Ecophysiological field studies of phosphorus nutrition of beech (*Fagus sylvatica*)

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List of abbrevations and acronyms

°C	degree Celsius
1wk	one week
^{32}P	radioactive isotope of phosphorus
^{33}P	radioactive isotope of phosphorus
8wk	eight weeks
a	year
ANOSIM	Analyses of similarity
ANOVA	Analyses of similarity
ASV	amplicon sequence variants
BBR	Bad Brückenau
BLAST	Basic Local Alignment Search Tool. Available at: https://blast.ncbi.nlm.nih.gov/Blast.cgi
bulk	bulk soil
C	carbon
cm	centimeter
Con	control
CWM	community-weighted mean
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide
dw	dry weight
EMF	ectomycorrhizal fungal species or ectomycorrhizal fungi/fungal
et al.	and others (et alii)
FAMEs	Fatty acid methyl esters
fw	fresh weight
g	gram
G	girdling
Gram -	gram negative bacteria
Gram +	gram positive bacteria
h	hour
ha	hectar
HP	high P
ICP-OES	Inductively coupled plasma optical emission spectrometer
ITS	internal transcribed spacer
K	kalium
KOSI	Kompetenzzentrum für Stabile Isotope; Center for Stable Isotope Research and Analysis
LARI	Laboratory for Radio-Isotopes
L-Leu	L-leucine peptidase
lmer	linear mixed effect models
LP	low P
LUE	Unterlüss
m	meter
m.a.s.l.	meters above sea level
MES	2-(N-morpholino)ethanesulfonic acid

mg	milligramm
min	minute
MIT	Mitterfels
ML	mineral layer
mm	milimeter
MP	medium P
MT	morphotype
MUB	Modified Universal Buffer
MUF	4-methylumbelliferone
N	nitrogen
n	number of replicates
N-Ace	N-Acetyl-glucosaminidase
NCBI	National Center of Biotechnology Information. Available at: https://www.ncbi.nlm.nih.gov/
NMDS	non-metric multidimentaional scaling
OL	organic layer
OTU	operational taxonomic unit
P	phosphorus
p	probability of error
PCA	principal component analysis
PCR	polymerase chain reaction
P-Die 6.1	phosphodiesterase with MES buffer at pH 6.1
PEPC	phosphoenolpyruvate carboxylase
Per	peroxidase
pH	negative log of the activity of the hydrogen ion
Phe	phenoloxidase
P_i	inorganig phosphate
P_{lab}	labile P
PLFA	Phospholipid-derived fatty acids
P_{mic}	microbial P
P-Mo 11	phosphomonoesterase with MUB buffer at pH 11
P-Mo 6.1	phosphomonoesterase with MUB buffer at 6.1
P_{sol}	soluable P
P_{tot}	total P
RAF	Root associated fungi
Rhizo	rhizosphere
RNA	ribonucleic acid
RWC	relativ water content
SAF	Soil associated fungi
SE	standard error
ß-Glu	β-glucosidase
UNITE	nordic ITS ectomycorrhiza database. Available at: http://unite.ut.ee/
WEOC	water-extractable organic carbon
Xylo	xylosidase
α-Glu	α-glucosidase

Summary

Phosphorus (P) is an essential element for plant nutrition because it is required for many essential biomolecules such as nucleotides, membrane lipids and ATP. Plants take up P as inorganic phosphate (P_i) from soil solutions. This P resource is usually scarcely available in soil due to low diffusion rates, sequestration by soil minerals or binding to organic matter. An important aspect of P nutrition is the symbiosis of plants with ectomycorrhizal fungal species (EMF). EMF contribute to P nutrition by secretion of chemical compounds and extending soil exploration by extrametrical hyphae. Although forest trees are generally adapted to low nutrient in soil, recent studies detected decreasing P concentrations of beech leaves across Europe (*Fagus sylvatica* L.). The reasons for the trend of declining P in beech are unknown.

Here, we analysed the P uptake strategies and nutritional status of beech and EMF under low P (LP), medium P (MP) and high P (HP) conditions to examine plant and mycorrhizal P efficiencies, as well as disorders of P supply by abiotic interferences to beech. Specifically, we examined aspects of P uptake including phosphatase activities, uptake by radioactively labeled P, as well as community analyses of EMF under different availabilities of macronutrients.

The following questions were addressed: a) Is the efficiency for P uptake and allocation to beech roots higher under LP than under HP conditions? Since the vertical distribution of P stocks differs between HP and LP forests, we also separated soil layers in the organic layer and the mineral topsoil. b) How important is recently photoassimilated carbon for P nutrition? Does carbohydrate depletion leads to decreased root P concentrations, with stronger effects under LP than under HP conditions? c) How do P and N availabilities affect soil and root associated fungal communities and functional composition in different compartments (soil, root) under different P conditions?

This study used the infrastructure of the International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests). From Level II site of ICP Forests basic parameters for soils and stands are accessible. We used three Level II forest sites across Germany, which are stocked with more than 100-year-old mono-specific beech stands. LP is located at the district Celle in Lower Saxony (52°50'21.7"N 10°1.6'2.3"E, 115 m a.s.l.), MP is located at the district Mitterfels in Bavaria (48°58'34.1"N 12°52'46.7"E, 1023 m a.s.l.) and HP is located at the biosphere reservation ‘Bayerische Rhön’ in Bavaria (50°21'7.2"N 9°55'44.5"E, 801 to 850 m a.s.l.).

The sites differ significantly in their soil P concentration ranging from 195 mg kg^{-1} to 2966 mg kg^{-1} of the A horizon (0-5 cm). The sites differ in the texture of the mineral topsoils as well as the humus form and thickness. The P gradient is a result of different parent materials of the soils. Despite the P differences the beeches maintain similar concentrations of foliar P ranging from 1.21mg g^{-1} dw to 1.66 mg g^{-1} dw.

a) To investigate the P uptake efficiency, we used intact soil cores including the organic and mineral layers from LP and HP sites. These intact soil cores were labeled with ^{33}P and incubated for 24 hours.
The main result was that the main pool of P acquired by beech, was taken up from different layers between contrasting soil P concentrations. While beech from LP sites acquires most P from the organic layer, beech from HP sites acquire most P from the mineral layer. This result was also reflected by the P enrichment of ectomycorrhizal root tips at the HP and LP sites. The importance of ectomycorrhizal root tips for P uptake was shown in contrast to non-mycorrhizal root tips, which were rare and exhibited lower enrichment with new P. Beech P uptake correlated with the enrichment efficiency of ectomycorrhizal root tips, demonstrating the relevance of mycorrhizal P acquisition for root P supply.

b) To investigate the impact of photoassimilated carbon on P uptake, we interrupted the transport to the roots by girdling of young beech trees under LP and HP conditions. We studied the consequences of the girdling in roots, soil, and microbes one week and two months after the treatment. To test the influence of girdling on P uptake we conducted a ^{32}P uptake experiment with young beech trees in the laboratory.
The radioactive tracer experiment showed that P uptake of fine roots was reduced after girdling. Girdling resulted in increased acid phosphatase and PEPC activity in fine roots. The girdled trees had a decreased number of vital root tips and less fine root biomass, thus, reducing P uptake capacity compared with controls. At the LP site, ectomycorrhizas exhibited increased extracellular acid phosphatase activity in response to girdling. In addition, gene abundances of P-transporters and of genes for P mineralization were increased in the soil as a consequence of P and C starvation at the LP site in response to girdling. Among the tested variables, at HP only increased phosphatase and PEPC activities in roots were observed, while at LP many adaptation responses were detected, even in the surrounding soil. We conclude that P concentrations of microbes and fine roots can be maintained due to increased P uptake, internal P relocation and a reduction of biomass at HP and LP conditions.

c) To investigate the role of P and N availability in soil as drivers for fungal communities in soil and roots, we used a fertilization experiment three years after addition of P (50 kg ha^{-1}) and regular additions of N (5 times 30 kg ha^{-1}) or the combination of the P+N treatments. The experimental treatments were conducted on plots in the HP, MP and LP forests and KCl treated plots served as the controls. We analysed the changes of fungal communities with Illumina MiSeq of soil and fine roots and morphotyping of EMF from roots grown in the organic and the mineral layer in spring and fall.
Fertilization with P increased the labile P concentration in soil and the total P concentration in roots. Fertilization with N only affected ammonia at LP in the organic layer, whereas no changes occurred at MP and HP or in the mineral topsoils of the

forests. N further decreased total P of root at the low P site. P and N fertilization didn’t affect ectomycorrhizal species richness but increased their relative abundance compared to that of saprotrophic fungi. However, the fungal communities and taxa associated with roots or soil (analysed by Illumina sequencing) did not show significant compositional changes, when studied at the level of 97% sequence identity (operational taxonomic units). At the phylogenetic classification of orders, we observed moderate decreases in the abundances of Russulales (P+N), whereas Boletales (P) increased. Russulales and Boletales contain mainly mycorrhizal forming fungi. Short-term changes in N and P inputs were resisted by fungal communities, whereas chronic exposure to enhanced nutrient inputs may lead to significant changes of the resident mycobiome structures.

In conclusion, the results of this thesis disentangle adaptation of mycorrhizal P efficiencies to soil P availability and highlight the complexity of biotic and abiotic factors that govern P supply to trees in forest ecosystems. Beech forest ecosystems revealed distinct strategies depending on the P availability to cope with varying environmental conditions. We provide insight in P acquiring and P recycling strategies by the comparison of LP and HP conditions of beech forests and their respective EMF community. Recycling strategies are characterized by P uptake of beech and P enrichment of EMF from the organic layer, where fine root mass and vital ectomycorrhized root tips are accumulated. Acquiring strategies are identified by P uptake of beech and P enrichment of EMF from the mineral topsoil, in which fine root mass and vital ectomycorrhized root tips are clustered. Each strategy has its unique fungal EMF communities, improving solubilization from organic matter under LP condition or from mineral soil under HP conditions.

Zusammenfassung

Phosphor (P) ist ein wesentliches Element für die Pflanzenernährung, da es für viele essentielle Biomoleküle wie Nukleotide, Membranlipide und ATP benötigt wird. Pflanzen nehmen P als anorganisches Phosphat (Pi) aus Bodenlösungen auf. Diese P-Ressource ist im Boden aufgrund geringer Diffusionsraten, Sequestrierung durch Bodenmineralien oder Bindung an organische Stoffe häufig begrenzt. Ein wichtiger Aspekt der P-Ernährung ist die Symbiose von Pflanzen mit Ektomykorrhizapilzarten (EMF). EMF tragen zur P-Ernährung bei, indem sie chemische Verbindungen sekretieren und die Bodenexploration durch extrametrische Hyphen erweitern. Obwohl Waldbäume im Allgemeinen an einen niedrigen Nährstoffgehalt im Boden angepasst sind, wurde in jüngsten Studien ein Rückgang der P-Konzentrationen von Buchenblättern in ganz Europa festgestellt (*Fagus sylvatica* L.). Die Gründe für den Trend zur Abnahme von P in Buche sind unbekannt.

Daher analysierten wir die P-Aufnahmestrategien und den Ernährungszustand von Buchen und EMF unter Bedingungen mit niedrigem P (LP), mittlerem P (MP) und hohem P (HP), um die P-Effizienz von Buche und Mykorrhiza, sowie Störungen der Buchen P-Versorgung durch abiotische Veränderungen zu untersuchen. Insbesondere untersuchten wir Aspekte der P-Aufnahme, einschließlich Phosphataseaktivitäten, die Aufnahme von radioaktiv markiertem P, sowie Gesellschafts-Analysen von EMF unter verschiedenen Verfügbarkeiten von Makronährstoffen.

Die folgenden Fragen wurden behandelt: a) Ist die Effizienz der P-Aufnahme und Allokation zu Buchenwurzeln unter LP höher als unter HP-Bedingungen? Da die vertikale Verteilung der P-Bestände zwischen HP- und LP-Wäldern unterschiedlich ist, haben wir auch die Bodenschichten in der organischen Schicht und in mineralischen Boden getrennt. b) Wie wichtig ist neuer photoassimilierter Kohlenstoff für die P-Ernährung? Führt der Kohlenhydratmangel zu einer Verringerung der P-Wurzelkonzentrationen mit stärkeren Auswirkungen unter LP- als unter HP-Bedingungen? c) Wie wirken sich P- und N-Verfügbarkeiten unter verschiedenen P-Bedingungen auf boden- und wurzelassoziierte Pilzgemeinschaften und die funktionelle Zusammensetzung dieser in verschiedenen Kompartimenten (Boden, Wurzel) aus?

Diese Studie nutzte die Infrastruktur des Internationalen Kooperationsprogramms zur Bewertung und Überwachung der Auswirkungen der Luftverschmutzung auf Wälder (ICP-Wälder). Vom Level II-Standort von ICP Forests sind grundlegende Parameter für Böden und Bestände zugänglich. Wir haben drei Waldstandorte der Stufe II in ganz Deutschland genutzt, die mit mehr als 100 Jahre alten monospezifischen Buchenbeständen bestückt sind. LP befindet sich im Landkreis Celle in Niedersachsen (52°50'21.7" N 10°16'2.3" O, 115 m ü.M.), MP befindet sich im Distrikt Mitterfels in Bayern (48°58'34.1“ N 12°52'46.7“ O, 1023 m ü.M.) und HP befinden

sich im Biosphärenreservat 'Bayerische Rhön' in Bayern (50°21'7.2" N 9° 55'44.5" O, 801 bis 850 m ü.M.).
Die Standorte unterscheiden sich signifikant in ihrer Boden-P-Konzentration im Bereich von 195 mg kg^{-1} bis 2966 mg kg^{-1} des A-Horizonts (0-5 cm). Die Standorte unterscheiden sich in der Textur der mineralischen Oberböden sowie in der Humusform und -dicke. Der P-Gradient ergibt sich aus unterschiedlichen Ausgangsmaterialien der Böden. Trotz der P-Unterschiede behalten die Buchen eine ähnliche Konzentration an Blatt-P bei, die von 1,21 mg g^{-1} dw bis 1,66 mg g^{-1} dw reicht.

a) Um die Effizienz der P-Aufnahme zu untersuchen, verwendeten wir intakte Bodenkerne, einschließlich der organischen und mineralischen Schicht von LP- und HP-Standorten. Diese intakten Bodenkerne wurden mit ^{33}P markiert und 24 Stunden inkubiert.
Das Hauptergebnis war, dass der Hauptpool von aufgenommenem P durch Buchen aus verschiedenen Schichten zwischen kontrastierenden Boden-P-Konzentrationen aufgenommen wurde. Während Buchen von LP-Standorten das meiste P aus der organischen Auflage gewinnen, gewinnen Buchen von HP-Standorten das meiste P aus dem mineralischen Oberboden. Dieses Ergebnis spiegelte sich auch in der P-Anreicherung der Ektomykorrhizawurzelspitzen der HP- und LP-Standorte wider. Die Bedeutung von Ektomykorrhizawurzelspitzen für die P-Aufnahme wurde im Gegensatz zu nicht-Mykorrhizawurzelspitzen gezeigt. Diese waren selten und zeigten eine geringere Anreicherung mit neuem P. Die P-Aufnahme der Buchen korrelierte mit der Anreicherungseffizienz von Ektomykorrhizawurzelspitzen und bestärkt die Relevanz von Mykorrhizawurzelspitzen für die Wurzel-P-Versorgung.

b) Um den Einfluss von photoassimiliertem Kohlenstoff auf die P-Aufnahme zu untersuchen, haben wir den Transport zu den Wurzeln unterbrochen, indem wir junge Buchen unter LP- und HP-Bedingungen geringelt haben. Wir untersuchten die Folgen des Ringelns in Wurzeln, Boden und Mikroben eine Woche und zwei Monate nach der Behandlung. Um den Einfluss des Ringelns auf die P-Aufnahme zu testen, führten wir im Labor ein ^{32}P-Aufnahmeexperiment mit jungen Buchen durch.
Das Experiment mit radioaktivem Tracer quantifizierte die verringerte P-Aufnahme von Feinwurzeln. Das Ringeln führte zu einer erhöhten sauren Phosphatase- und PEPC-Aktivität in Feinwurzeln. Zusätzlich wurden Buchen durch Ringeln mit einer abnehmenden Anzahl lebenswichtiger Wurzelspitzen und Feinwurzelbiomasse belastet, wodurch die P-Aufnahmekapazität verringert wurde. An dem LP-Standort zeigten Ektomykorrhizen eine erhöhte Aktivität der extrazellulären sauren Pphosphatase als Reaktion auf das Ringeln. Darüber hinaus war die Genhäufigkeit von P-Transportern und von Genen für die P-Mineralisierung im Boden infolge des P- und C-Mangels an dem LP-Standort als Reaktion auf das Ringeln erhöht. Während bei HP nur erhöhte Phosphatase- und PEPC-Aktivitäten von Pflanzenmaterial beobachtet wurden, wurden bei LP selbst im umgebenden Boden viele Anpassungen

festgestellt. Wir schließen daraus, dass die P-Konzentrationen von Mikroben und Feinwurzeln aufgrund einer erhöhten P-Aufnahme, einer internen P-Verlagerung und einer Verringerung der Biomasse unter HP- und LP-Bedingungen aufrechterhalten werden können.

c) Um die Rolle der Verfügbarkeit von P und N im Boden als Treiber für Pilzgemeinschaften in Boden und Wurzeln zu untersuchen, verwendeten wir ein drei jähriges Düngeexperiment nach Zugabe von P (50 kg ha-1) und regelmäßigen Zugaben von N (5 mal 30 kg ha-1) oder die Kombination der P+N-Behandlungen. Die experimentellen Düngungen wurden an Parzellen in den HP-, MP- und LP-Wäldern durchgeführt, und mit KCl behandelte Parzellen dienten als Kontrollen. Wir analysierten die Veränderungen der Pilzgemeinschaften des Bodens und der Feinwurzeln mit Illumina MiSeq und die nutzten die Morphotypisierung von EMF von Wurzeln, aus der organischen Auflage und des mineralischen des Frühjahrs und Herbstes.
Die Düngung mit P erhöhte die labile P-Konzentration im Boden und die gesamt P-Konzentration der Wurzeln. Die Düngung mit N wirkte sich nur auf Ammonium bei LP in der organischen Schicht aus, während bei MP und HP oder in den mineralischen Oberböden der Wälder keine Veränderungen auftraten. Weiterhin verringerte die N Düngung den gesamt P Gehalt der Wurzeln des P-armen Standortes. Weder die P- noch die N-Düngung erhöhte den Pilzreichtum (Richness) der Ektomycorrhizen, aber ihre relative Abundanz wurde durch die Zugabe von P oder N im Vergleich zu saprotrophen Pilzen erhöht. Die mit Wurzeln oder Boden assoziierten Pilzgemeinschaften und Arten (analysiert durch Illumina-Sequenzierung) zeigten jedoch keine signifikanten Veränderungen der Zusammensetzung, wenn sie auf der Ebene von 97% Sequenzidentität (operative taxonomische Einheiten) untersucht wurden. Auf der phylogenetischen Klassifikation der Ordnungen beobachteten wir eine moderate Abnahme der Häufigkeiten der Ordnungen Russulales (P + N), während Boletales (P) zunahm. Russulales und Boletales enthalten hauptsächlich mykorrhizalbildende Pilze. Kurzzeitige Änderungen der N und P Einträge hatten somit keine Einflüsse auf die Pilzgemeinschaften, wohingegen langfristige Belastungen mit Nährstoffeinträgen, möglicherweise zu signifikanten Änderungen des vorhandenen Mykobioms führen könnten.

Zusammenfassend lässt sich sagen, dass die Ergebnisse dieser Arbeit die Anpassung der P-Effizienz von Mykorrhizapilzen an die Verfügbarkeit von P im Boden entwirren und die Komplexität biotischer und abiotischer Faktoren hervorheben, die die P-Versorgung von Bäumen in Waldökosystemen steuern. Die Ökosysteme der Buchenwälder zeigten je nach Verfügbarkeit von P unterschiedliche Strategien, um mit verschiedensten Umweltbedingungen ihre P Versorgung aufrecht zu erhalten. Wir bieten Einblicke in P-Akquirierungs- und P-Recycling-Strategien durch den Vergleich der LP- und HP-Bedingungen von Buchenwäldern und ihrer jeweiligen EMF-Gemeinschaft. Recyclingstrategien sind gekennzeichnet durch die P-

Aufnahme von Buchen und die P-Anreicherung von EMF aus der organischen Auflage, in der sich die Feinwurzelmasse und lebenswichtige ektomykorrhisierte Wurzelspitzen ansammeln. Akquisitionsstrategien werden durch P-Aufnahme von Buche und P-Anreicherung von EMF aus dem mineralischen Oberboden identifiziert, in dem sich Feinwurzelmasse und lebenswichtige ektomykorrhisierte Wurzelspitzen zusammenballen. Jede Strategie hat ihre eigenen Pilz-EMF-Gemeinschaften, die die P Solubilisierung aus organischer Substanz unter LP-Bedingungen oder aus mineralischem Boden unter HP-Bedingungen verbessern.

1 Introduction

1.1 Beech forests

In temperate Europe, beech (*Fagus sylvatica* L.) is the most widespread naturally occurring forest tree, which occurs on a broad range of different soil types (Fig. 1.1) (Leuschner et al., 2006). Its distribution area is mainly located in the Central and Western Europe and covers various habitats ranging from mountainous regions in Southern and Eastern Europe to lowlands in Central Europe (Bolte et al., 2007). An analysis of pollen records indicate that the species has spread across Europe from small scattered populations left after the last glaciation and is currently probably at its maximum post-glacial spread (Magri, 2008). Beech needs a growing season of at least 140 days and for this reason cannot survive too far north in Scandinavia (Magri, 2008). High summer temperatures, drought and moisture availability are limiting factors for the distribution of beech in Europe, but continentalism is also associated with limiting beeches spread in north-western regions (Fang et al., 2006).

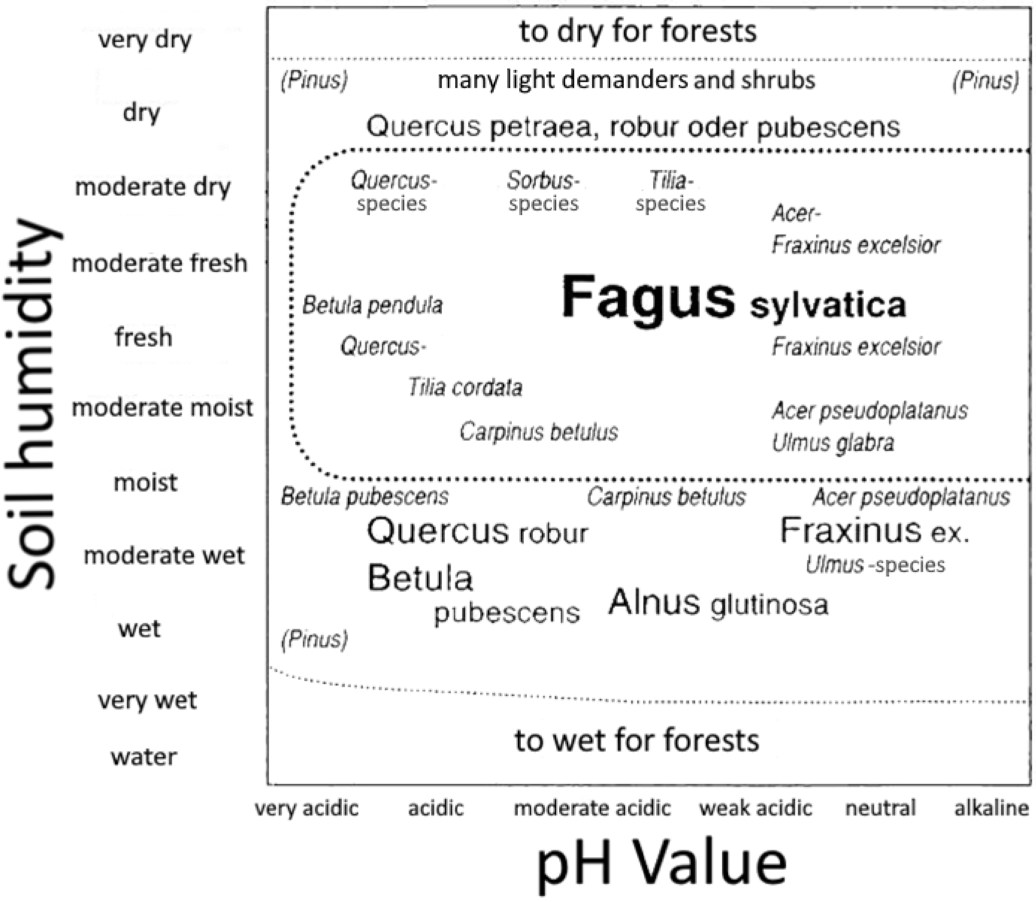

Fig. 1.1: Ecogram of the forest creating tree species of the submontane level of Central Europe. The dashed line roughly contours the domain of *Fagus sylvatica* L.. Source: Leuschner and Ellenberg, 2017

In general, forests provide many ecosystem services ranging from ecological to economic importance. This includes a diverse habitat for many plants and animals, and resources for humans, including wood and recreational options, amongst others (Cardenas et al., 2015; Wood et al., 2017). Due to the high biomass and variation of plants, forests are important for primary productivity and a significant sink of carbon (Bonan, 2008).

Beech has a finely grained and knot-free wood, which is hard, has a pale cream color and shows good workability (Goldstein et al., 1995). Beech trees yield valuable wood (von Wühlisch and Muhs, 2011), its wear resistance, strength and excellent bending capabilities make it ideal for boatbuilding, flooring, stairs, furniture, musical instruments (piano pinblocks), plywood, panels, veneering and cooking utensils such as bowls, platters and wooden spoons (Houston Durrant et al., 2016). It is also used for pulp and can be coppiced for fire wood and charcoal due to its relatively high energetic potential (Horgan et al., 2003; Packham et al., 2012).

Beech forests are of high ecological relevance as potential natural vegetation in Central European Lowland (Fig. 1.2) (Leuschner and Ellenberg, 2017). Several recent studies have shown that beech forests might retreat from vulnerable ecosystems, especially at the edge of their natural range (Jump et al., 2006; Geßler et al., 2007). Climate change may have impacts on the future distribution of the European beech, particularly at the extremes of its range, in the south and east (primarily because of drought) (Kramer et al., 2010). Also beech could expand its spread into Scandinavia and the Baltic (Kramer et al., 2010).

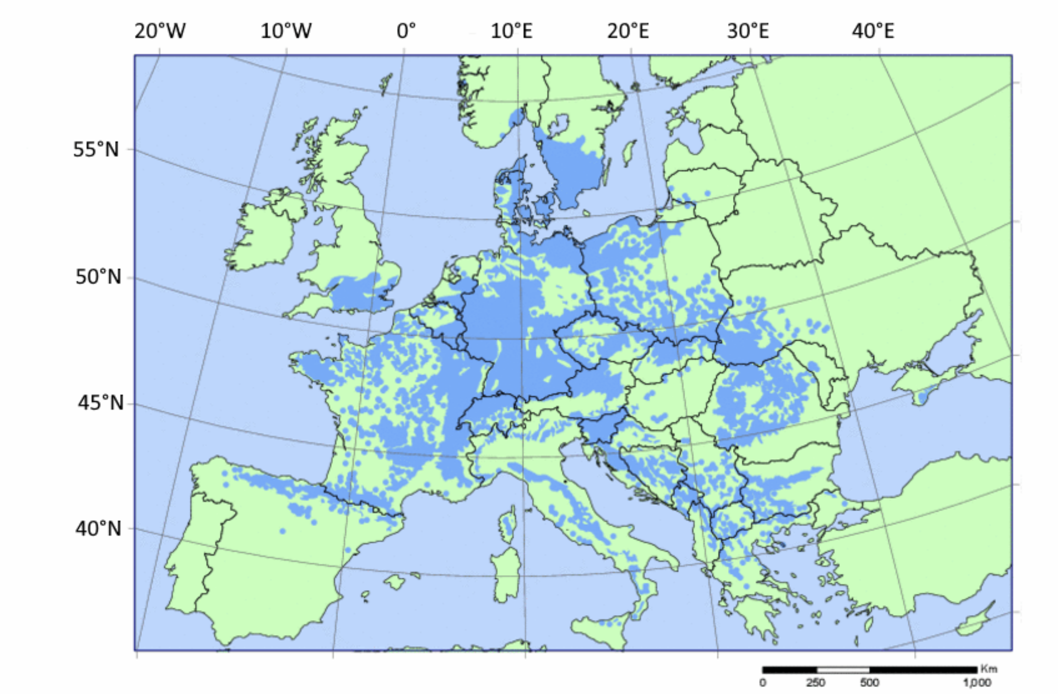

Fig. 1.2: Natural distribution area of *Fagus sylvatica* L.. Blue: occurrence of beech. Source: EUFORGEN, 2009

1.2 Phosphorus

Phosphorus (P) is an essential element for plant nutrition. P is a component of nucleic acids, nucleotides, membrane lipids and ATP. The concentration of total P can be up to 1% of the plant's dry weight (Bieleski, 1973). Plants take up P as inorganic phosphate (P_i, HPO_4^{2-} or PO_4^{3-}) from soil solutions (Schachtman et al., 1998; Plassard and Dell, 2010). In most soils, P is among the least available nutrients for plants (Holford, 1997) because of the formation of precipitates with cations (calcium, iron, aluminum) and absorption to soil particles (Attiwill and Adams, 1993; Holford, 1997; Lambers et al., 2015b; Prietzel et al., 2016) and its low diffusion rates (Shen et al., 2011). In temperate forests, P is bound to organic matter (Ilg et al., 2008; Lambers et al., 2008). Under most conditions this results in a concentration of P_i in the soil below 10 µM, whereas the concentration in the plant is between 5 to 20 mM (Bieleski, 1973).

1.3 Forest ecosystem nutrition strategies

P cycles between the lithosphere, hydrosphere and biosphere in ecosystems. Forest ecosystems have evolved from acquiring systems to recycling systems (Fig. 1.3), the latter present under P-poor soil conditions (Lang et al., 2016). Acquiring systems are characterized by high P inputs from the lithosphere, whereas recycling systems acquire less P from soil but recycle from the biosphere.

The process of soil geological aging leads to reduced P levels (Turner and Condron, 2013). Furthermore the P input into the soil by P deposition is extremely low (Peñuelas et al., 2013). In addition, a chemical shift of plant available to unavailable organic bound phosphate (Walker and Syers, 1976; Callaway and Nadkarni, 1991; Chadwick et al., 1999; Vitousek et al., 2010; Vincent et al., 2013) diminishes the plant available P in the soil.

Sequentially, during plant evolution several morphological, physiological and molecular strategies have been developed to overcome this limitation (Vance et al., 2003; Lambers et al., 2008, 2015a,b), resulting in tighter nutrient cycles on P-poor than on P-rich sites (Lang et al., 2016). Thereby, P wastage from the biogeochemical cycle is minimized, because organic fractions like leaves, twigs and fruits are degraded and the released P is being used by plants and microbes (Jobbágy and Jackson, 2004; George et al., 2011; Jansa et al., 2011: Lang et al., 2017). Thus, HP and LP sites differ significantly in the turnover rate of the organic matter ranging from 1/5 a^{-1} for high-P to 1/40 a^{-1} for low-P, due to different soil pH (Lang et al., 2016).

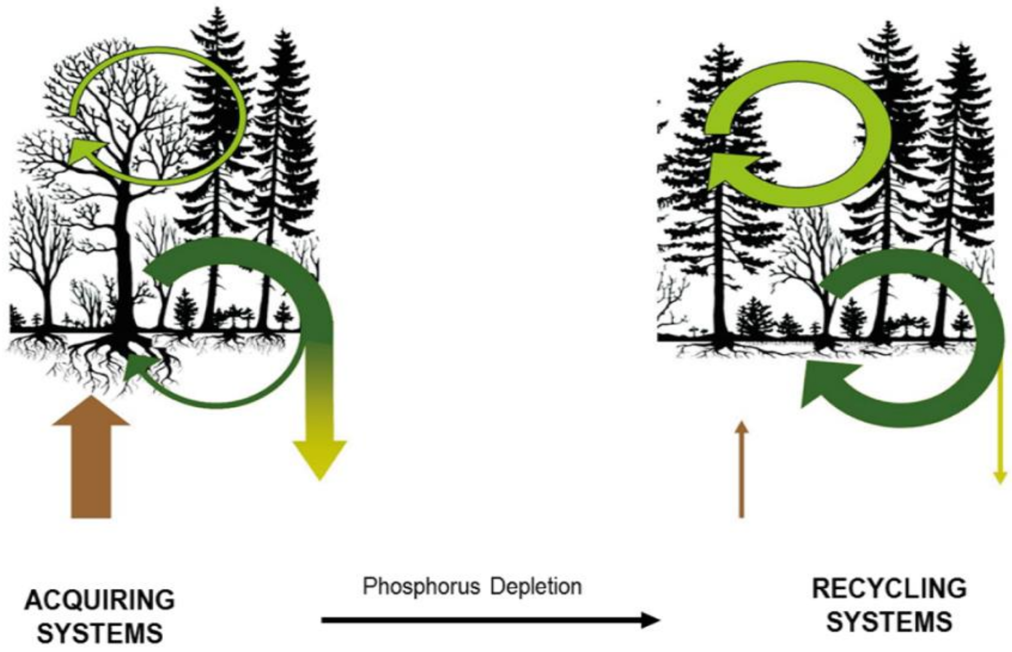

Fig. 1.3: Model of P-acquiring and P-recycling systems. Brown: P from soil, which is taken up into the biological circle of soil microbes (bacteria and fungi); Dark Green: P in roots and in the forest floor; Bright Green: P in plants; Yellowish Green: P bound to the lithosphere. Thickness of the arrows indicates the amount of P. Source: Lang et al. 2016

1.4 Plant adaptations to low P

Plants are able to sense the P availability locally at the root tips and systemically in the whole plant but a P receptor is not known until now (López-Arredondo et al., 2014; Baker et al., 2015). A signaling system causes plants to react when the P availability is not sufficient to maintain metabolism and growth (P starvation).

Cellular changes in metabolism, physiology, hormonal balance and gene expression lead to two categories of P starvation responses: 1) enhanced acquisition of P and 2) increased efficiency of P (Lajtha and Harrison, 1995; Vance et al., 2003). To economize the available P, plants reduce their growth rate, re-use and recycle P from cell compounds like membrane lipids (Nakamura, 2013; Siebers et al., 2015; Xu et al., 2020) and storage tissues (Netzer et al., 2017; Zavisic and Polle 2018). Increased acquisition of P includes a restructuring of the root system as shown for *Arabidopsis* with longer primary and lateral roots, accompanied by an increased density of first-order lateral roots (Gruber et al., 2013). Restructured root systems for enhanced P acquisition often lead to an increased root:shoot ratio with higher branching intensity of the roots, accelerated root proliferation (Lambers et al., 2006), increased root surface area by root hair initiation and elongation (Hammond and White, 2008). Restructured root systems involve an increased sucrose transport from shoot to root (Xu et al., 2020) by enhanincg the transcription levels of genes related to sucrose transport (Koren et al., 2017) to sufficiently satisfy the carbon demand of the root system (Cheng et al., 2014) and to induce the root system architectural adjustments

(Lemoine et al., 2013). Sucrose further acts as a global regulator for the expression of phosphate starvation-induced genes, which are involved in P_i signaling, transport, mobilization and allocation (Lei et al., 2011; Kavka and Polle, 2017). Plants increase the availability of recalcitrant P by exudation of organic acids and by the secretion of phosphatases, which release organically bound P (Neumann and Römheld, 2007; Hofmann et al., 2016). These mechanisms are especially efficient when coupled with an elevated expression of high-affinity P-transporter activities (Smith et al., 2002; Vance et al., 2003; Kavka and Polle, 2017).

1.5 Study sites

The priority program SPP 1685 ‘Ecosystem Nutrition, Forest Strategies for limited Phosphorus Resources’ (Lang et al., 2016) uses five temperate forest sites across Germany. The three selected sites of this thesis (Fig. 1.4 and Fig. 1.5) are Level II sites (monitoring program ICP Forests for comprehensive information on forest condition in Europe), which have been operated by federal forest institutions for about 15 years. All sites sustain mature mono-specific beech stands but differ in their P stocks from 2966 mg per kg to 195 mg per kg of the A-horizon (0-5 cm) (Lang et al., 2017). The location of the study sites, climate, stand and soil characteristics are shown in table 1.1, which is based on data provided by Lang et al. (2017).

Fig. 1.4: The three selected forest sites of this thesis in Germany. Source: Simon Clausing, 2018

The gradient (BBR > MIT > LUE) in P stocks and availability is a consequence of different parent materials of the soils (Lang et al., 2017). The soils at BBR have developed from basaltic rock, those at MIT originate from paragneiss rock and those at LUE from sandy glacial till (Lang et al., 2017). In Lüss, organic layers and soil types are classified as a mor-like moder forest floor on hyperdystric folic cambisols developed from sandy till. At the Mitterfels site, organic layers and soil types are classified as a moder forest floor on hyperdystric chromic folic cambisol developed from paragneis. In Bad Brückenau site, mull-like moder forest floors on a dystric skeletic Cambisols developed on alkaline igneous rocks/metamorphites (Haußmann and Lux 1997). Furthermore the sites differ in P input by the leaf litter ranging from 0.229 g per m² and year to 0.156 g per m² and year showing a P gradient pattern similar to that in the soil P (BBR > MIT > LUE) (Lang et al., 2016). The thickness of the forest floor layer shows the opposite pattern, ranging from 7cm to 5cm to 3cm (LUE>MIT>BBR) with slow turnover rates at LUE (39 years), medium turnover rates at MIT (14 years) and fast rates at BBR (5 years) (Lang et al., 2016). Until now it is unclear if the different sites maintain their P supply from the same soil layers and if the P uptake efficiency differs between the sites.

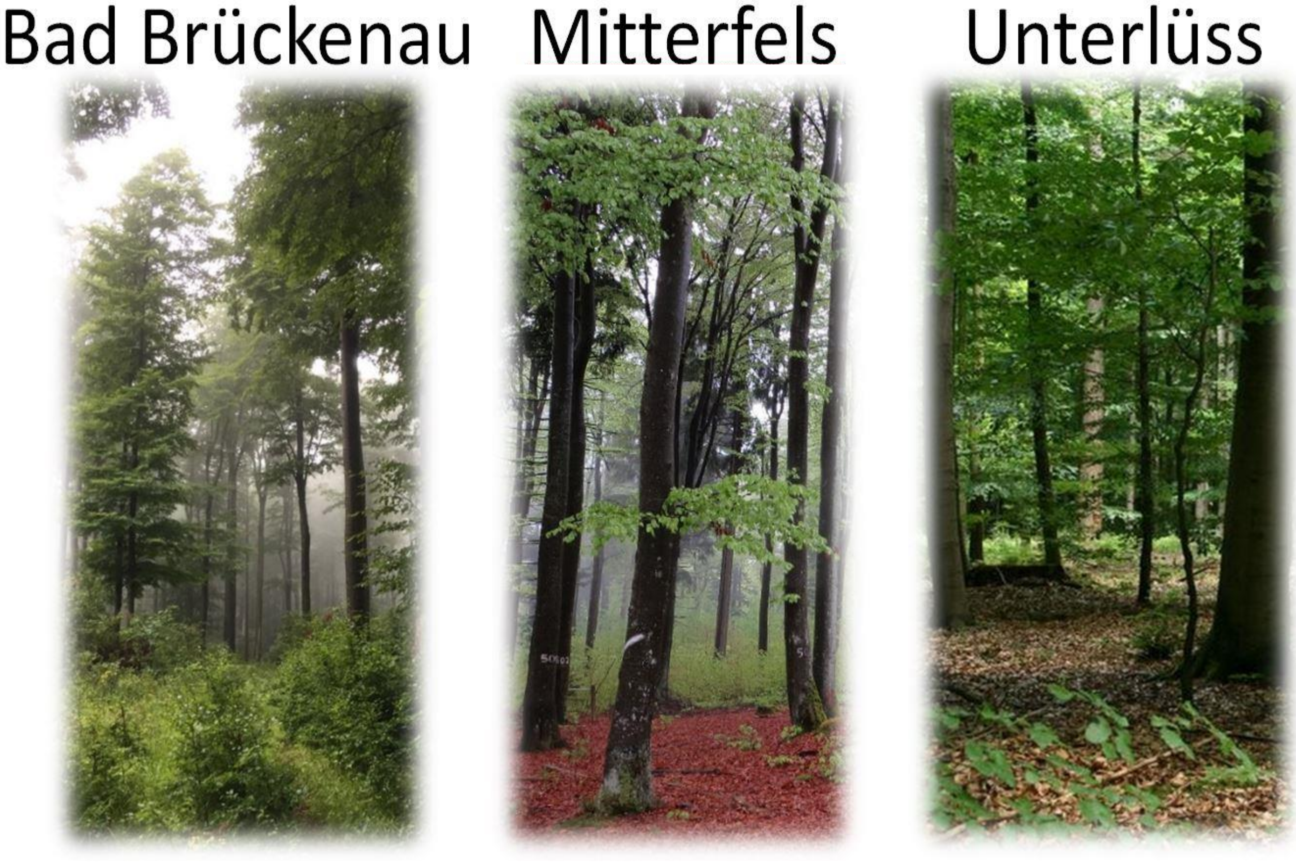

Fig. 1.5: The experimental forest sites Bad Brückenau (HP), Mitterfels (MP) and Unterluess (LP) forming a natural P gradient. Source: Simon Clausing, 2017

Table 1.1: Characteristics of the research sites in the high-P (HP, Bad Brückenau), the medium-P (MP, Mitterfels) and the low-P 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. Extractable P was determined with the resin method.

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°1.6'2.3"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 (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	3.6	3.5
Total P (mg kg^{-1})	2966	1375	195
Extractable P (mg kg^{-1})	116	70	11
P in leaf litter (g m^{-2} a^{-1})	0.229	0.213	0.156
P in leaves (mg g^{-1} dry mass)	1.41	1.66	1.21

1.6 Soil Fungi

Soil fungi are essential components of ecosystems and consist of three key trophic guilds: symbiotrophs, saprotrophs and pathotrophs (Kernaghan, 2013). Saprotrophs enable nutrient cycles (Větrovský and Baldrian, 2013; Treseder and Lennon, 2015), pathogens manage species composition (van Agtmaal et al., 2017) and symbiotrophs provide mutualistic benefits to their host species. Therefore, fungi occupy a key position in biogeochemical processes (Tedersoo et al., 2014) and in soil health (Raaijmakers and Mazzola, 2016).

Sugars directly usable as energy source are scarcely available in soil (Nehls et al., 2007), but are exudated by plant roots into the rhizosphere and crucial for root-associated fungal communities. Saprotrophs obtain carbon mainly from leaf litter but also from dead roots or dead ectomycorrhizal hyphae, while pathogenic fungi obtain carbon from living or dead root cells depending on biotrophic or necrotrophic types (Kernaghan, 2013). Symbiotrophic fungi that form associations with plants obtaining their carbohydrates from the photo assimilated carbon of the plant. It is unknown how a shortage of this exchange affects the host P supply.

Mycorrhizal, endosymbiotic relationships with roots have evolved for almost 90% of the land plants (Smith and Read, 2008).

Ectomycorrhizal fungi (EMF) are associated with high amounts of forest tree biomass across the northern hemisphere (>50%) and lower amounts at the equatorial zone (Fig. 1.6) and benefit by photosynthetic carbon in exchange for increased nutrient supply (Smith and Read, 2008; Courty et al., 2010).

In natural ecosystems, which are limited by N, K or P, EMF play a critical role in improving the uptake of plant nutrients (Johri et al., 2015). In boreal and temperate forests specific EMF communities are formed to mobilize sparse soil nutrients (Buscot et al., 2000; Read and Perez-Moreno, 2003). Tracer studies revealed not only nutrient mobilization by EMF from soil resources (Uroz et al., 2013; Lindahl and Tunlid, 2015) but also enhanced host plant nutrition due to EMF symbiosis (Finlay et al., 1988; Ek et al., 1997).

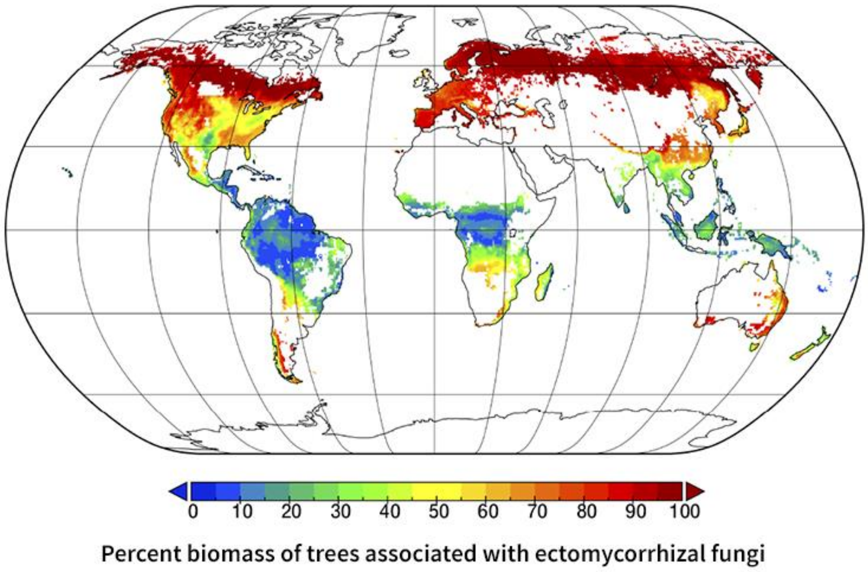

Fig. 1.6: Biomass of trees associated with ectomycorrhizal fungi across the globe. Color gradient from dark blue: low ratio; to dark red: high ratio. Source: Steidinger et al. 2019

The exudation of organic acids by microbes and roots improve P solubilization (Plaxton and Tran, 2011) and is crucial for Al- and Fe-bound soil P (Jones and Oburger, 2011; Prietzel et al., 2016). Furthermore, extracellular phosphatases secreted by microbes and plant roots cleave organic-bound P (Tran et al., 2010; Hinsinger et al., 2015; Smith et al., 2015; Chen and Liao, 2016). The amount of secreted acid phosphatase into the rhizosphere depends on the P content in the soil, with higher levels at P-poor than at P-rich forest soils (Hofmann et al., 2016). EMF obtain P and K from minerals by bioweathering (Wallander, 2000; Balogh-Brunstad et al., 2008; Jansa et al., 2011). This includes hyphal tunneling (Jongmans et al., 1997; Hoffland et al., 2003) as well as the secretion of extracellular polymeric substances

(EPS) that enhances the effective surface area of contact between fungi and minerals (Gazzè et al., 2013).

Environmental factors such as soil properties and vegetation type affect root associated fungal communities of *Quercus* (Bougoure et al., 2007), *Ericaceae* (Maghnia et al., 2017) and *Fagus* (Lang et al., 2017; Pena et al., 2017; Nguyen et al., 2020). Root associated fungi are recruited from the near soil in beech forests and poplar plantations (Danielsen et al., 2012; Goldmann et al., 2016). Soil associated fungal communities are mostly influenced by soil properties, geographic location, climatic conditions and vegetation (Goldmann et al., 2016).

1.7 N deposition and global change

Nitrogen (N) can be a limiting growth factor in terrestrial ecosystems for organisms (Vitousek and Howarth, 1991). N is converted into multiple chemical forms, while it circles between the atmosphere, terrestrial and marine ecosystems (Fowler et al., 2013). Important steps are fixation, ammonification, nitrification and denitrification.

The usage of chemical N fertilizers in agriculture and fossil fuel combustion lead to an increased N deposition on a global scale (Fig. 1.7)(Galloway et al., 2004, 2008).

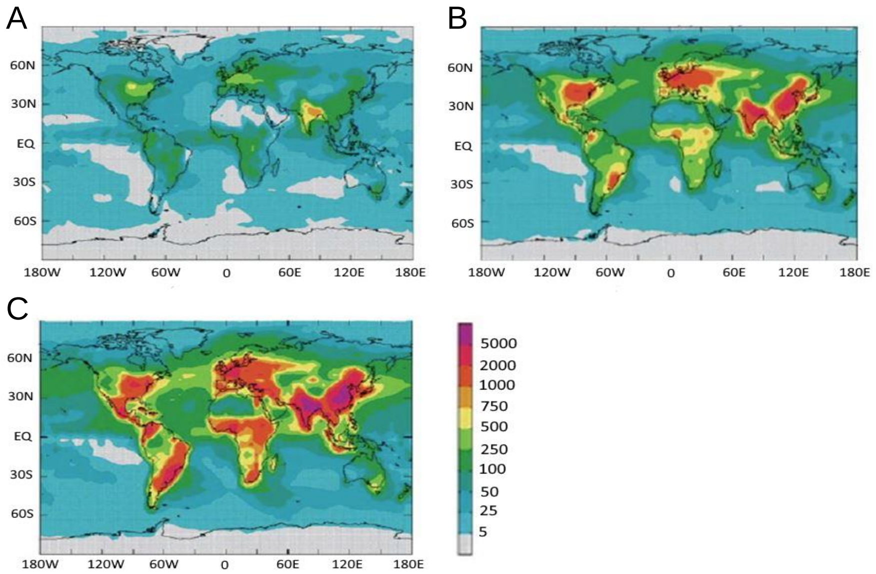

Fig. 1.7: Global spatial patterns of total inorganic nitrogen deposition in 1860 (A), 1990 (B) and 2050 (C). Values of 2050 depend on a developed model based on various datasets. Source: Galloway et al., 2004

The dominant contribution of the annual release of N into the atmosphere is based on human activities (Galloway et al., 2004; Fowler et al., 2015). Terrestrial ecosystem processes, for example the soil N cycle, in particular the mineralization-immobilization turnover, are directly modified by elevated N levels (Lu et al., 2011). N deposition is boosting the primary production in N-limited forest ecosystems (Du and De Vries, 2018; Schulte-Uebbing and De Vries, 2018). The stimulating effect on forest growth subsides with increasing time due to additional effects, such as unbalanced nutrient ratios (Crowley et al., 2012).

N in combination with P surpasses the enhanced effect of N on primary production in terrestrial ecosystems (Elser et al., 2007). Since N accumulation can stimulate phosphatase activity the availability of P for plants may increase (Marklein and Houlton, 2012) and can induce soil acidification, which enhances concentrations of inorganic phosphate and extractable P (Malhi et al., 2003; Stroia et al., 2011). Soil acidification is mainly caused by the oxidation of N, Carbon and Sulphur, which result in the release of protons (Bolan et al., 1991).

Many studies showed a decrease of soil microbial biomass (Castro et al., 2010; Liu and Greaver, 2010) and a shift of composition and functional capacity of bacterial communities (Cheng et al., 2017; Melillo et al., 2017; Li et al., 2018) by N deposition. In addition, reductions of soil fungal biomass (Frey et al., 2014; Bahr et al., 2015) as well as an altered fungal community composition are reported (Morrison et al., 2016). Altered communities are a consequence of the proliferation of nitrophylic fungal species (Morrison et al., 2016).

N availability has strong effects on composition, function and diversity of EMF communities (Kranabetter et al., 2009; Lilleskov et al., 2011) depending on the N concentration (de Witte et al., 2017). Increased N deposition leads to decreased richness and shifts in community composition of EMF for beech, conifer, oak and spruce forests (Lilleskov et al. 2002a, 2008; Cox et al. 2010; Bahr et al. 2013; Suz et al. 2014; de Witte et al., 2017). Naturally N-rich soils can also show high mycorrhizal diversity (Kranabetter et al., 2009). In contrast, moderate N increases cause higher fungal richness of symbiotrophic species (Cox et al., 2010; van der Linde et al., 2018; Nguyen et al., 2020), whereas He et al. (2016) detected stable fungal species richness under N treatment for alpine meadows. Excessively high inorganic N additions are reported to change EMF community structures (Peter et al., 2001; Lilleskov et al., 2002b). Nevertheless, it is important to keep in mind that fungi use different forms of N. Fungi preferring inorganic N tended to be positively influenced by N deposition and fungi preferring organic N tended to be negatively affected by N deposition (van der Linde et al., 2018). Shifts result in altered rates of soil nutrient cycling which regulate nutrient availability to plants. At the plant level, fine root length, EMF root colonization, EMF diversity and production of extramatrical mycelium decreased significantly in response to enhanced N availability (de Witte et al., 2017).

In contrast to N deposition the P deposition is quite low and typically confined to specific regions in the world (Fig. 1.8) (Peñuelas et al., 2012). This results in unbalanced human-induced N and P inputs into the biosphere, although being extremely variable geographically. The inputs increase continuously in the Northern Hemisphere (Peñuelas et al., 2012) and these unbalanced nutrient inputs are very likely to alter the environment (Peñuelas et al., 2013).

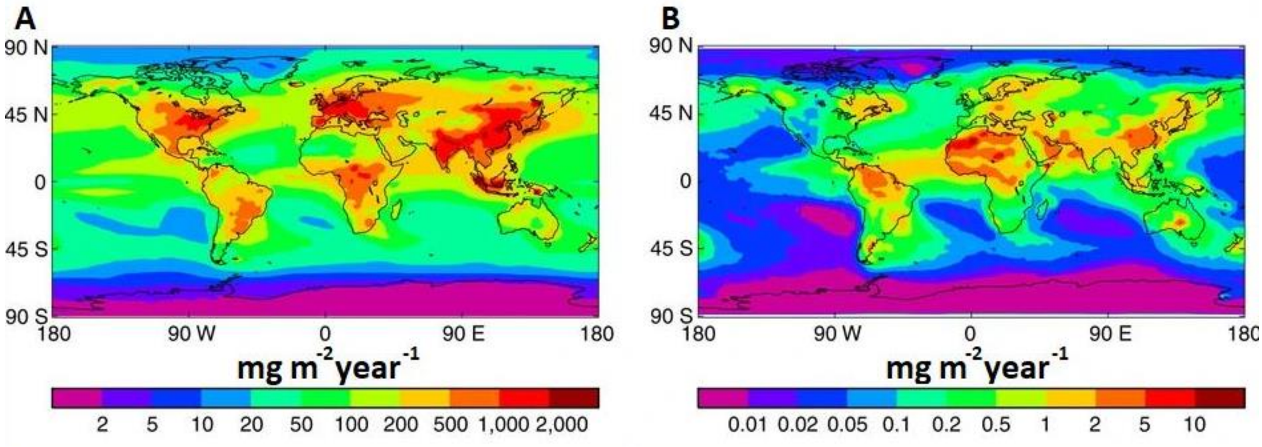

Fig 1.8: Total nitrogen (A) and total phosphorus (B) deposition between 2000 – 2010. Only phosphorus as phosphate is considered. Source: Peñuelas et al., 2013

1.8 Objectives

Recent large-scale surveys across the European continent detected declining foliar P concentrations of beech (Talkner et al., 2015) as well as N, P and K throughout the last decades (Penuales et al., 2020) with site specific heterogeneity (Lang et al., 2017). For this reasons it is important to research the reactions of beech forest ecosystems and their respective ectomycorrhizal community under different P regimes. Especially the vertical distribution of P stocks between the different experimental sites and consequences of limitation or addition of macronutrients as well as changes of climatic conditions are important concerning the maintance and management of forest ecosystems.

The main objective of this thesis was to characterize the P acquisition strategies of beech (*Fagus sylvatica* L.) under different P availabilities in soil solution.

In “Mycorrhizal phosphorus efficiencies and microbial competition drive root P uptake“ we address the following question: “Is the P uptake efficiency and allocation to beech roots higher under LP than under HP conditions?” Since the vertical distribution of P stocks differs between HP and LP forests, we examined the soil layers separately. We quantified differences of the main soil layer for beech P supply, the contributions of mycorrhizal and non-mycorrhizal root tips to P_i acquisition and the competition between beech roots, microbes and other plants roots for P uptake. Therefore, we used intact soil cosms from a P-rich and a P-poor beech forest with intact organic layer and mineral topsoil including the natural root system and traced P uptake of roots and microbes with ^{33}P (chapter 2).

In “Carbohydrate depletion of roots impedes phosphorus nutrition of forest trees” we approach the following questions: “How important is recent photoassimilated carbon for P nutrition? Does carbohydrate depletion leads to decreased root P concentrations, with stronger effects under LP than under HP conditions?” We examined the P concentrations and enzyme activities (internal P mobilization in roots and P mobilization from soil) under C shortage. We installed plots in a P-rich and a P-poor beech forest with young beech trees by trenching and trees were girdled under the lowest branches. We harvested one and eight weeks after the girdling treatment. (chapter 3).

In “Impact of nitrogen and phosphorus amendments on resident soil and root mycobiomes in beech forests ” we deal with the following questions: “How does P and N fertilization affect soil and root associated fungal communities under different P conditions and functional composition in belowground habitats with increasing distance from the roots?” We wanted to reveal the effects of P and N fertilization on soil and root associated fungal communities of the organic and mineral top soil. We evaluated shifts in community and functional compositions and their diversity parameters (richness, evenness, diversity), whereas fungal orders reveal distinct patterns depending on their habitat. However, those specializations fade on trophic guild level. To address these goals, we excised soil cores from three well-characterized beech forests, which constitute the high-P, medium-P and low-P ends of a geosequence. The plots have been fertilized regularly with either P, N or P & N over a period of two years (chapter 4).

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Chapter 2

Mycorrhizal phosphorus efficiencies and microbial competition drive root P uptake

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Summary Chapter 2

Phosphorus (P) concentrations and contents show large differences among different soil types, affecting P nutrition of forest trees across different ecosystems. Chemical binding of P to soil particles and different organic fractions affect distribution of P between soil and solution, governing soluble P concentrations upon which plants, their associated mycorrhizal symbionts, and microbes feed. The aim of this research was to quantifiy root P uptake by mycorrhizal and non-mycorrhizal root tips in rivalry with microbes *in situ* in the organic and mineral layer of a P-rich and a P-poor forest. We used intact soil cores (0.2m depth) including the organic and mineral layer from two beech (*Fagus sylvatica* L.) forests to trace ^{33}P in different fractions (soil, plant and microbial). We used the dilution of ^{33}P to naturally P in the rhizosphere of each soil layer separately to rate the accumulation with new P in mycorrhizal and non-mycorrhizal root tips and P uptake of fine roots. Root P uptake occurred under HP conditions with 25% and 75% in the organic and mineral layer, respectively, whereas under LP conditions, 60% occurred in the organic and 40% in the mineral layer. Enrichment of new P in mycorrhizal root tips was differed between soil layers and was analysed as mycorrhizal P efficiency. Root tip abundance didn’t correlate with root P uptake, whereas mycorrhizal P efficiency correlated well. This analyse punctuates the meaning of the regulation of mycorrhizal P acquisition for root P supply. Further the mycorrhizal communities differed between forests but not between soil layers. Hence, different P efficiencies resulted from physiological customizations of the symbionts. In general, non-mycorrhizal root tips were scarce and revealed lower enrichment with new P than mycorrhizal root tips. Their share to root P sustenance was inconsiderably low. Microbes were no rivals for P in P-rich soil but in P-poor soil. Understory roots occured in P-rich soil but did not compete for P resources. Our results expose regulations of mycorrhizal P efficiencies and accentuate the complexity of abiotic and biotic factors that regulate P nourishment of trees in forest ecosystems.

Chapter 3

Carbohydrate depletion in roots impedes phosphorus nutrition in young forest trees

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Summary Chapter 3

Tree health in European forests is impaired by nutrient imbalances but the subjacent physiological mechanisms are unclear. We assayed the ramifications of shrinking root carbohydrate pools for phosphorus (P) mobilization and uptake by forest trees. We performed our study in deciduous beech (*Fagus sylvatica*) forests grown on P-rich or P-poor soil. In an area with young, naturally grown trees, half of the saplings were girdled and the other half was used as controls in a divided plot. We ascertained P mobilization acticities of roots, ectomycorrhiza and microbes in the organic layer and mineral top soil. Root carbohydrate reserves decelined and phosphoenolpyruvate carboxylase activities, connecting carbon and P metabolism, increased. Acid phosphatase activities of roots and ectomycorrhizal species increased, as well as the abundance of bacteria catalyzing steps in P turnover. Whereas soil enzymes involved in P mobilization remained stable. The feedbacks to girdling were stronger in P-poor than in P-rich forests. P uptake, traced by ^{33}P, was reduced after girdling. Nevertheless, soluble and total P concentrations of roots were constant, but fine root biomass dropped. Our results endorse that carbohydrate fading leads to constraint P uptake, amplified internal P remobilization to handle P limitation, and root biomass trade-off for upkeep of P homeostasis. Since loss of root biomass make trees more drought vulnerable, our results connect tree impairment with malfunction of P supply as the results of decreased belowground carbohydrate distribution.

Chapter 4

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

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Summary Chapter 4

In forest soils, the pools of N and P available for soil microbes and plants are highly dependent on atmospheric inputs and soil properties. Here, we ran a P and N fertilization experiment to enlighten the effects of nutrient availability from those of other habitat factors on soil fungal assemblages at three scales (soil-residing, root-associated and ectomycorrhizal fungi) along a natural P gradient in beech (*Fagus sylvatica*) forests. We checked the hypothesis that in P-poor forests, P fertilization generates enhanced fungal diversity across the three levels, resulting in increased P nutrition of beech and that N fertilization leads to P shortage, changing the fungal communities towards nitrophilic species. In the third year after the begin of the fertilization treatments (1x 50kg ha^{-1} P, 5x 30kg ha^{-1} N), the labile P soil fraction and total P in roots were enhanced. Total P of roots decreased by N treatment and increased by P addition at the low P site. Ectomycorrhizal species richness was unaffected by P or N addition in any habitat, but their relative abundance increased compared to that of saprotrophic fungi. At the phylogenetic level of taxa, fungal communities were unaltered by fertilization treatments, but at the classification of orders, contrasting reaction patterns for saprotrophic soil and ectomycorrhizal root oders were detected. Russulales decreased in response to N+P and Boletales increased under P addition. In conclusion, our study shows resistance of fungal community structures to short-term variation in N and P inputs. The shifts in fungal orders suggest that chronic exposure to increased nutrient inputs into forest ecosystem may overcomes the resistance of the resident mycobiome structures.

5. Synopsis

5.1 Conclusion

Our studies reveal different importance of the separate soil layers at the different study sites for tree P nutrition. While the fractions of a thick organic layer soak up newly added P (LP conditions) and only small amounts reach the mineral layer, we observed the opposite under HP conditions, where the organic layer is rather thin. This finding must be taken into account for the fertilization treatment. Here we analysed shifts of fungal communities by applied P and N. With the results from the soil core experiment in mind, it may be, that the applied elements allocate in different layers at the different sites. Under LP conditions most of the fertilizer should accumulate in the fractions of the organic layer, especially in rhizosphere and microbe fractions, as well as insoluble P in the bulk soil. This can be an explanation of the resilient EMF community at the LP site under P fertilization. The major amount of the applied P is directly bound. In contrast to HP conditions, where newly added P leaches into the mineral layer and distributes evenly between rhizosphere, microbes and roots. Also the fraction bound as insoluble P in bulk soil is smaller. We revealed only little effects in NH_4^+ and NO_3^- concentrations by the treatment, occurring only in the OL of LP and MP. No effects were detected in the ML of all sites.

An intact undisturbed forest floor is of tremendous importance under nutrient-poor conditions for uptake and recycling of P. Our study provides insights into the mechanisms that contribute to the adaptation of ecosystems to LP availability. Obviously, forests that rely on P recycling with the majority of roots in the upper layer are at risk under climate change. When the duration and intensity of summer droughts increase, the roots and microbes of top soil layers will be the first to be negatively affected by water shortage. The weather conditions during the NxP experiment resulted in decreased vitality of root tips between the seasons at the LP site of the mineral layer. If the climatic condition persists, the beech roots of the organic layer may also suffer under low water availability.

Carbon assimilation and translocation to belowground biomass, which is essential for P mobilization, is retarded at low water availability (Ruehr et al., 2009) and will increase due to global warming. Additionally reduced levels of carbon by photosynthesis lead to reduced levels of available energy for the whole plant, resulting in reduced plant performance. This vulnerability can manifest in reduced fine root biomass with lower numbers of vital root tips, which are crucial for P uptake due to the symbiosis with EMF. A sufficient P supply is necessary for tree nutrition and resistance against drought (Sardans and Peñuelas, 2007). Depletion of P is expected in the future due to lower P mineralization rates (Schimel et al., 2007), lower P mobility in drier soil (Schachtman et al., 1998; Kreuzwieser and Gessler, 2010), N deposition and soil acidification (Vitousek and Howawth., 1991; Peñuelas et al., 2013). P metabolism and uptake of plants and associated EMF, as well as their community composition, are more susceptible to limitation of carbohydrates and changes of macronutrients than the associated soil-located processes and fungal communities. Here we only found small effects by the NxP fertilization.

Our results propose that trait conservatism in related species granted resistance of the resident mycobiome community to nutritional changes. We observed that P fertilization leads to a decrease in RAF richness and increase in the relative abundance of Boletales. At the scale of distinct taxa (OTU-based) these changes were not detectable, implying that individual responses were small but dispers across a group of related species, thus, accumulating to measurable effects at higher phylogenetic levels. Considering the increase of the relative abundance of symbiotrophic fungi of SAF and RAF, while root P decreased regardless of the soil P contents under N fertilization, N additions may lock P in fungal biomass with negative consequences for tree P nutrition.

This result emphazises the relevance to differentiate habitats and to include the major nutrients to better understanding the driving factors of fungal communities in relation to nutrient cycling.

In total, forest types with different P stocks revealed distinct reactions controlling P nutrition, but extra soil factors, including chemical parameters, soil texture, microbial composition and vegetation should be included in future analysis.

5.2 Outlook

Dirks et al. (2019) suggested that drought and N deposition will likely decrease the P availability and that it is unclear how plant strategies for decreased P resources respond to elevated N deposition and summer droughts. Furthermore, the secretion of phosphatases and expression of high affinity P_i transporters are expected to be negatively mediated by drought (Sardans and Peñuelas, 2007; Lang et al., 2016) as well as the mycorrhizal symbiosis (Kleczewski et al., 2010). Due to less pronounced effects for N uptake by drought, plant P storages will be reduced (Sardans and Peñuelas, 2007) and a further divergence of the N:P ratio will appear (He and Dijkstra, 2014).

Therefore it is essential to gain deeper insights into P acquisition and nutritional strategies. It would be very interesting to analyse the transcriptomes of beech fine roots of the different layers (organic layer and mineral layer) and leaves of HP and LP forests.

This could complete the model of the importance of the different layers for the contrary acquisition strategies. Especially worthwhile are P transporter genes like PHT1-1, a high-affinity transporter for external inorganic phosphate, and PHT2;2, a sodium-phosphate symporter which plays a fundamental housekeeping role in phosphate transport. Several studies showed that the root specific PHT1-1 gene

expression was enhanced under P deficiency for tobacco (Mitsukawa et al., 1997), arabidopsis (Shin et al., 2004) and poplar (Kavka and Polle, 2016), whereas PHT2-2 showed higher expression levels in leaves compared to roots (Kavka and Polle, 2016).
Stress indicative genes like abiscisic acid (ABA), 9-cis-epoxycarotenoid dioxygenase (NCED) and protein phosphatase 2C (PP2C) should also be analysed. ABA is known to respond to P deficiency in arabidopsis and barley (Vysotskaya *et al.*, 2016; Yu et al., 2016) and levels increase upon P starvation (Zhang et al., 2014). Fang Zhu et al. (2018) showed that an ABA mechanism regulates P re-utilization of cell walls under P-deficient conditions in rice. Due to this impact of ABA, also NCED as key limiting enzyme of the ABA biosynthetic pathway (Huang et al. 2018) and PP2C as negative regulator to the ABA signaling pathway (Manohar et al., 2017) are of high interest. In addition, PP2C is enriched under P deficiency in arabidopsis (Lan et al., 2013) and related to P starvation tolerance in sorghum (Zhang et al., 2019).
Furthermore, N responsive genes like nitrate reductase (NR), glutamine oxoglutarate aminotransferase (GOGAT) and glutamate dehydrogenase (GDH) under different P conditions or especially N addition treatments are worth analysing under increased N depositions in the future resulting in shifted soil N:P ratios. Gan et al. (2016) showed for poplar that reduced P availability leads to higher N concentrations in roots and lower N concentrations in leaves resulting in reduced activity of N-related enzymes (NR, GOGAT, GDH).
The NxP setup would allow analysing the impact of P and N addition on beech itself. Mostly EMF rich genera were influenced by the fertilization treatments. This observation hints at shifting chemical and morphological parameters of beech. Thus P concentration and fine root biomass can result in valuable information, as well as the total P content of roots, which already revealed insights in nutrition strategies, during the girdling experiment. Supplementary data on fine root growth over the year could yield insights into the balance between P uptake and root production.

Of similar importance are the ectomycorrhizal fungi. Here it would be interesting to analyse the P uptake of individual species with radioactive isotopes. I expect similar uptake behavior by similar species from the organic layer regardless site conditions, whereas those from mineral top soil exhibit highly divergent P uptake depending on soil P concentrations. The ideal experimental setup would be the use of intact young beech trees from different forest types in the laboratory with ectomycorrhized root tips. Those can be either used for radioactive P tracer studies or morphotyping and ITS sequencing.

Additionally, it would be very interesting to investigate even more different forest types like calcareous forests. This thesis deals only with silicate forest types. Due to differences in nitrogen uptake compared to acidic soils (Leberecht et al., 2016) it is worthwhile to investigate fungal community compositions as well as P uptake.

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