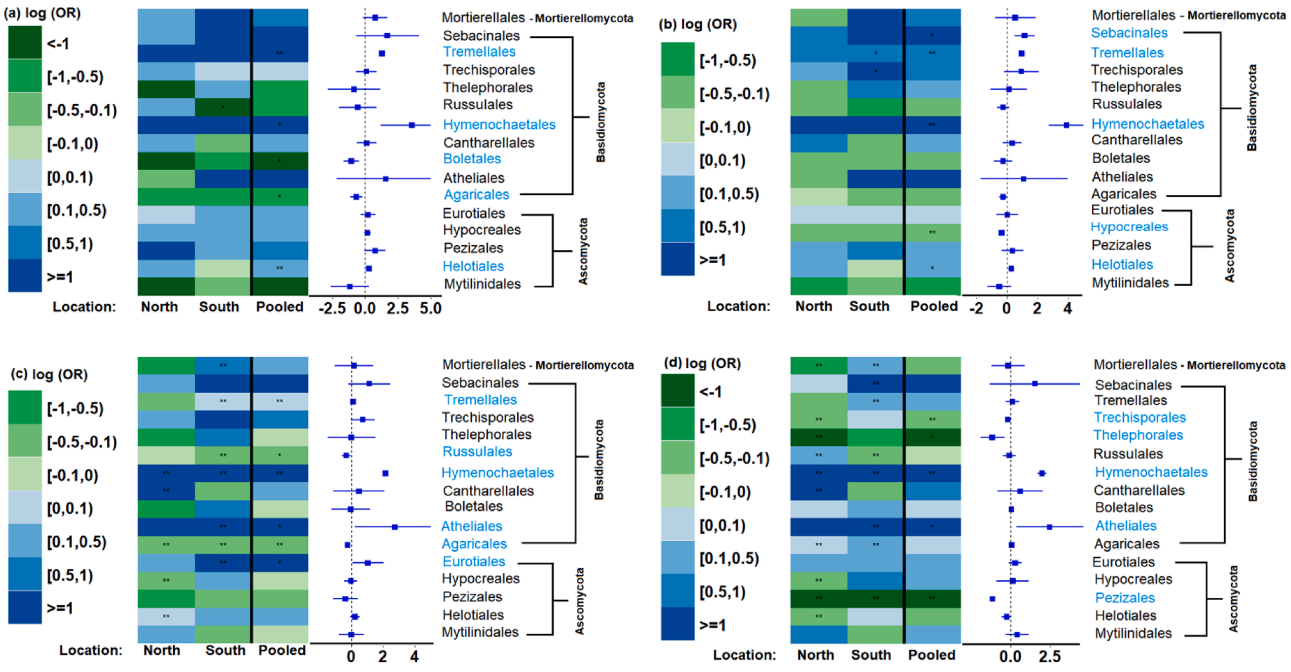


Fig. 5. Changes in the relative abundance of fungal orders in conifer and mixed stands compared with beech:(a) Douglas-fir/ beech, (b) mixture of beech with Douglas-fir/ beech, (c) spruce/beech, (d) mixture of beech with spruce/beech. Data are shown as log ratio of odds (log(OR)). Green cells (negative values) indicate enrichment of a fungal order in beech forests and blue (positive values) indicate enrichment in the conifer forest or mixture. Significant differences at $p \leq 0.05$ (GAMLSS model with BEZI family, n = 4 for the fungal orders in stands in the north and south location, n = 8 for the pooled data) are indicated with stars (* $p < 0.05$, ** $p < 0.01$). Significant orders are highlighted in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

However, the enrichment of Russulales was only observed in the south, where soil N contents were higher than in the north. Russulales appears to be specialized for ammonium acquisition (Nygren et al., 2008). Since they descent from white-rot fungi, Russulales may be able to attack lignin (Looney et al., 2018) but may not be capable of accessing C from cellulose and other C-rich biopolymers (Wolfe et al., 2012). Thus, variation of the Russulales appears to be driven by resource availability. Similarly, Boletales, which contain ectomycorrhizal fungi with oxidative enzymes able to degrade lignin (Op De Beeck et al., 2018), were enriched in beech compared with Douglas-fir but were variable in mixed and spruce forests. Atheliales was the only ectomycorrhizal fungal order that showed a positive effect in a conifer forest.

Overall, the commonalities of Douglas-fir and spruce were confined to saprotrophic taxa, while the influence on other orders was context-dependent and affected saprotrophic and ectomycorrhizal guilds likewise. The importance of substrate quality for fungal populations has been highlighted (Bossuyt et al., 2001; Schröter et al., 2019; Nguyen et al., 2020). The phylogenetic positions of the affected fungal orders and genomic information from reference species suggest that mechanistic understanding of fungal assemblies comes into reach.

5. Conclusion

In agreement with our hypothesis (ii), distinct fungal assemblages were observed in beech and conifer tree stands. We found that the fungal assemblages in spruce stands were similar to Douglas-fir stands, which is an indication that Douglas-fir, a non-native tree species in Europe, is able to integrate native soil fungal networks similar to native spruce. In contrast to our initial hypothesis (i), forest mixtures did not result in enhanced fungal species richness in soil. In addition to forest types, we unraveled the strong impact of soil properties and climatic factors (pH, C/N ratio, Ca, P and soil moisture) on fungal community composition. Douglas-fir and beech-Douglas-fir stands demonstrated a shift in fungal functional guilds, exhibiting low and high proportions of symbiotrophic and saprotrophic fungi, respectively. In concurrence with our hypothesis (iii), we observed higher proportions of saprotrophs in conifer than beech stands. The shifts in taxonomic and functional structures of soil fungi observed in the forest types and different site conditions can potentially affect vital ecosystem processes such as decomposition, C sequestration and nutrient cycling.

CRediT authorship contribution statement

Likulunga Emmanuel Likulunga: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Carmen Alicia Rivera Pérez:** Conceptualization, Methodology, Project administration, Writing – review & editing. **Dominik Schneider:** Data curation, Writing – review & editing. **Rolf Daniel:** Data curation, Writing – review & editing. **Andrea Polle:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank R. Thoms and M. Reichel for assistance with sampling, T. Klein, G. Lehmann, and M. Smiatacz for processing samples, M. Franke-Klein for element analyses, T. Klein for DNA extraction and library preparation.

Funding

This work was made possible through the Research Training Group

2300 (RTG2300: Enrichment of European Beech Forests with Conifers) financially supported by the German Research Foundation (Grant ID: 316045089). LEL was financially supported by the Special Research Fellowship of the University of Zambia (Staff Development Office), Bernhard-Ulrich-Stiftung and as an associated PhD student of RTG2300.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2021.119709.

References

Ammer, C., Annighöfer, P., Balkenhol, N., Hertel, D., Leuschner, C., Polle, A., Lamersdorf, N., Scheu, S., Glatthorn, J., 2020. RTG 2300-study design, location, topography and climatic conditions of research plots in 2020. PANGAEA. https://doi.pangaea.de/10.1594/PANGAEA.923125.

Asplund, J., Kauserud, H., Ohlson, M., Nybakken, L., 2019. Spruce and beech as local determinants of forest fungal community structure in litter, humus and mineral soil. FEMS Microbiol. Ecol. 95 (2), fiy232. https://doi.org/10.1093/femsec/fiy232.

Augusto, L., De Schrijver, A., Vesterdal, L., Smolander, A., Prescott, C., Ranger, J., 2015. Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. Biol. Rev. Cambridge Philos. Soc. 90 (2), 444–466. https://doi.org/10.1111/brv.12119.

Augusto, L., Ranger, J., Binkley, D., Rothe, A., 2002. Impact of several common tree species of European temperate forests on soil fertility. Ann. For. Sci. 59 (3), 233–253. https://doi.org/10.1051/forest:2002020.

Awad, A., Majcherczyk, A., Schall, P., Schröter, K., Schöning, I., Schrumpf, M., Ehbrecht, M., Boch, S., Kahl, T., Bauhus, J., Seidel, D., Ammer, C., Fischer, M., Kües, U., Pena, R., 2019. Ectomycorrhizal and saprotrophic soil fungal biomass are driven by different factors and vary among broadleaf and coniferous temperate forests. Soil Biol. Biochem. 131, 9–18. https://doi.org/10.1016/j.soilbio.2018.12.014.

Bahnmann, B., Mašínová, T., Halvorsen, R., Davey, M.L., Sedlák, P., Tomšovský, M., Baldrian, P., 2018. Effects of oak, beech and spruce on the distribution and community structure of fungi in litter and soils across a temperate forest. Soil Biol. Biochem. 119, 162–173. https://doi.org/10.1016/j.soilbio.2018.01.021.

Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiol. Rev. 41, 109–130. https://doi.org/10.1093/femsre/fuw040.

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

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

Bolte, A., Hilbrig, L., Grundmann, B., Kampf, F., Brunet, J., Roloff, A., 2010. Climate change impacts on stand structure and competitive interactions in a southern Swedish spruce–beech forest. Eur. J. Forest Res. 129 (3), 261–276. https://doi.org/10.1007/s10342-009-0323-1.

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Appl. Soil Ecol. 16 (3), 195–208. https://doi.org/10.1016/S0929-1393(00)00116-5.

Brimecombe, M.J., De Leij, F.A., Lynch, J.M., 2000. The effect of root exudates on rhizosphere microbial populations. Biochemistry and Organic Substances at the Soil-Plant Interface. The Rhizosphere, Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp. 95–140.

Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220 (4), 1108–1115. https://doi.org/10.1111/nph.14976.

Cannon, F.F., Kirk, P.M., 2007. Fungal families of the world. CABI, Wallingford UK. https://doi.org/10.1079/9780851998275.0000.

Clausing, S., Likulunga, E.L., Janz, D., Feng, H., Schneider, D., Daniel, R., Krüger, J., Lang, F., Polle, A., 2021. Impact of nitrogen and phosphorus addition on resident soil and root mycobiomes in beech forests. Biol. Fertil. Soils. https://doi.org/10.1007/s00374-021-01593-x.

Cornelissen, J., Aerts, R., Cerabolini, B., Werger, M., van der Heijden, M., 2001. Carbon cycling traits of plant species are linked with mycorrhizal strategy. Oecologia 129 (4), 611–619. https://doi.org/10.1007/s004420100752.

Cremer, M., Kern, N.V., Prietzel, J., 2016. Soil organic carbon and nitrogen stocks under pure and mixed stands of European beech, Douglas-fir and Norway spruce. For. Ecol. Manage. 367, 30–40. https://doi.org/10.1016/j.foreco.2016.02.020.

Cremer, M., Prietzel, J., 2017. Soil acidity and exchangeable base cation stocks under pure and mixed stands of European beech, Douglas fir and Norway spruce. Plant Soil 415 (1-2), 393–405. https://doi.org/10.1007/s11104-017-3177-1.

Dawud, M.S., Vesterdal, L., Raulund-Rasmussen, K., 2017. Mixed-species effects on soil C and N stocks, C/N ratio and pH using a transboundary approach in adjacent common garden Douglas-fir and beech Stands. Forests 8 (4), 95. https://doi.org/10.3390/f8040095.

Dučić, T., Berthold, D., Langenfeld-Heyser, R., Beese, F., Polle, A., 2009. Mycorrhizal communities in relation to biomass production and nutrient use efficiency in two varieties of Douglas fir (*Pseudotsuga menziesii* var. *menziesii* and var. *glauca*) in different forest soils. Soil Biol. Biochem. 41 (4), 742–753. https://doi.org/10.1016/j.soilbio.2009.01.013.

Essl, F., 2005. Verbreitung, Status und Habitatbindung der subspontanen Bestände der Douglasie (*Pseudotsuga menziesii*) in Österreich. Phyton-Ann. Rei Botanicae 45 (1), 117–144.

Fernandez, C.W., Kennedy, P.G., 2016. Revisiting the 'Gadgil effect': do interguild fungal interactions control carbon cycling in forest soils? New Phytol. 209 (4), 1382–1394. https://doi.org/10.1111/nph.2016.209.issue-410.1111/nph.13648.

Foltran, C.E., Ammer, C., Lamersdorf, N., 2020. Douglas fir and Norway spruce admixtures to European beech along a site gradient in Northern Germany – Are soil nutrient conditions affected? bioRxiv. https://doi.org/10.1101/2020.09.25.313213.

Foudyl-Bey, S., Brais, S., Drouin, P., 2016. Litter heterogeneity modulates fungal activity, C mineralization and N retention in the boreal forest floor. Soil Biol. Biochem. 100, 264–275. https://doi.org/10.1016/j.soilbio.2016.06.017.

Gadgil, P.D., Gadgil, R.L., 1975. Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. N. Z. J. For. Sci. 5, 35–134.

Gessler, A., Keitel, C., Kreuzwieser, J., Matyssek, R., Seiler, W., Rennenberg, H., 2007. Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. Trees 21 (1), 1–11. https://doi.org/10.1007/s00468-006-0107-x.

Haichar, FeZ., Santaella, C., Heulin, T., Achouak, W., 2014. Root exudate mediated interactions belowground. Soil Biol. Biochem. 77, 69–80. https://doi.org/10.1016/j.soilbio.2014.06.017.

Harrell Jr, F.E., Dupont, C., et al., 2020. Hmisc: Harrell Miscellaneous. R package version 4.4-2. https://CRAN.R-project.org/package=Hmisc.

Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80, 1150–1156. https://doi.org/10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2.

Hermann, R.K., 1987. North American tree species in Europe. J. Forest. 85 (12), 27–32.

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

Isaac-Renton, M.G., Roberts, D.R., Hamann, A., Spiecker, H., 2014. Douglas-fir plantations in Europe: a retrospective test of assisted migration to address climate change. Glob. Change Biol. 20 (8), 2607–2617. https://doi.org/10.1111/gcb.2014.20.issue-810.1111/gcb.12604.

Jülich, W., 1981. Higher taxa of Basidiomycetes. Bibliotheca Mycologica 85, 1–485.

Kardol, P., De Long, J.R., 2018. How anthropogenic shifts in plant community composition alter soil food webs. F1000 Res. 7, 4. https://doi.org/10.12688/f1000research.13008.1.

Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A., 2008. Dictionary of the fungi, 10th ed. CABI, Wallingford, p. 22.

Kivlin, S.N., Winston, G.C., Goulden, M.L., Treseder, K.K., 2014. Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. Fungal Ecol. 12, 14–25. https://doi.org/10.1016/j.funeco.2014.04.004.

Kohler, A., Kuo, A., Nagy, L.G., Morin, E., Barry, K.W., Buscot, F., Canbäck, B., Choi, C., Cichocki, N., Clum, A., Colpaert, J., Copeland, A., Costa, M.D., Doré, J., Floudas, D., Gay, G., Girlanda, M., Henrissat, B., Herrmann, S., Hess, J., Högberg, N., Johansson, T., Khouja, H.-R., LaButti, K., Lahrmann, U., Levasseur, A., Lindquist, E. A., Lipzen, A., Marmeisse, R., Martino, E., Murat, C., Ngan, C.Y., Nehls, U., Plett, J. M., Pringle, A., Ohm, R.A., Perotto, S., Peter, M., Riley, R., Rineau, F., Ruytinx, J., Salamov, A., Shah, F., Sun, H., Tarkka, M., Tritt, A., Veneault-Fourrey, C., Zuccaro, A., Tunlid, A., Grigoriev, I.V., Hibbett, D.S., Martin, F., 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat. Genet. 47 (4), 410–415. https://doi.org/10.1038/ng.3223.

Kubartová, A., Ranger, J., Berthelin, J., Beguiristain, T., 2009. Diversity and decomposing ability of saprophytic fungi from temperate forest litter. Microb. Ecol. 58 (1), 98–107. https://doi.org/10.1007/s00248-008-9458-8.

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

Leake, J.R., Donnelly, D.P., Boddy, L., 2002. Interactions between ectomycorrhizal and saprotrophic fungi. In: van der Heijden, M.G.A., Sanders, I.R. (Eds.), Mycorrhizal Ecology, Ecological Studies. Springer, Berlin, Heidelberg, pp. 345–372. https://doi.org/10.1007/978-3-540-38364-2_14.

Leinonen, R., Sugawara, H., Shumway, M., 2011. The Sequence Read Archive. Nucleic Acids Res. 39 (Database), D19–D21. https://doi.org/10.1093/nar/gkq1019.

Leuschner, C., Ellenberg, H., 2017. Ecology of Central European forests. Springer Verlag, Cham Switzerland.

Leuschner, C., Meier, I.C., Hertel, D., 2006. On the niche breadth of *Fagus sylvatica*: soil nutrient status in 50 Central European beech stands on a broad range of bedrock types. Ann. For. Sci. 63, 335–368. https://doi.org/10.1051/forest:2006016.

Likulunga, L.E., Rivera Pérez, C.A., Schneider, D., Daniel, R., Polle, A., 2021. The abundance of soil fungi with associated functional and taxonomic guilds in pure and mixed beech-conifer tree stands. PANGAEA. https://doi.org/10.1594/PANGAEA.933499.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., Finlay, R. D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytol. 173 (3), 611–620. https://doi.org/10.1111/nph.2007.173.issue-310.1111/j.1469-8137.2006.01936.x.

Looney, B.P., Meidl, P., Piatek, M.J., Miettinen, O., Martin, F.M., Matheny, P.B., Labbé, J. L., 2018. Russulaceae: a new genomic dataset to study ecosystem function and evolutionary diversification of ectomycorrhizal fungi with their tree associates. New Phytol. 218 (1), 54–65. https://doi.org/10.1111/nph.2018.218.issue-110.1111/nph.15001.

Lu, J.-Z., Scheu, S., 2021. Response of soil microbial communities to mixed beech-conifer forests varies with site conditions. Soil Biol. Biochem. 155, 108155. https://doi.org/10.1016/j.soilbio.2021.108155.
Lwila, A.S., Mund, M., Ammer, C., Glatthorn, J., 2021. Site conditions more than species identity drive fine root biomass, morphology and spatial distribution in temperate pure and mixed forests. For. Ecol. Manage. 499, 119581. https://doi.org/10.1016/j.foreco.2021.119581.
Moeller, H.V., Dickie, I.A., Peltzer, D.A., Fukami, T., 2015. Mycorrhizal co-invasion and novel interactions depend on neighborhood context. Ecology 96 (9), 2336–2347. https://doi.org/10.1890/14-2361.110.1890/14-2361.1.sm.
Nacke, H., Goldmann, K., Schöning, I., Pfeiffer, B., Kaiser, K., Castillo-Villamizar, G.A., Schrumpf, M., Buscot, F., Daniel, R., Wubet, T., 2016. Fine spatial scale variation of soil microbial communities under European beech and Norway spruce. Front. Microbiol. 7, 2067. https://doi.org/10.3389/fmicb.2016.02067.
Nguyen, D.Q., Schneider, D., Brinkmann, N., Song, B., Janz, D., Schöning, I., Daniel, R., Pena, R., Polle, A., 2020. Soil and root nutrient chemistry structure root-associated fungal assemblages in temperate forests. Environ. Microbiol. 22 (8), 3081–3095. https://doi.org/10.1111/emi.v22.810.1111/1462-2920.15037.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006.
Nygren, C.M.R., Eberhardt, U., Karlsson, M., Parrent, J.L., Lindahl, B.D., Taylor, A.F.S., 2008. Growth on nitrate and occurrence of nitrate reductase-encoding genes in a phylogenetically diverse range of ectomycorrhizal fungi. New Phytol. 180, 875–889. https://doi.org/10.1111/j.1469-8137.2008.02618.x.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Macglinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan.
Op De Beeck, M., Troein, C., Peterson, C., Persson, P., Tunlid, A., 2018. Fenton reaction facilitates organic nitrogen acquisition by an ectomycorrhizal fungus. New Phytol. 218 (1), 335–343. https://doi.org/10.1111/nph.14971.
Parlade, J., Alvarez, I., Pera, J., 1995. Ability of native ectomycorrhizal fungi from northern Spain to colonize Douglas-fir and other introduced conifers. Mycorrhiza 6 (1), 51–55. https://doi.org/10.1007/s005720050105.
Pena, R., Lang, C., Lohaus, G., Boch, S., Schall, P., Schöning, I., Ammer, C., Fischer, M., Polle, A., 2017. Phylogenetic and functional traits of ectomycorrhizal assemblages in top soil from different biogeographic regions and forest types. Mycorrhiza 27 (3), 233–245. https://doi.org/10.1007/s00572-016-0742-z.
Põlme, S., Bahram, M., Jacquemyn, H., Kennedy, P., Kohout, P., Moora, M., Oja, J., Öpik, M., Pecoraro, L., Tedersoo, L., 2018. Host preference and network properties in biotrophic plant–fungal associations. New Phytol. 217 (3), 1230–1239. https://doi.org/10.1111/nph.14895.
Prada-Salcedo, L.D., Goldmann, K., Heintz-Buschart, A., Reitz, T., Wambsganss, J., Bauhus, J., Buscot, F., 2020. Fungal guilds and soil functionality respond to tree community traits rather than to tree diversity in European forests. Mol. Ecol. 30 (2), 572–591. https://doi.org/10.1111/mec.v30.210.1111/mec.15749.
Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. For. Ecol. Manage. 309, 19–27. https://doi.org/10.1016/j.foreco.2013.02.034.
R Core Development Team., 2020. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna.
Rayner, A.D.M., Boddy, L., 1988. Fungal decomposition of wood, its biology and ecology. John Wiley & Sons Ltd, Chichester, UK.
Rinaldi, A.C., Comandini, O., Kuyper, T.W., 2008. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Diversity 33, 1–45.
Rothe, A., Binkley, D., 2001. Nutritional interactions in mixed species forests: A synthesis. Can. J. For. Res. 31 (11), 1855–1870. https://doi.org/10.1139/x01-120.
Schlyter, P., Stjernquist, I., Bärring, L., Jönsson, A.M., Nilsson, C., 2006. Assessment of the impacts of climate change and weather extremes on boreal forests in northern Europe, focusing on Norway spruce. Climate Research 31, 75–84. https://doi.org/10.3354/cr031075.
Schmit, J.P., Mueller, G.M., 2007. An estimate of the lower limit of global fungal diversity. Biodivers. Conserv. 16 (1), 99–111. https://doi.org/10.1007/s10531-006-9129-3.
Schröter, K., Wemheuer, B., Pena, R., Schöning, I., Ehbrecht, M., Schall, P., Ammer, C., Daniel, R., Polle, A., 2019. Assembly processes of trophic guilds in the root mycobiome of temperate forests. Mol. Ecol. 28 (2), 348–364. https://doi.org/10.1111/mec.2019.28.issue-210.1111/mec.14887.
Sicard, C., Saint-Andre, L., Gelhaye, D., Ranger, J., 2006. Effect of initial fertilization on biomass and nutrient content of Norway spruce and Douglas-fir plantations at the same site. Trees 20, 229–246. https://doi.org/10.1007/s00468-005-0030-6.
Smith, S.E., Read, D.J., 1997. Mycorrhizal symbiosis. USA Academic Press Inc., San Diego, CA.
Sterkenburg, E., Bahr, A., Brandström Durling, M., Clemmensen, K.E., Lindahl, B.D., 2015. Changes in fungal communities along a boreal forest soil fertility gradient. New Phytol. 207 (4), 1145–1158. https://doi.org/10.1111/nph.2015.207.issue-410.1111/nph.13426.
Stoutjesdijk, P., Barkman, J.J., 1992. Microclimate, Vegetation and Fauna. Opulus Press, Knivsta, Sweden.
Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T. W., Harend, H., Guo, L.-D., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. Science 346 (6213), 1256688. https://doi.org/10.1126/science:1256688.
Tedersoo, L., May, T.W., Smith, M.E., 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20 (4), 217–263. https://doi.org/10.1007/s00572-009-0274-x.
Teng, J., Tian, J., Barnard, R., Yu, G., Kuzyakov, Y., Zhou, J., 2021. Aboveground and belowground plant traits explain latitudinal patterns in topsoil fungal communities from tropical to cold temperate forests. Front. Microbiol. 12, 1308. https://doi.org/10.3389/fmicb.2021.633751.
Toju, H., Tanabe, A.S., Yamamoto, S., Sato, H., 2012. High-coverage ITS primers for the DNA-based identification of Ascomycetes and Basidiomycetes in environmental samples. PLoS ONE 7, e40863. https://doi.org/10.1371/journal.pone.0040863.
Treseder, K.K., Bent, E., Borneman, J., McGuire, K.L., 2014. Shifts in fungal communities during decomposition of boreal forest litter. Fungal Ecol. 10, 58–69. https://doi.org/10.1016/j.funeco.2013.02.002.
Treseder, K.K., Lennon, J.T., 2015. Fungal traits that drive ecosystem dynamics on land. Microbiol. Mol. Biol. Rev. 79 (2), 243–262. https://doi.org/10.1128/MMBR.00001-15.
Urbanová, M., Šnajdr, J., Baldrian, P., 2015. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. Soil Biol. Biochem. 84, 53–64. https://doi.org/10.1016/j.soilbio.2015.02.011.
Uroz, S., Oger, P., Tisserand, E., Cébron, A., Turpault, M.-P., Buée, M., De Boer, W., Leveau, J.H.J., Frey-Klett, P., 2016. Specific impacts of beech and Norway spruce on the structure and diversity of the rhizosphere and soil microbial communities. Sci. Rep. 6 (1), 27756. https://doi.org/10.1038/srep27756.
Veen, G.F.(., ten Hooven, F.C., Weser, C., Hannula, S.E., Singh, B., 2021. Steering the soil microbiome by repeated litter addition. J. Ecol. 109 (6), 2499–2513. https://doi.org/10.1111/jec.v109.610.1111/1365-2745.13662.
Venables, W.N., Ripley, B.D., 2002. Modern applied statistics with S, fourth ed. Springer, New York.
Větrovský, T., Kohout, P., Kopecký, M., Machac, A., Man, M., Bahnmann, B.D., Bradcová, V., Choi, J., Meszárošová, L., Human, Z.R., Lepinay, C., Lladó, S., López-Mondéjar, R., Martinović, T., Mašínová, T., Morais, D., Navrátilová, D., Odriozola, I., Štursová, M., Švec, K., Tláskal, V., Urbanová, M., Wan, J., Žifčáková, L., Howe, A., Ladau, J., Peay, K.G., Storch, D., Wild, J., Baldrian, P., 2019. A meta-analysis of global fungal distribution reveals climate-driven patterns. Nat. Commun. 10, 5142. https://doi.org/10.1038/s41467-019-13164.
von Lüpke, B., 2009. Überlegungen zur Baumartenwahl und Verjüngungsverfahren bei fortschreitender Klimaänderung in Deutschland. Forstarchiv 80, 67–75.
Wambsganss, J., Freschet, G.T., Beyer, F., Goldmann, K., Prada-Salcedo, L.D., Scherer-Lorenzen, M., Bauhus, J., 2021. Tree species mixing causes a shift in fine-root soil exploitation strategies across European forests. Funct. Ecol. 35 (9), 1886–1902. https://doi.org/10.1111/fec.v35.910.1111/1365-2435.13856.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols. Elsevier, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.
Wolfe, B.E., Tulloss, R.E., Pringle, A., 2012. The irreversible loss of a decomposition pathway marks the single origin of an ectomycorrhizal symbiosis. PLoS ONE 7, e39597. https://doi.org/10.1371/journal.pone.0039597.
Wubet, T., Christ, S., Schöning, I., Boch, S., Gawlich, M., Schnabel, B., Fisher, M., Buscot, F., 2012. Differences in soil fungal communities between European beech (*Fagus sylvatica* L.) dominated forests are related to soil and understory vegetation. PLoS ONE 7 (10). https://doi.org/10.1371/journal.pone.0047500.
Xue, C., Hao, Y., Pu, X., Penton, C.R., Wang, Q., Zhao, M., Zhang, B., Ran, W., Huang, Q., Shen, Q., Tiedje, J.M., 2019. Effect of LSU and ITS genetic markers and reference databases on analyses of fungal communities. Biol. Fertil. Soils 55, 79–88. https://doi.org/10.1007/s00374-019-01408-0.

Supplement (Chapter 4)

TREE SPECIES COMPOSITION AND SOIL PROPERTIES IN PURE AND MIXED BEECH-CONIFER STANDS DRIVE SOIL FUNGAL COMMUNITIES

Likulunga Emmanuel Likulunga, Carmen Alicia Rivera Pérez, Dominik Schneider, Rolf Daniel, Andrea Polle

Published in: Forest Ecology and Management, 2021 (502), 119709

https://doi.org/10.1016/j.foreco.2021.119709

Supplement Table S1. Confusion matrix based on linear discriminant analysis (LDA) of all measured soil properties. The plots with completely correct classification are indicated in bold.

Study sites	**Harz**	**Dassel**	**Winnefeld**	**Nienover**	**Nienburg**	**Unterlüss**	**Göhrde II**	**Göhrde I**
Harz	**5**	0	0	0	0	0	0	0
Dassel	0	**5**	0	0	0	0	0	0
Winnefeld	0	0	4	0	0	0	0	0
Nienover	0	0	1	**5**	0	0	0	0
Nienburg	0	0	0	0	4	0	0	0
Unterlüss	0	0	0	0	1	**5**	1	0
Göhrde II	0	0	0	0	0	0	4	1
Göhrde I	0	0	0	0	0	0	0	4

Stand type	D	BD	B	BS	S
D	5	0	0	0	0
BD	1	6	0	0	0
B	1	2	7	0	1
BS	0	0	0	7	2
S	1	0	1	1	5

Location	**North**	**South**
North	**20**	0
South	0	**20**

Supplement Table S2. Correlation of explanatory variables to all, symbiotrophic and saprotrophic fungi based on envifit function. The explanatory variables soil properties (pH, MC: moisture, Ca: total calcium, P: total phosphorous, N: nitrogen and C/N: C/N ratio) and the proportion of conifers among the tree stands (Conifer_proportion) were included for analysis while variables C, Na, K, Mg, Fe, Mn and Al were excluded due to multicollinearity with exception of N, C/N and P.

Variable	All fungi		SYM		SAP	
	R^2	p	R^2	p	R^2	p
pH	0.3931	**0.002**	0.3789	**0.001**	0.271	**0.003**
MC	0.3606	**0.001**	0.3944	**0.001**	0.4118	**0.001**
C/N	0.6599	**0.001**	0.6524	**0.001**	0.6244	**0.001**
N	0.1568	**0.046**	0.1188	0.099	0.1978	**0.015**
Ca	0.5050	**0.001**	0.469	**0.001**	0.3604	**0.003**
P	0.4419	**0.001**	0.4556	**0.001**	0.5204	**0.001**
Conifer_proportion	0.7936	**0.001**	0.6701	**0.001**	0.7266	**0.001**

Supplement Table S3. Relative abundance (%) of soil fungal orders in tree stands of Douglas-fir (D), beech (B), spruce (S), mixture of beech with Douglas-fir (BD) and mixture of beech with spruce (BS). Relative abundance was determined based on the abundance of all fungal orders in each tree stand. Fungal orders with abundances > 1% of the total counts were included. Data indicate means (n = 3, ± SE). Differences in relative abundance were analyzed with GAMLSS-BEZI model (Fig. 5, Supplement Fig. S4).

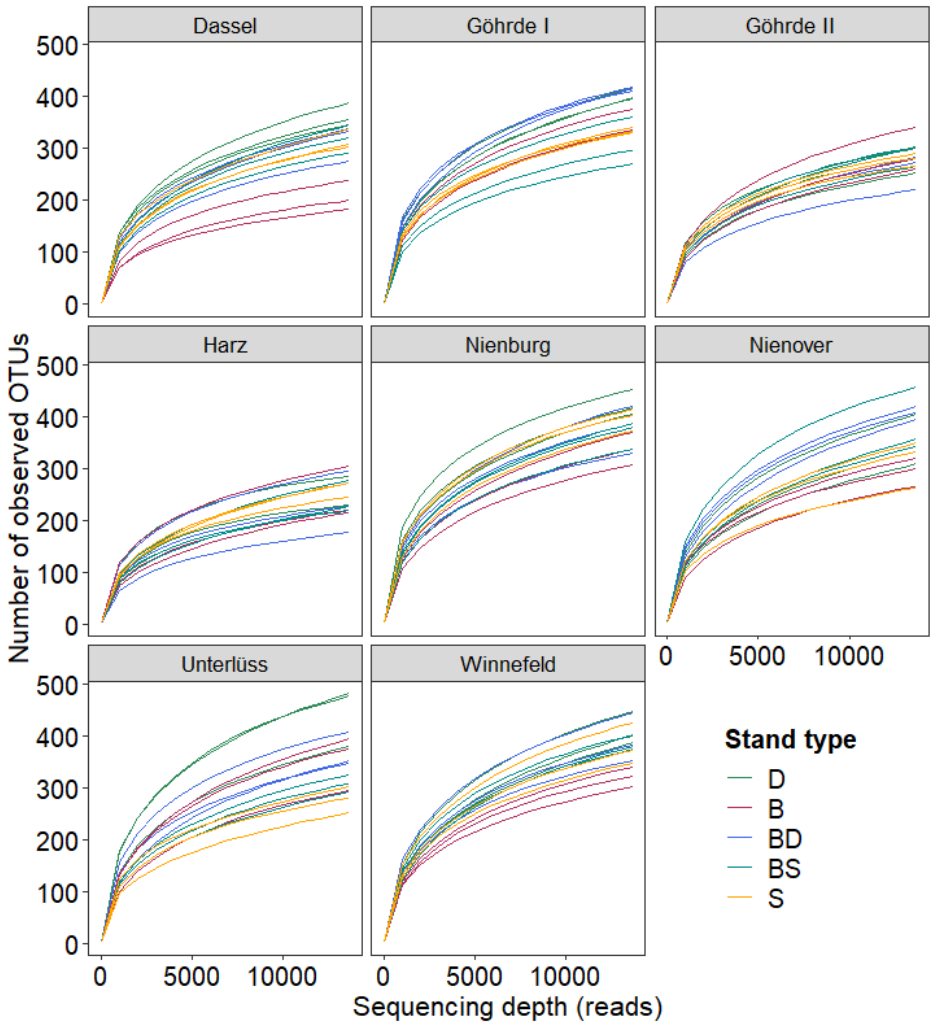
Site	**Harz**										**Dassel**									
Stand type	D	SE	BD	SE	B	SE	BS	SE	S	SE	D	SE	BD	SE	B	SE	BS	SE	S	SE
Eurotiales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.06	0.02	0.01	0.00	0.00	0.00	0.00	0.02	0.01	0.06	0.03
Helotiales	0.26	0.11	0.32	0.22	1.12	0.62	0.70	0.10	0.93	0.21	2.20	0.48	2.06	0.60	1.12	0.27	1.46	0.17	4.38	1.40
Hypocreales	0.00	0.00	0.00	0.00	0.10	0.09	0.04	0.01	0.03	0.01	0.47	0.13	0.11	0.03	0.04	0.02	0.61	0.02	0.75	0.20
Mytilinidales	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.34	0.23	0.00	0.00	0.03	0.03	0.09	0.08	0.03	0.01
Pezizales	0.02	0.00	0.55	0.43	2.46	0.72	0.10	0.06	0.03	0.01	1.09	0.03	0.33	0.15	0.07	0.03	0.37	0.23	0.71	0.23
Agaricales	6.53	1.16	4.65	1.32	9.50	3.25	8.29	1.49	7.23	0.58	3.28	0.28	6.37	1.49	6.61	3.35	5.52	1.15	4.32	0.98
Atheliales	0.12	0.11	0.39	0.39	0.01	0.01	0.39	0.38	0.51	0.30	3.28	1.03	0.29	0.19	0.09	0.06	5.20	1.18	5.10	1.17
Boletales	0.10	0.02	0.07	0.03	0.03	0.03	0.32	0.17	0.23	0.17	0.58	0.16	0.39	0.15	0.80	0.01	0.80	0.23	3.58	1.44
Cantharellales	1.08	0.57	0.42	0.35	0.17	0.11	1.96	1.45	0.13	0.02	0.29	0.10	0.49	0.23	0.44	0.19	0.09	0.02	0.01	0.00
Hymenochaetales	0.06	0.05	7.49	7.48	0.01	0.00	0.11	0.08	0.04	0.02	0.27	0.17	0.03	0.02	0.01	0.01	0.10	0.06	0.13	0.08
Russulales	2.82	1.68	2.49	2.39	6.81	1.42	6.20	1.71	6.34	1.66	0.85	0.52	7.40	2.63	11.73	1.56	5.46	2.28	4.73	1.69
Sebacinales	0.66	0.63	0.32	0.24	0.01	0.01	2.68	1.46	0.17	0.08	1.63	1.41	0.34	0.23	0.02	0.01	0.07	0.07	0.00	0.00
Thelephorales	0.16	0.09	1.21	0.98	1.88	0.30	0.51	0.15	4.64	1.52	3.32	0.90	3.27	1.74	0.21	0.05	0.67	0.32	1.70	1.02
Trechisporales	0.00	0.00	0.00	0.00	0.27	0.18	0.02	0.01	0.10	0.08	0.33	0.05	0.03	0.02	0.02	0.01	0.58	0.49	0.35	0.05
Tremellales	1.21	0.57	1.28	0.72	0.34	0.11	0.75	0.32	0.37	0.13	3.18	0.92	0.91	0.11	0.81	0.23	0.42	0.10	0.56	0.16
Mortierellales	10.65	1.00	8.10	2.63	2.69	0.21	5.19	1.09	6.19	1.54	5.54	0.36	3.32	0.51	0.50	0.13	0.75	0.26	0.81	0.13
	Winnefeld										**Nienover**									
Eurotiales	0.14	0.02	0.08	0.04	0.01	0.00	0.05	0.03	0.04	0.01	0.05	0.01	0.03	0.00	0.10	0.06	0.02	0.00	0.06	0.02
Helotiales	2.42	0.27	2.58	0.15	1.79	0.21	2.67	0.20	2.27	0.44	2.86	0.51	2.33	0.23	2.89	0.75	2.46	0.54	2.67	0.17
Hypocreales	1.19	0.08	0.97	0.23	0.63	0.07	2.05	0.86	0.85	0.08	2.60	1.08	0.68	0.07	0.47	0.03	0.90	0.06	0.90	0.19
Mytilinidales	0.02	0.01	0.25	0.13	0.87	0.40	0.03	0.01	0.30	0.01	0.30	0.19	0.49	0.34	0.42	0.25	0.58	0.28	0.06	0.01
Pezizales	1.38	0.20	2.57	0.38	0.97	0.11	0.17	0.02	0.90	0.19	3.29	1.22	3.26	0.90	0.32	0.10	0.19	0.05	0.70	0.54
Agaricales	6.77	3.38	3.62	0.43	4.77	0.70	8.72	3.51	3.74	1.26	1.01	0.31	4.33	0.33	4.59	0.63	5.37	1.59	5.89	1.96
Atheliales	2.73	0.23	0.69	0.37	0.02	0.01	2.43	0.78	1.83	0.44	5.15	1.35	2.82	2.48	0.24	0.14	2.80	0.21	6.67	1.62
Boletales	0.18	0.03	0.44	0.35	0.99	0.40	0.51	0.15	0.58	0.21	0.10	0.03	0.20	0.09	0.37	0.15	0.77	0.28	0.34	0.03
Cantharellales	0.02	0.01	0.03	0.01	1.11	0.64	0.03	0.01	0.04	0.02	0.19	0.17	0.95	0.78	0.21	0.16	0.54	0.36	3.39	3.38
Hymenochaetales	3.01	2.44	0.03	0.02	0.01	0.00	0.03	0.02	0.08	0.04	4.09	3.64	0.53	0.25	0.02	0.01	0.05	0.03	0.10	0.07
Russulales	5.04	1.53	6.20	2.75	5.17	0.74	5.37	1.24	8.44	3.60	2.44	0.99	5.92	2.38	10.11	4.05	10.04	0.57	4.75	3.12
Sebacinales	0.52	0.23	0.10	0.08	0.08	0.07	0.15	0.07	1.13	0.55	0.36	0.13	0.03	0.01	0.07	0.06	1.04	1.01	0.61	0.31
Thelephorales	0.77	0.12	1.66	1.05	1.02	0.42	0.37	0.20	1.98	1.11	2.35	1.58	2.04	1.71	1.05	0.65	0.26	0.16	0.63	0.22
Trechisporales	0.93	0.20	5.00	2.84	0.40	0.07	0.82	0.28	3.49	0.15	2.89	1.85	1.14	0.60	0.76	0.20	0.20	0.09	1.18	0.49
Tremellales	1.65	0.02	0.85	0.15	0.42	0.13	0.67	0.25	0.48	0.05	0.77	0.22	1.00	0.26	0.33	0.07	0.72	0.16	0.62	0.05
Mortierellales	1.78	0.19	2.72	0.25	0.81	0.10	0.88	0.16	2.74	0.14	0.71	0.17	1.79	0.31	1.16	0.17	1.24	0.32	0.89	0.36

Continuation of Supplement Table S3.

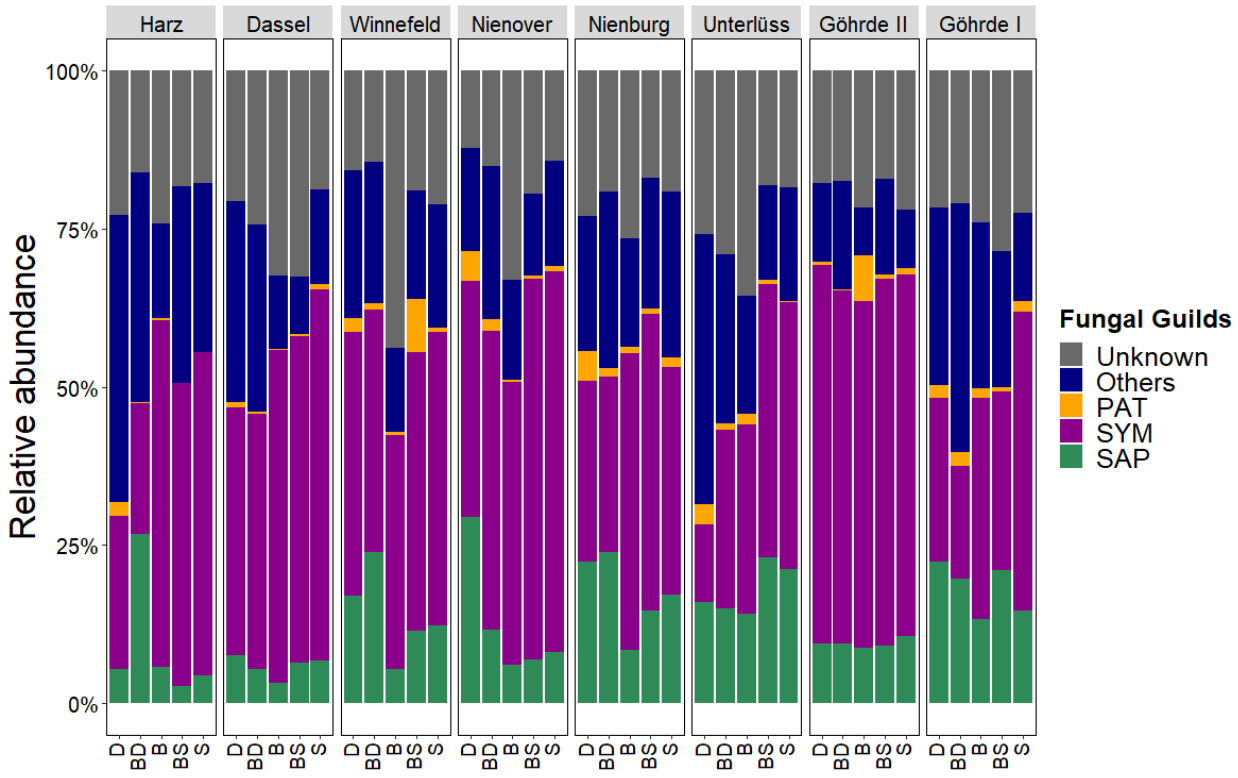
Site	Nienburg										Unterlüss									
Stand type	D	SE	BD	SE	B	SE	BS	SE	S	SE	D	SE	BD	SE	B	SE	BS	SE	S	SE
Eurotiales	1.04	0.25	0.75	0.29	0.06	0.01	0.16	0.05	0.62	0.51	0.65	0.18	1.52	0.81	1.89	1.57	5.06	1.69	2.16	0.74
Helotiales	3.43	0.92	2.16	0.60	2.59	0.20	2.08	0.25	2.32	0.53	3.40	0.43	6.47	2.59	2.44	0.47	2.28	0.36	4.47	0.28
Hypocreales	3.22	0.87	1.29	0.43	0.96	0.17	0.95	0.21	2.78	0.44	2.10	0.23	1.00	0.21	1.33	0.10	1.71	0.19	1.04	0.18
Mytilinidales	0.24	0.10	1.03	0.29	0.63	0.20	2.20	0.92	0.74	0.40	0.15	0.01	0.32	0.19	0.37	0.19	1.75	0.77	2.76	1.00
Pezizales	0.41	0.22	0.20	0.07	0.04	0.01	0.03	0.02	0.46	0.07	2.18	0.42	0.34	0.17	0.19	0.06	0.03	0.03	0.04	0.03
Agaricales	3.09	0.44	4.50	1.41	7.13	0.21	8.15	1.96	6.47	1.99	2.22	0.76	3.20	0.17	5.07	1.68	5.08	0.16	3.81	0.64
Atheliales	0.12	0.12	0.04	0.03	1.21	0.37	2.03	0.66	2.46	0.72	0.54	0.45	1.34	0.93	1.93	1.83	2.46	1.20	2.07	0.54
Boletales	0.19	0.12	1.59	0.66	1.62	0.17	0.83	0.19	0.57	0.18	0.17	0.10	1.36	0.50	0.90	0.27	0.43	0.11	0.42	0.15
Cantharellales	0.65	0.43	2.54	2.52	0.11	0.02	0.04	0.03	0.02	0.01	0.23	0.12	0.03	0.02	0.06	0.03	1.97	1.95	2.67	0.67
Hymenochaetales	0.47	0.24	1.22	1.17	0.03	0.01	0.28	0.27	0.15	0.05	0.05	0.02	0.06	0.03	0.01	0.00	0.18	0.08	0.37	0.31
Russulales	6.62	1.35	3.09	0.40	6.86	0.94	5.97	2.01	2.49	1.50	1.67	1.00	4.29	1.08	3.30	0.29	5.21	0.40	4.30	0.24
Sebacinales	0.10	0.09	1.05	0.82	0.53	0.22	0.44	0.17	0.36	0.20	0.09	0.03	0.15	0.07	0.20	0.12	0.07	0.02	0.20	0.01
Thelephorales	0.72	0.62	1.62	0.32	1.36	0.74	1.47	0.35	1.39	0.33	0.10	0.05	0.73	0.23	0.75	0.46	0.09	0.03	0.43	0.17
Trechisporales	1.38	0.07	0.29	0.08	0.86	0.49	1.58	1.36	1.17	0.66	0.42	0.14	0.94	0.33	0.26	0.10	0.12	0.03	0.10	0.07
Tremellales	1.72	0.15	0.62	0.03	0.60	0.15	0.59	0.11	1.06	0.47	1.26	0.21	1.05	0.33	0.28	0.07	0.28	0.06	0.03	0.01
Mortierellales	1.04	0.28	1.43	0.07	1.07	0.20	0.58	0.17	3.55	0.73	7.45	0.87	1.41	0.49	2.31	0.56	0.73	0.24	0.61	0.27
	Göhrde II										**Göhrde I**									
Eurotiales	0.74	0.19	0.91	0.37	0.70	0.58	1.09	0.35	0.62	0.19	0.26	0.06	0.09	0.02	0.96	0.26	0.46	0.15	0.87	0.24
Helotiales	3.14	0.56	3.47	0.33	3.59	1.33	1.59	0.12	2.59	0.35	5.08	1.28	3.36	0.48	2.75	0.20	2.43	0.14	3.03	0.20
Hypocreales	0.74	0.20	0.84	0.16	2.78	1.92	1.07	0.09	1.16	0.05	1.70	0.25	1.33	0.26	1.34	0.31	1.06	0.20	0.83	0.15
Mytilinidales	0.14	0.02	1.47	0.24	1.94	0.91	1.35	0.17	1.26	0.18	0.18	0.10	0.06	0.02	1.42	0.30	2.10	0.37	1.76	0.22
Pezizales	0.01	0.01	0.01	0.00	0.03	0.03	0.01	0.01	0.00	0.00	0.14	0.02	0.25	0.06	0.25	0.13	0.06	0.04	0.02	0.01
Agaricales	0.98	0.37	4.86	1.96	3.03	1.46	4.06	0.88	2.97	0.75	4.81	2.46	6.98	1.58	5.61	1.21	4.07	2.52	2.98	0.67
Atheliales	0.34	0.18	1.00	0.50	0.94	0.94	3.74	0.95	7.20	0.96	0.17	0.16	0.01	0.00	0.01	0.00	1.42	0.39	1.61	0.17
Boletales	0.50	0.16	0.75	0.37	0.25	0.17	0.70	0.17	0.59	0.18	0.54	0.02	0.35	0.12	2.68	0.82	5.00	3.18	0.83	0.15
Cantharellales	0.01	0.00	0.01	0.00	0.07	0.04	0.16	0.14	0.33	0.33	0.05	0.00	0.19	0.07	0.15	0.06	0.10	0.09	0.10	0.07
Hymenochaetales	0.07	0.02	0.03	0.01	0.01	0.00	0.03	0.00	0.10	0.06	0.15	0.07	0.97	0.95	0.02	0.00	0.09	0.05	0.03	0.01
Russulales	18.39	1.60	11.58	0.68	7.48	3.08	12.18	1.09	7.61	0.83	4.16	1.96	3.66	1.91	5.80	2.04	3.85	1.58	8.95	0.97
Sebacinales	0.10	0.03	0.20	0.04	0.00	0.00	0.01	0.00	0.04	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Thelephorales	0.13	0.08	0.28	0.15	3.18	1.62	0.06	0.01	0.08	0.03	0.02	0.01	0.46	0.35	0.19	0.07	0.20	0.10	0.46	0.16
Trechisporales	0.04	0.00	0.10	0.08	0.07	0.03	0.19	0.09	0.23	0.14	0.69	0.23	1.40	0.80	0.59	0.29	0.14	0.08	1.05	0.40
Tremellales	0.94	0.07	0.91	0.31	0.49	0.09	0.38	0.04	0.22	0.03	4.10	0.68	3.27	0.27	0.79	0.23	0.64	0.08	1.57	0.07
Mortierellales	0.72	0.10	1.35	0.48	1.08	0.27	0.91	0.07	0.47	0.13	2.01	0.35	1.80	0.34	2.70	0.20	1.47	1.32	0.47	0.08

Supplement Table S4. Relative abundance of soil fungal orders in pure (European beech; Douglas-fir; Spruce) and mixed (European beech with Douglas-fir; European beech with Spruce) tree stands. Relative abundance compiled out of total abundance (1,640,160 reads) comprising all orders.

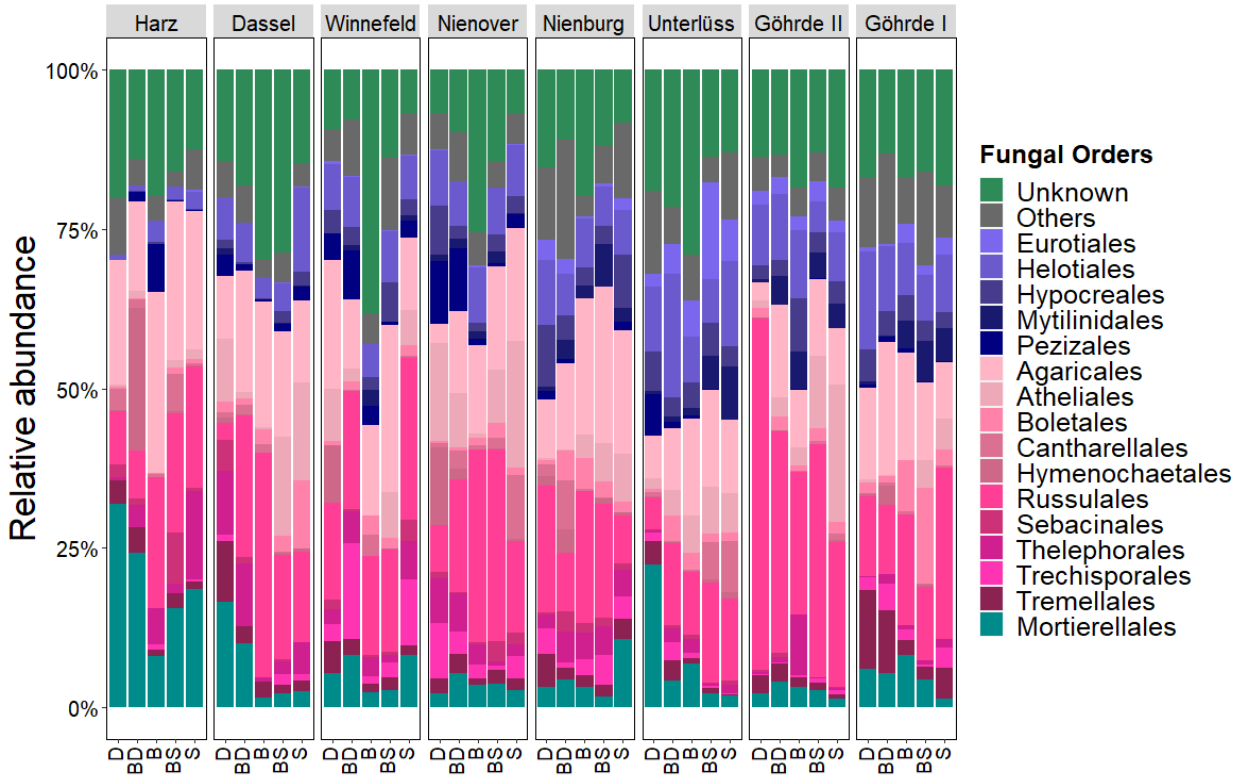
Order (> 1 %)	RA (%)	Order (0.003 % - 0.005 %)	RA (%)	Order (< 0.003 %)	RA (%)
Russulales	18.43	Dacrymycetales	0.042	Annulatascales	0.0029
Agaricales	15.01	Erysiphales	0.037	Umbilicariales	0.0028
Helotiales	7.66	GS20	0.036	Jaapiales	0.0025
Mortierellales	6.87	Filobasidiales	0.036	Mucoromycotina	0.0022
Atheliales	5.36	Leucosporidiales	0.035	Exobasidiales	0.0019
Thelephorales	3.26	Geoglossales	0.035	Pachnocybales	0.0017
Hypocreales	3.20	Microbotryomycetes	0.034	Diversisporales	0.0016
Tremellales	2.86	Orbiliales	0.034	Phaeomoniellales	0.0016
Boletales	2.37	Agaricomycetes	0.032	GS37	0.0015
Trechisporales	2.26	Glomerellales	0.025	Urocystidales	0.0015
Mytilinidales	1.93	Rhizophydiales	0.024	Erythrobasidiales	0.0014
Pezizales	1.81	Spizellomycetales	0.024	Malasseziales	0.0014
Eurotiales	1.60	Phacidiales	0.022	GS07	0.0013
Cantharellales	1.58	Endogonales	0.0182	Pleurotheciales	0.0013
Hymenochaetales	1.54	Basidiobolales	0.0182	GS23	0.0012
Sebacinales	1.02	Glomerales	0.0181	GS03	0.0012
		Corticiales	0.0170	Lecanorales	0.0012
Order (0.05 % - 1 %)	**RA (%)**	Tremellodendropsidales	0.0145	Ustilaginales	0.0008
		Mytilinidiales	0.0143	Archaeosporales	0.0007
Chaetothyriales	0.872	Kriegeriales	0.0137	Calcarisporiellales	0.0007
Auriculariales	0.829	Kickxellales	0.0135	Dothideomycetes	0.0007
Pleosporales	0.602	Onygenales	0.0134	GS09	0.0007
Polyporales	0.406	Atractiellales	0.0132	Georgefischeriales	0.0006
Archaeorhizomycetales	0.392	GS04	0.0130	Gigasporales	0.0005
Sordariales	0.366	Cystofilobasidiales	0.0109	Microbotryales	0.0005
Mucorales	0.353	Myrmecridiales	0.0098	Peltigerales	0.0005
Trichosporonales	0.327	Diaporthales	0.0096	Taphrinales	0.0005
Umbelopsidales	0.293	Ostropales	0.0084	GS06	0.0005
Geastrales	0.242	GS34	0.0079	Olpidiales	0.0005
Xylariales	0.215	Coniochaetales	0.0070	Lulworthiales	0.0004
Capnodiales	0.201	GS05	0.0068	Amylocorticiales	0.0004
Chaetosphaeriales	0.197	Dothideales	0.0059	Sclerococcales	0.0003
Zoopagales	0.168	GS21	0.0056	Barbatosporales	0.0002
Venturiales	0.144	Microascales	0.0053	Caliciales	0.0002
Sporidiobolales	0.133	Candelariales	0.0050	Coryneliales	0.0002
GS22	0.125	Lobulomycetales	0.0050	Incertae	0.0001
Rhytismatales	0.104	Ophiostomatales	0.0048	Teloschistales	0.0001
Saccharomycetales	0.093	Hysterangiales	0.0046	Harpellales	0.0001
Phallales	0.090	Acarosporales	0.0042	Platygloeales	0.0001
Pezizomycotina	0.058	Pyxidiophorales	0.0038		
GS11	0.053	Hysteriales	0.0037		
Thelebolales	0.051	Chytridiales	0.0035		
		Tubeufiales	0.0030		



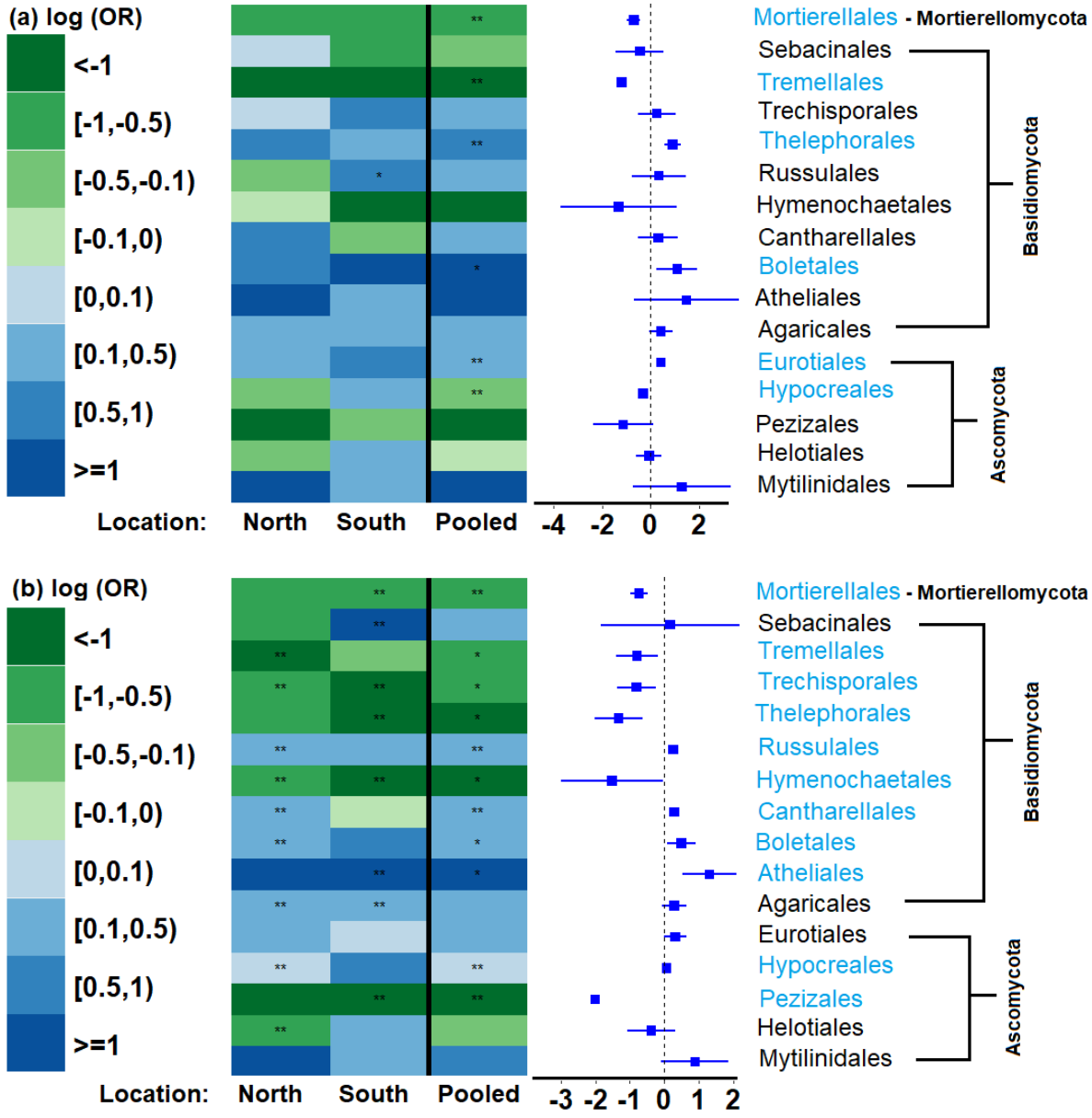
Supplement Fig S1. Rarefaction curves for soil fungal OTUs. Curves show three replicates per plot in beech (B), spruce (S), and Douglas-fir (D) stands and mixture of beech with Douglas-fir (BD) and mixture of beech with spruce (S) according to study sites (Harz, Dassel, Winnefeld, Nienover, Nienburg, Unterlüss, Göhrde I and II.



Supplement Fig. S2. Relative abundance of symbiotrophic (SYM), saprotrophic (SAP), pathotrophic (PAT), others and unidentified (unknown) fungi in soil of the study sites Harz, Dassel, Winnefeld, Nienover, Nienburg, Unterlüss, Göhrde II and Göhrde I in different tree stands of Douglas-fir (D), beech (B), spruce (S), mixture of beech with Douglas-fir (BD) and mixture of beech with spruce (BS). Other fungi are all fungi for which no clear guild annotation was obtained. Unknown fungi lack phylogenetic information. Data show means (n = 3 per site and stand).



Supplement Fig. S3. Relative abundance of abundant fungal orders (>1 %) among the different study sites of Harz, Dassel, Winnefeld, Nienover, Nienburg, Unterlüss, Göhrde II and Göhrde I in different tree stands of Douglas-fir (D), beech (B), spruce (S), mixture of beech with Douglas-fir (BD) and mixture of beech with spruce (BS). Orders are arranged according to phyla Ascomycota (blue), Basidiomycota (pink to violet) and Mortierellomycota (dark cyan). Other fungi represent the sum of all fungi with abundances < 1% per order. Unknown fungi lack phylogenetic information. Data show means (n = 3 per site and stand).



Supplement Fig S4. Changes in the relative abundance of fungal orders in conifer and mixed stands: (a) spruce/Douglas-fir, (b) beech-spruce/beech-Douglas-fir. Data are shown as log ratio of odds (log(OR)). Green cells (negative values) indicate enrichment of a fungal order in Douglas-fir and beech-Douglas-fir forests and blue (positive values) indicate enrichment in spruce and beech-spruce forests. Significant differences at $p \leq 0.05$ (GAMLSS model with BEZI family, n = 4 for the fungal orders in stands in the north and south location, n = 8 for the pooled data) are indicated with stars (* $p < 0.05$, ** $p < 0.01$). Significant orders are highlighted in blue.

CHAPTER 5: GENERAL DISCUSSION

5.1. General overview and conclusions

In this thesis, the factors contributing to seasonal fine root trajectories in European beech dominated stands along climatic and soil fertility gradients are highlighted. This thesis also highlights the impact of tree species composition on soil fungi in pure and mixed beech-conifer tree stands (Fig. 5.1).

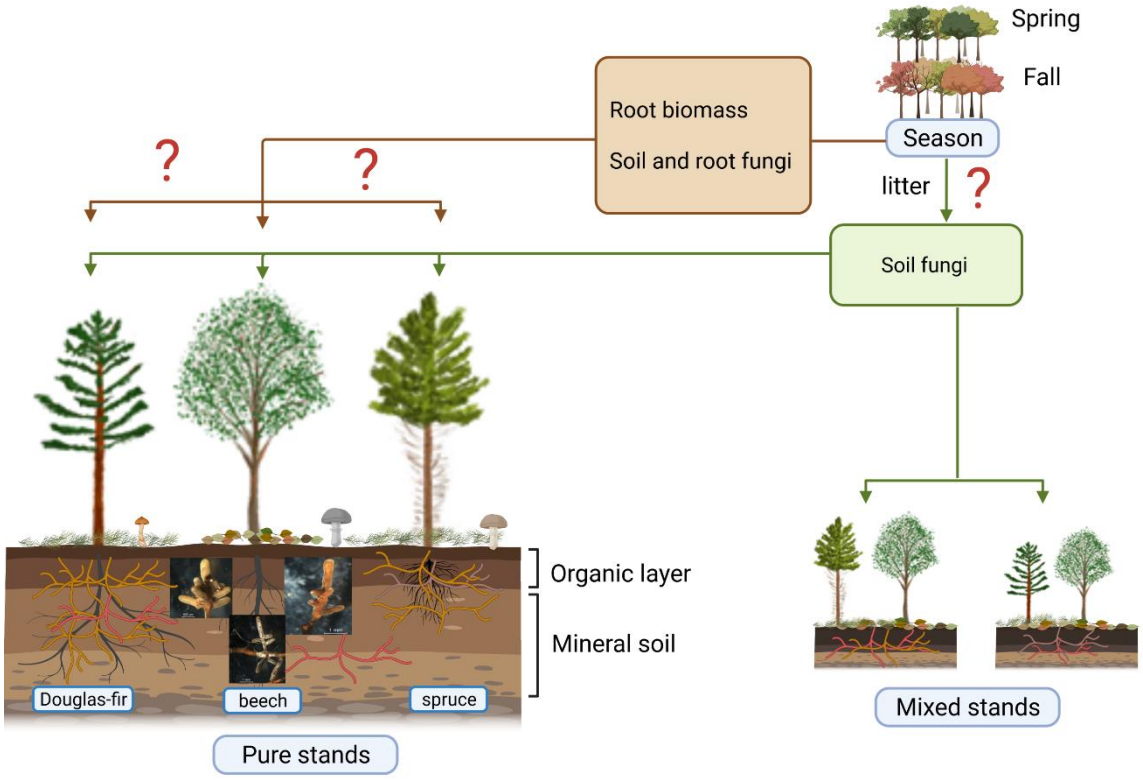


Figure 5.1. Schematic diagram illustrating the synopsis of this thesis. The green arrows show the study on the impact of mixing beech with either Douglas-fir or Norway spruce on soil fungal composition while the brown arrows portray the study on seasonal trends of root biomass and fungi in pure beech stands. The question marks indicate research questions to be investigated in future.

In this thesis, the first goal was to determine which factors influence seasonal patterns of fine root biomass in beech dominated stands on sites that differ in climate and soil P content. Addressing this goal, the present results highlighted that beech fine root biomass seasonal trends are variable depending on the site. For example, at the P-low site, root biomass was higher in spring than fall, contrary to what was observed at the P-high site (both organic layer and mineral soil). In this thesis, it has been shown that climate explained a higher portion of variance of fine root biomass in the mineral

soil than in the organic layer, whereas soil water content explained a higher portion of root biomass variation in the organic layer (i.e. ~2 times more in organic layer that mineral soil). Soil moisture has been elaborated as the key factor affecting root biomass and turnover (Gill and Jackson, 2000; Leuschner and Hertel, 2003). Root biomass patterns were studied in pure and mixed beech-conifer tree stands by Lwila et al. (2021). They showed that root biomass at the dry and poor nutrient was higher than at the humid and nutrient-rich region, irrespective of the tree stand type but significant differences were only detected for pure beech stands: root biomass was nearly twice greater in nutrient poor and dry region than in the humid and nutrient-rich region (Lwila et al. 2021). Although the study by Lwila et al. (2021) was only done in spring, it reflects the findings in this thesis that root biomass is driven by differences in climate factors and nutrients. Considering that the organic layer is important for tree nutrition, especially on sites limited with P (Lang et al. 2017; Clausing and Polle, 2020), fine root biomass may be severely impacted by limited water content. The results on beech response to nutrients and climate in this thesis and the study by Lwila et al. (2021) are particularly important because they show beech root system plasticity in response to nutrients and climate factors. These findings are important for understanding beech adaptation to changing environmental conditions, contributing to information on sustainable forest management.

In addition, mycobiomes are important for organic matter decomposition, release of mineral nutrients and nutrient uptake by trees (Baldrian, 2017; Brundrett and Tedersoo, 2018). This thesis provided insights on the impact of shifts in P and N nutrition on ectomycorrhizal fungi (EMF), which support tree nutrition (Clausing et al. 2021). The results from this study show that EMF composition, richness and diversity were not affected by nutrient inputs, an indication of relative stability of EMF communities to moderate nutrient input, as also shown for N deposition by Lilleskov et al. (2019). Therefore, beech trees can still rely on symbiotic associations with EMF to enhance nutrient uptake and tree productivity. The insights gained from collaborative research in this thesis (Clausing et al. 2021) showed that EMF of the orders Russulales were negatively and Boletales positively impacted by P and P+N additions in the mineral soil in fall, implying that fungal structures are influenced by nutrient dynamics. Whether the seasonal effects of root biomass, soil and root associated fungi elucidated in this thesis can be extrapolated to conifer stands needs

investigation in future (question marks in Fig. 5.1). Given various factors such as litter input, litter quality and root exudation which impact microbial communities (Stoutjesdijk and Barkman, 1992; Augusto et al., 2002), it is likely that seasonal patterns in root biomass and fungal assemblages along climate and nutrient gradients in conifers will be different from what has been found in the pure beech tree stands in this thesis. Moreover, nutrients such as P and N showed a general pattern of being higher in spring than fall. Studies (Netzer et al. 2017; Zavišić and Polle, 2018) also support P mobilization from stem and root in spring to support leaf flushing. Therefore, all these changes in nutrient cycling and litter input may show different patterns in root biomass and fungal composition in conifers or mixed beech-conifer stands, hence the need for future investigation (Fig. 5.1).

In this thesis, the further goal addressed the impact of mixing beech with non-native (Douglas-fir) and native (Norway spruce) conifers on soil fungi (Likulunga et al. 2021). The inclusion of Douglas-fir in this study was important because this tree species has been planted in Germany since 1820s (Booth,1877; Knoerzer and Reif, 2002), yet its impact on native soil fungal communities is unknown. Moreover, concerns about negative effects of non-native Douglas-fir on local diversity are discussed (Schmid et al. 2014). The study (Likulunga et al. 2021) in this thesis showed that Douglas-fir can accommodate local soil fungal communities, i.e. similar assemblages as native spruce. In this thesis, it has also been shown in agreement with other studies (Pena et al. 2017; Asplund et al. 2019) that conifers form fungal communities different from beech. This finding reinforces the previous conclusion from the first and second thesis goals that seasonal changes in root biomass and fungal composition may differ in conifers. The results from this thesis (Likulunga et al. 2021) further indicated a reduction in abundance of symbiotrophs in Douglas-fir and mixed beech-Douglas-fir tree stands. Whether the reduction in abundance of symbiotrophs translates into negative effects on forest ecosystem functions resulting in less nutrient coupling between soil and plants needs investigation. Positive effects such as an enhancement in nutrient cycling and provision to the trees without mycorrhizal intervention may also be envisaged. Further, it should be noted that here fungal communities were only studied in the upper 10 cm depth after removal of non-decomposed litter (leaves, twigs, etc.) (Likulunga et al. 2021). Considering the different rooting patterns of beech and conifers (flat root system in conifers than beech), different type of litter input (i.e.

conifers have lower foliar nutrients of P, N, K, Ca and Mg: Augusto et al. 2002; Augusto et al. 2015) and variation of fungal composition in litter, humus and mineral soil (Asplund et al. 2019, Khokon et al. 2021), it is likely that conifers and beech-conifers will portray different fungal structures, a suggestion that requires further investigation. Moreover, we found that in pure beech stands, fungal communities in organic layer and mineral soil were distinct (Clausing et al. 2021). The strong stratification of fungal communities between the organic layer and mineral soil has also been demonstrated across large-scale biogeographic regions in Germany stocked with beech, spruce and other tree species (Khokon et al. 2021). In addition, soil nutrients are impacted by season as demonstrated from the study in this thesis (Clausing et al. 2021) (generally high P and N in spring than fall) and therefore, may influence seasonal shifts in fungal assemblages in conifers and beech-conifer mixed stands. Whether conifer stands, in which needle litter is shed throughout the year (Portillo-Estrada et al. 2013; Fu et al. 2017), show different seasonal patterns of fungal communities than beech forests needs to be investigated (Fig. 5.1).

5.2. References

Asplund, J., Kauserud, H., Ohlson, M., Nybakken, L., 2019. Spruce and beech as local determinants of forest fungal community structure in litter, humus and mineral soil. FEMS Microbiology Ecology 95 (2), fiy232. doi: 10.1093/femsec/fiy232

Augusto, L., De Schrijver, A., Vesterdal, L., Smolander, A., Prescott, C., Ranger, J., 2015. Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. Biological Reviews of the Cambridge Philosophical Society 90 (2), 444–466. doi: 10.1111/brv.12119

Augusto, L., Ranger, J., Binkley, D., Rothe, A., 2002. Impact of several common tree species of European temperate forests on soil fertility. Annals of Forest Science 59 (3), 233–253. doi: 10.1051/forest:2002020

Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiology Reviews 41, 109–130. doi: 10.1093/femsre/fuw040

Booth, J., 187). Die Douglas-Fichte und einige andere Nadelhölzer namentlich aus dem nordwestlichen Amerika in Bezug auf ihren forstlichen Anbau in Deutschland. Berlin, Springer

Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220 (4), 1108–1115. doi: 10.1111/nph.14976

Clausing, S., Likulunga, E.L., Janz, D., Feng, H., Schneider, D., Daniel, R., Krüger, J., Lang, F., Polle, A., 2021. Impact of nitrogen and phosphorus addition on resident soil and root mycobiomes in beech forests. Biology and Fertility of Soils. doi: 10.1007/s00374-021-01593-x

Clausing, S., Polle, A., 2020. Mycorrhizal Phosphorus Efficiencies and Microbial Competition Drive Root P Uptake. Frontiers in Forests and Global Change 3, 54. doi: 10.3389/ffgc.2020.00054

Fu, C., Yang, W., Tan, B., Xu, Z., Zhang, Y., Yang, J., Ni, X., Wu, F., 2017. Seasonal dynamics of litterfall in a sub-alpine spruce-fir forest on the Eastern Tibetan plateau: Allometric scaling relationships based on one year of observations. Forests 8(9), 314. doi: 10.3390/f8090314

Gill, R.A., Jackson, R.B., 2000. Global patterns of root turnover for terrestrial ecosystems. New Phytologist 147, 13–31

Khokon, A.M., Schneider, D., Daniel, R., Polle, A., 2021. Soil layers matter: vertical stratification of root-associated fungal assemblages in temperate forests reveals differences in habitat colonization. Microorganisms 9, 2131. doi: 10.3390/microorganisms9102131

Knoerzer, D., Reif, A., 2002. “Fremdländische Baumarten in deutschen Wäldern,” in Biologische Invasionen - eine Herausforderung zum Handeln? Neobiota 1, eds I. Kowarik and U. Starfinger (Berlin: Springer), 27–35

Lang, F., Krüger, J., Amelung, W., Willbold, S., Frossard, E., Bünemann, E. K., et al., 2017. Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. Biogeochemistry 136, 5–29. doi: 10.1007/s10533017-0375-0

Leuschner C., Hertel, D., 2003. Fine root biomass of temperate forests in relation to soil acidity and fertility, climate, age and species. In: Progress in Botany, Vol.

64 (eds Esser K, Lüttge U, Beyschlag W, Hellwig F), pp. 405–438. Springer, Berlin, Heidelberg

Likulunga, E.L., Rivera Pérez, C.A., Schneider, D., Daniel, R., Polle, A., 2021. Tree species composition and soil properties in pure and mixed beech-conifer stands drive soil fungal communities. Forest Ecology and Management, 119709. doi: 10.1016/j.foreco.2021.119709

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

Lwila, A.S., Mund, M., Ammer, C., Glatthorn, J., 2021. Site conditions more than species identity drive fine root biomass, morphology and spatial distribution in temperate pure and mixed forests. Forest Ecology and Management 499, 119581. doi: 10.1016/j.foreco.2021.119581

Netzer, F., Schmid, C., Herschbach, C., Rennenberg, H., 2017. Phosphorus nutrition of European beech (*Fagus sylvatica* L.) during annual growth depends on tree age and P-availability in the soil. Environmental and Experimental Botany 137, 194–207. doi: 10.1016/j.envexpbot.2017.02.009

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

Portillo-Estrada, M., Korhonen, J.F.J., Pihlatie, M., Pumpanen, J., Frumau, A.K.F., Morillas, L., Tosens, T., Niinemets, Ü., 2013. Inter- and intra-annual variations in canopy fine litterfall and carbon and nitrogen inputs to the forest floor in two European coniferous forests. Annals of Forest Science 70, 367–379. doi: 10.1007/s13595-013-0273-0

Schmid, M., Pautasso, M., Holdenrieder, O., 2014. Ecological consequences of Douglas fir (Pseudotsuga menziesii) cultivation in Europe. European Journal of Forest Research 133, 13—29. doi: 10.1007/s10342-013-0745-7

Stoutjesdijk, P., Barkman, J.J., 1992. Microclimate, Vegetation and Fauna. Opulus Press, Knivsta, Sweden

Zavišić, A., Polle, A., 2018. Dynamics of phosphorus nutrition, allocation and growth of young beech (*Fagus sylvatica* L.) trees in P-rich and P-poor forest soil. Tree Physiology 38, 37-51. doi: 10.1093/treephys/tpx146

DECLARATION OF AUTHOR'S CONTRIBUTION

CHAPTER 2

Andrea Polle, Simon Clausing, Likulunga Emmanuel Likulunga, Jaane Krüger and Friederike Lang contributed to the design of the study. Jaane Krüger and Friederike Lang maintained the plots and the fertilization experiment. Likulunga Emmanuel Likulunga and Simon Clausing conducted field sampling. Simon Clausing conducted measurements. Andrea Polle supervised the measurements. Likulunga Emmanuel Likulunga analyzed the data and wrote the chapter. Andrea Polle supervised data analysis and writing of this chapter.

CHAPTER 3

Author's contribution has been provided in the published manuscript.

CHAPTER 4

Author's contribution is provided in the published manuscript.

DECLARATION OF ORIGINALITY AND CERTIFICATE OF AUTHORSHIP

I, Likulunga Emmanuel Likulunga, hereby declare that I am the sole author of this dissertation entitled “Influence of mycorrhiza on nutrient physiology of trees in mixed and mono-specific stands along climatic and land use gradients”. I further declare that this work has not been submitted elsewhere for the award of the degree. To the best of my knowledge, I also state that all references and data sources utilized in this dissertation have been acknowledged appropriately.

Place and date: Goettingen, 20.05.2022 Signature:

Likulunga Emmanuel Likulunga

ACKNOWLEDGEMENTS

I would like to express my profound gratitude to my principal supervisor Prof. Dr. Andrea Polle for granting me the opportunity to work on this research topic, support and for the relentless meticulous job of supervision. I extend my gratitude to members of my thesis committee Prof. Dr. Stefan Scheu and Prof. Dr. Christian Ammer for excellent guidance and support during my thesis committee meetings as well as the entire period of my study.

I am also grateful to all members of Forest Botany and Tree Physiology department for their support during my studies. In this regard, I am thankful to Dr. Rodica Pena for useful scientific advice during the course of my study. I thank Dr. Johannes Ballauff for helpful discussions on data analysis. I am thankful to Dr. Christian Eckert for support and translation of my thesis abstract into German language. My appreciation extends to the technicians Gabriele Lehmann, Thomas Klein, Monika Franke-Klein, Merle Fastenrath, Bernd Kopka, Thoms Ronny and Michael Reichel for their technical support. My thanks further go to Dr. Simon Clausing and Carmen Alicia Rivera Pérez for the collaborative research work and field work memories. I thank Ashkan Amirkhosravi for his support, encouragement and company during my studies and extracurricular activities. I am grateful to all my PhD student colleagues for support and encouragement during my studies.

My appreciation goes to the University of Zambia Staff Development Office for granting me study leave and financial support to undertake my studies. I am thankful to the German Research Foundation for funding this research work through the Research Training Group RTG 2300 (Grant ID:316045089) and Ecosystem Nutrition project SPP 1685 (Po362/22–2). I am grateful to Bernhard-Ulrich-Stiftung for additional financial assistance towards my studies and family. My profound appreciation goes to the entire members of the Research Training Group RTG 2300 and particularly Dr. Serena Müller for excellent coordination and providing relentless support.

I am highly indebted to my family especially my wife Nchimunya Handila, my son Likulunga Alexander Likulunga, my elder brother Likando Likulunga, my mum Nolia Mwisi

Likulunga, my niece Inonge Sitenge and my dad Francis Nguya Likulunga (RIP) for the motivation and tremendous support towards my PhD journey. I am equally grateful to Prof. Kasuka Mwauluka for the encouragement and mentorship.

Last but not the least, I am grateful to Alexander Hafner, Coach Christian Dreher and the entire SG Niedernjesa team and management for giving me mental strength through football activities, enabling me to cope with my studies.