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PLASTICITY OF BEECH SAPLINGS (*FAGUS SYLVATICA* L.) IN RESPONSE TO SOIL P AVAILABILITY.

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presented by

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Table of contents

Abbreviations

ANOVA	Analysis of Variance
BBR	Bad Brückenau (high P site)
C	Carbon
CAP	Canonical Analysis of Principal Coordinates
LUE	Unterlüss (low P site)
MUF	4-methylumbelliferone
N	Nitrogen
P	Phosphorus
PA	Potential phosphatase activity
PCO	Principal Coordinate Analysis
PERMANOVA	Permutational Analysis of variance
PL	Plasticity index
RE	Resorption
RGB	color model (Red, Green, Blue)
OTU	Operational taxonomic unit

Abstract

Forests dominated by beech (*Fagus sylvatica* L.) cover a large part of Europe and occur on a wide variety of soils, realizing a broad ecological niche in terms of soil chemical properties including pH, base saturation, and plant-available phosphorus (P) pools in the mineral topsoil. On the other hand, a decrease in foliar P concentrations and a corresponding increase in the nitrogen (N) to P ratio during the last decades all over Europe raised questions about a possible reduction in forest health and productivity due to P limitation. These changes in leaf nutrient status have been attributed to continuing high N deposition and increasing atmospheric carbon dioxide concentrations. Both accelerated tree growth due to high N and carbon (C) input and adverse effects of elevated N on fine root biomass, mycorrhization, and litter mineralization appear to have created a higher need for other nutrients that cannot be met by the supply from the soil. Surprisingly little is known about internal P allocation of beech and its capacity to deal with changing soil P availability in the short-term. Considering the distribution of beech forests on soils encompassing a broad range of nutrient availability, and thus proven ability to adapt to different soil conditions in the long-term, we hypothesized that this tree species exhibits a high phenotypic plasticity allowing it to alter multiple traits in response to local nutrient availability leading to adaptive acclimation and resulting in a phenotype tailored for local soil conditions.

This PhD-thesis aimed to investigate acclimation of beech from high to low and from low to high soil P availability in terms of plant internal P cycling and plant-soil interactions in the rhizosphere.

For this purpose, we grew two groups of 12–15-year-old beech saplings originating from sites with high and low soil P availability in mineral soil (Bh/Bv horizon) from their own site and soil from the other site in rhizoboxes for two years in the greenhouse. We assumed that our saplings were adapted to their soil of origin and that their P nutritional status thus reflected the nutrient availability in the soil of origin.

First, we assessed the plasticity of mass and P allocation in the beech saplings aiming to identify the traits most responsive to current soil conditions. After two growing seasons, P concentrations in leaves and stem, as well as mass allocation to leaves and fine roots were affected by both soil and plant origin. By contrast, P allocation to leaves and fine roots, as well as P concentrations in

fine roots, were determined almost entirely by the experimental soil. Independent of the P nutritional status defined as average concentration of P in the whole plant, which still clearly reflected the soil conditions at the site of plant origin, P allocation to leaves was a particularly good indicator of P availability in the experimental soil. Furthermore, the high plasticity of this plant trait was indicated by a large difference between plants growing in the two experimental soils. This suggests a strong ability of beech to alter resource allocation in response to specific soil conditions.

Second, we investigated the plasticity of root traits and rhizosphere properties of young beech trees from populations, that are adapted to either high or low nutrient supply, when growing in soils differing in their fertility. Fine root traits related to growth (biomass, length), architecture (branching) and morphology (diameter) responded strongly to the properties of the soil. This response was modified by an effect of provenance, that was consistent with an influence of the plant status in those nutrients, which were not in sufficient supply in the soil. However, an additional genotypic difference in the sensitivity of the beech saplings to different soil nutrient supply could not be excluded. Fine root parameters normalized for length, mass, or volume (root tip density and frequency, specific root length and area, root tissue density) did not differ among the treatments. Differences in mycorrhizal colonization and rhizosphere parameters related to phosphorus mobilization potential (pH, an abundance of organic acid anions, phosphatase activity) were small and mainly determined by the soil. Provenance had only a minor modifying effect, possibly due to differences in the ability to transfer carbon compounds from the shoot to the root and the fungal partner. Our results indicate the high plasticity of young beech trees to adapt root growth, architecture, and morphology to different soil nutrient supply, thereby also taking into account internal nutrient reserves.

Third, amplicon sequencing was used to assess to which degree beech saplings with a different P nutritional status can shape their rhizosphere microbial community along the root axis. After the first growing season, we determined the community composition and relative abundance of bacteria and fungi in bulk soil and different zones of the rhizosphere of freshly grown roots defined by root segments potentially differing in their functionality (root tip, elongation zone,

side root, old roots). We found that soil was the main factor determining microbial diversity and composition irrespective of plant origin and zone. Overall, OTU (operational taxonomic unit; an operational term for groups of closely related biota) numbers of bacteria and fungi, correlating well with soil microbial biomass, were higher in the soil with high P availability than in the soil with low P availability. Based on the relative abundance of OTUs, the bacterial and fungal communities in the different rhizosphere zones grouped distinctly indicating a succession from bulk soil to older roots via root tip, elongation zone, and side root zone demonstrating the often-postulated gradual development of rhizosphere microbial communities along the root axis. There was a significant effect of plant nutritional status on bacterial community diversity (beta diversity) differentiating elongation zone and on fungal community in the elongation and root tip zones. Furthermore, local average microbial species diversity (alpha diversity quantified with Shannon index) in the bulk soil and active rhizosphere zones (root tip, elongation zone) was higher in treatments with beech saplings acclimating to altered soil P availability than in treatments with plants adapted to the respective conditions. The only indication of a plant effect potentially offering an advantage concerning P nutrition, was a higher relative abundance of the bacterial order Solibacterales, potentially important for the mobilization of inorganically bound P, in some zones in treatments with beech saplings exhibiting a low P nutritional status. We conclude that overall, the P nutritional status of beech saplings had only a small influence on the soil microbial community in the rhizosphere. However, the related plant effects were strongest in the rhizosphere of the elongation zone, i.e., the potentially most active root segment in terms of root exudation.

This Ph.D. thesis demonstrated that beech saplings are capable of fast acclimation to changes in soil P availability involving alterations of internal P allocation patterns, root architecture, and interactions with local ectomycorrhizal fungi and bacteria in the rhizosphere. Comparing the results for treatments representing plants adapted to low or high soil P availability and for treatments representing plants with a given P nutritional status acclimating to altered soil P availability suggests that alterations of the same sets of multiple traits are involved in both long-term adaptation and short-term acclimation to given soil conditions. Together, the traits define respective growth strategies of adapted plants, i.e., a conservative strategy in soils with low P

availability prioritizing nutrient storage and internal recycling and thus limiting losses, and a strategy prioritizing fast growth in soils with high P availability leading to high P acquisition rates but also high losses. These strategies can be considered analogous to and being part of "recycling" and "acquiring" strategies on the ecosystem level. However, our results also indicate that during the first phases of acclimation to lower soil P availability, beech saplings with a high P nutritional status use their reserves to grow as much as possible.

Zusammenfassung

Buchenwälder (*Fagus sylvatica* L.) bedecken grosse Teile Europas und kommen auf einer Vielzahl von Böden vor, die einerseits eine breite ökologische Nische in Bezug auf bodenchemische Eigenschaften wie pH-Wert, Basensättigung und pflanzenverfügbaren Phosphor (P) im Oberboden bilden. Andererseits haben in Europa zurückgehende P Gehalte in Blättern und der damit einhergehende Anstieg des Stickstoff (N) zu P Verhältnisses während der letzten Jahrzehnten die Frage über einen möglichen Rückgang der Waldgesundheit und der Produktivität aufgrund von P Limitierung aufkommen lassen. Diese Veränderungen im Nährstoffgehalt der Blätter wurden hauptsächlich auf anhaltend hohe N Depositionen und ansteigende atmosphärische CO_2 Konzentrationen zurückgeführt. Sowohl beschleunigtes Baumwachstum durch hohe N und Kohlenstoff (C) Einträge als auch negative Effekte von erhöhtem N auf die Feinwurzelbiomasse, die Mykorrhizierung und die Streumineralisierung scheinen einen höheren Bedarf an anderen Nährstoffen ausgelöst zu haben, der nicht durch die Versorgung aus dem Boden gedeckt werden kann. Erstaunlicherweise ist sehr wenig bekannt über interne P Zuteilung in Buchen und deren Kapazität mit veränderter Phosphorverfügbarkeit im Boden auf kurze Sicht zurecht zu kommen.

In Anbetracht der Verbreitung von Buchenwäldern auf Böden, die ein breites Spektrum an Nährstoffverfügbarkeiten und damit eine nachgewiesene langfristige Anpassungsfähigkeit an unterschiedliche Bodenverhältnisse aufweisen, stellen wir die Hypothese auf, dass diese Baumart eine hohe phänotypische Plastizität aufweist. Dies ermöglicht ihr, mehrere Merkmale als Reaktion auf lokale Nährstoffverfügbarkeiten anzupassen, was zu einer Akklimatisierung führt und in einem Phänotyp resultiert, der angepasst ist auf lokale Bodenverhältnisse.

Das Ziel dieser Doktorarbeit ist es, die Akklimatisation von Buchen von hohen zu niedrigen und von niedrigen zu hohen Bodenphosphorverfügbarkeiten bezüglich Pflanzen-internen P Kreislauf und Pflanzen-Boden Interaktion in der Rhizosphäre zu untersuchen.

Dazu haben wir zwei Gruppen von 12-15 Jahre alten Buchensetzlingen von Standorten mit hoher und niedriger Phosphorverfügbarkeit im Mineralboden (Bh/Bv Horizont) in ihrem eigenen Boden und dem anderen Boden für zwei Jahre in Rhizoboxen im Gewächshaus gezogen. Unsere

Annahme war, dass die Setzlinge an ihren Herkunftsboden angepasst waren und dass ihr Phosphorversorgungsstatus daher die Phosphorverfügbarkeit des Herkunftsboden widerspiegelt.

Zuerst wurde die Plastizität der Masse und die P Zuteilung in Buchensetzlingen untersucht, mit dem Ziel die Merkmale zu identifizieren, die am ehesten auf die aktuellen Bodenbedingungen reagieren. Nach zwei Vegetationsperioden waren sowohl die P Konzentrationen in Blättern und Stängeln als auch die Massenverteilung zu Blättern und Feinwurzeln sowohl von Boden- als auch von Pflanzenherkunft beeinflusst. Im Gegensatz dazu wurden sowohl die P Zuteilung zu Blättern und Feinwurzeln als auch die P Konzentration in den Feinwurzeln fast ausschliesslich durch den Versuchsboden bestimmt. Unabhängig vom P Ernährungszustand (definiert als Mittelwert der P Konzentration der ganzen Pflanze), der immer noch klar die Bodenverhältnisse des Herkunftsstandorts widerspiegelte, war die P Zuteilung zu Blättern ein guter Indikator für P Verfügbarkeit im Versuchsboden. Zudem wies der grosse Unterschied zwischen dem Pflanzenwachstum in den beiden Böden auf hohe Plastizität dieses Pflanzenmerkmals hin. Dies legt nahe, dass Buchen die Fähigkeit haben, die Ressourcenverteilung als Reaktion auf spezifische Bodenbedingungen zu verändern.

Zweitens wurde die Plastizität von Wurzelmerkmalen und Rhizosphären-Eigenschaften junger Buchen aus Populationen untersucht, die entweder an eine hohe oder tiefe Nährstoffverfügbarkeit angepasst waren und dann in Böden mit unterschiedlicher Nährstoffverfügbarkeit gezogen wurden. Merkmale von Feinwurzeln mit Bezug zu Wachstum (Biomasse, Länge), Architektur (Verästelung) und Morphologie (Durchmesser) reagierten stark auf die Bodeneigenschaften. Diese Reaktion wurde durch einen Herkunftseffekt modifiziert, der mit einem Einfluss des Pflanzenzustandes auf jene Nährstoffe übereinstimmte, die im Boden nicht ausreichend vorhanden waren. Ein zusätzlicher genotypischer Unterschied in der Empfindlichkeit der junge Buchenpflanzen gegenüber einer unterschiedlichen Nährstoffversorgung des Bodens konnte jedoch nicht ausgeschlossen werden. Die auf Länge, Masse oder Volumen normalisierten Feinwurzelparameter (Wurzelspitzendichte und -häufigkeit, spezifische Wurzellänge und -fläche, Wurzelgewebedichte) unterschieden sich zwischen den

Behandlungen nicht. Die Unterschiede in der Mykorrhizabesiedlung und den Rhizosphärenparametern in Bezug auf das Phosphormobilisierungspotenzial (pH-Wert, Häufigkeit von Anionen organischer Säuren, Phosphataseaktivität) waren gering und wurden hauptsächlich durch den Boden bestimmt. Die Provenienz hatte nur einen geringen modifizierenden Effekt, möglicherweise aufgrund von Unterschieden in der Fähigkeit, Kohlenstoffverbindungen vom Spross zur Wurzel und zum Pilzpartner zu übertragen. Unsere Ergebnisse weisen auf die hohe Plastizität junger Buchen hin, um Wurzelwachstum, Architektur und Morphologie an die unterschiedliche Nährstoffversorgung des Bodens anzupassen und dabei auch die internen Nährstoffreserven zu berücksichtigen.

Drittens wurde mit Hilfe der Amplikon-Sequenzierung untersucht, inwieweit Buchensetzlinge mit unterschiedlichem P-Ernährungsstatus ihre mikrobielle Rhizosphärengemeinschaft entlang der Wurzelachse gestalten können. Nach der ersten Vegetationsperiode haben wir die Zusammensetzung der Mikrobengemeinschaft und die relative Häufigkeit von Bakterien und Pilzen im wurzelfreien Boden und in verschiedenen Zonen der Rhizosphäre von frisch gewachsenen Wurzeln bestimmt. Die verschiedenen Wurzelsegmente unterscheiden sich potentiell durch ihre Funktionalität (Wurzelspitze, Dehnungszone, Seitenwurzel, alte Wurzeln). Wir fanden heraus, dass der Boden der Hauptfaktor war, der die mikrobielle Vielfalt und Zusammensetzung unabhängig von der pflanzlichen Herkunft und Wurzelzone bestimmt. Die Gesamtzahl der OTUs von Bakterien und Pilzen, die gut mit der mikrobiellen Biomasse des Bodens korrelieren, war in dem Boden mit hoher P-Verfügbarkeit höher als in dem mit niedriger P-Verfügbarkeit. Basierend auf der relativen Häufigkeit von OTUs gruppierten sich die Bakterien- und Pilzgemeinschaften in den verschiedenen Rhizosphärenzonen und zeigten deutlich eine Sukzession vom wurzelfreien Boden über Wurzelspitze, Dehnungszone und Seitenwurzelzone zu älteren Wurzeln an, was die oft postulierte graduelle Entwicklung von mikrobiellen Gemeinschaften in der Rhizosphäre entlang der Wurzelachse zeigt. Innerhalb der Rhizosphäre gab es einen signifikanten Einfluss des Pflanzenernährungszustandes auf die bakterielle Beta-Diversität in der Verlängerungszone und auf die Pilz-Diversität in der Verlängerungs- und Wurzelspitzenzone. Darüber hinaus war die mikrobielle Alpha-Diversität im wurzelfreien Boden und in der aktiven Rhizosphäre bei Behandlungen mit Buchensetzlingen, die sich an die

veränderte Boden-P-Verfügbarkeit gewöhnen, höher als bei Behandlungen mit an die jeweiligen Bedingungen angepassten Pflanzen. Der einzige Hinweis auf einen Pflanzeneffekt, der potenziell einen Vorteil in Bezug auf die P-Ernährung bietet, war eine höhere relative Häufigkeit der bakteriellen Ordnung Solibacterales in einigen Zonen bei Behandlungen mit Buchensetzlingen, die einen niedrigen P-Ernährungsstatus aufweisen. Solibacterales sind potentiell wichtig für die Mobilisierung von anorganisch gebundenem P. Wir kommen zu dem Schluss, dass der P-Ernährungsstatus von Buchensetzlingen insgesamt nur einen geringen Einfluss auf die mikrobielle Bodengemeinschaft in der Rhizosphäre hatte. Die damit verbundenen Pflanzeneffekte waren jedoch am stärksten in der Rhizosphäre der Dehnungszone, d.h. dem potenziell aktivsten Wurzelsegment in Bezug auf die Wurzelexsudation.

Diese Doktorarbeit zeigte, dass Buchensetzlinge in der Lage sind, sich schnell an Veränderungen in der Boden-P-Verfügbarkeit zu gewöhnen. Das beinhaltet Veränderungen des Musters der internen P Zuteilung, der Wurzelarchitektur und der Interaktionen mit lokalen Ektomykorrhizapilzen und Bakterien in der Rhizosphäre. Der Vergleich der Ergebnisse der Behandlungen (Pflanzen, die an niedrige oder hohe Boden-P-Verfügbarkeit angepasst sind und Behandlungen bei der die Pflanzen mit einem bestimmten P-Nährstoffstatus sich an die veränderte Boden-P-Verfügbarkeit anpassen) deutet darauf hin, dass Veränderungen der gleichen Gruppen von mehreren Merkmalen sowohl an die langfristige Anpassung als auch an die kurzfristige Akklimatisierung an die gegebenen Bodenbedingungen beteiligt sind.

Zusammen definieren die Merkmale entsprechende Wachstumsstrategien angepasster Pflanzen, d.h. eine konservative Strategie in Böden mit geringer P-Verfügbarkeit, die der Nährstoffspeicherung und dem internen Recycling Priorität einräumt und damit Verluste begrenzt, und eine Strategie, die dem schnellen Wachstum in Böden mit hoher P-Verfügbarkeit Priorität einräumt, was zu hohen P-Akquisitionsraten, aber auch zu hohen Verlusten führt. Diese Strategien können als analog zu den "Recycling-" und "Acquiring"-Strategien auf Ökosystemebene angesehen werden. Unsere Ergebnisse deuten aber auch darauf hin, dass Buchensetzlinge mit einem hohen P-Ernährungsstatus in der ersten Phase der Akklimatisierung

an die niedrigere P-Verfügbarkeit des Bodens ihre Vorräte nutzen, um dennoch so viel wie möglich wachsen zu können.

CHAPTER 1
General introduction

Increasing awareness of the negative impacts of high atmospheric N inputs and climate change on ecosystems has raised interest in the stability of forest ecosystems in Central Europe. Apart from their role in climate change mitigation, as habitat for a variety of flora and fauna, and in soil and water protection, forests are of high economic value. The total roundwood production in the EU reached an estimated 470 million m^3 in 2017, and 2.75 billion euros was invested in forestry and logging in 2016 in 17 of the central wood-producing countries in Europe (Agriculture, forestry and fishery statistics, Eurostat, 2018). Multiple observations (e.g., Braun et al. 2010, Jonard et al. 2015) of a decrease in phosphorus (P) concentration accompanied by an increase in the nitrogen (N) to phosphorus ratio (N/P) in beech leaves during the last decades all over Europe raised questions about a possible reduction in forest health and productivity due to P limitation (Figure 1.1).

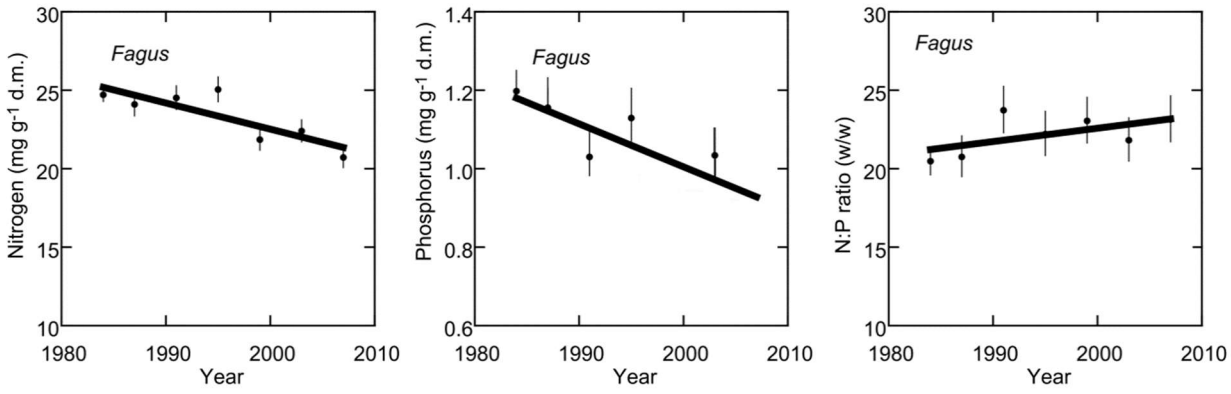

FIGURE 1.1| Trend of foliar concentrations of N (left), P (middle), and the N/P ratio (right) on mature beech (*Fagus sylvatica*) in permanent forest observation plots in different regions of Switzerland between 1980 and 2007. Values are corrected to account for an age trend. Bars = 95% confidence intervals of plot medians. Modified from Braun et al. (2010).

These changes in leaf nutrient status have been attributed to, on the one hand to accelerated tree growth resulting from high atmospheric N deposition and increasing carbon dioxide concentrations, and on the other hand to adverse effects of elevated N, including a decrease in fine root biomass, mycorrhization (ectomycorrhizal root tip abundance and mycelial production), and litter mineralization (Nadelhofer et al.,2000; Kjøller et al., 2012; Peñuelas et al., 2013). In consequence, these effects might have created an apparent greater need for other nutrients that cannot be met by the supply from the soil. This applies particularly to P, since in most soils plant-available P occurs at only low concentrations in the soil solution, as

most of this nutrient occurs in unavailable forms adsorbed to reactive surfaces of the soil solid phase or is bound in minerals or soil organic matter (Hinsinger, 2001; Lambers et al., 2008).

In contrast to N nutrition, the P nutrition of trees in forest ecosystems of the temperate zone is largely under-studied (Rennenberg and Herschbach, 2013). Little is known about internal P cycling and allocation, P acquisition, or the role of microbes in the P nutrition of forest ecosystems. To address these gaps we focused on European beech (*Fagus sylvatica* L.) because it is a common tree species covering a large part of Europe (ca 14–15 Mha) and occurring on a wide variety of soils, thus realizing a broad ecological niche in terms of climate and soil chemical properties (Brunet et al., 2010; Durrant et al., 2016). In particular, the plant-available P pool varies widely within beech geographical range, suggesting that beech could have a high acclimation potential/plasticity, which would potentially lower the risks posed by decreasing soil P availability (Leuschner et al., 2006; Batjes, 2011; Yang et al., 2013).

Role of phosphorus in plant nutrition

Phosphorus is a macronutrient involved in many physiological processes in plants, including energy transfer reactions, genetic control, and protein synthesis. Therefore, an adequate supply of P is vital for the functioning of fundamental processes like photosynthesis, N fixation, reproduction, immunity, and growth (e.g., Marschner, 2012; George et al., 2011). A deficiency in P can result in adverse reactions like a decrease in photosynthetic activity, shoot growth inhibition (Marschner, 2012; Yang et al., 2016), higher susceptibility to parasite attacks (Flückiger and Braun, 2003), and decreased seed production (Brady and Weil, 1999; Marschner, 2012; Güsewell, 2004), which in turn can lead to a decrease in plant productivity.

Internal P allocation of beech trees

A large amount of P in the forest ecosystem is stored in tree biomass. In beech it can reach 22 kg P ha^{-1} in stem wood, 6 kg P ha^{-1} in stem bark, 26 kg P ha^{-1} in branch wood and bark, and almost 6 kg P ha^{-1} in current-year twigs and leaves, resulting in a total of around 60 kg P ha^{-1} in aboveground biomass and approximately 19 kg P ha^{-1} in roots (Jacobsen et al., 2003). The assessment of all plant P pools is challenging from a practical perspective; therefore, foliar P is commonly used to monitor the P nutritional state of forests (Flückiger and Braun, 1998; Duquesnay et al., 2000; Jonard et al., 2009; Braun et al., 2010; Jonard et al., 2015; Talkner et al., 2015). Critical values for nutrient concentrations and ratios in leaves have been established based on a large number of studies comparing leaf values with deficiency

symptoms, growth, or reactions to fertilization (Mellert and Göttlein, 2012). However, the relationship between concentrations of P in leaves across different forest sites and measures of P availability in the soil remains unclear (Talkner et al., 2015; Lang et al., 2017). Further, the seasonality in foliar P concentrations (Zavišić and Polle, 2018) suggests complex internal P management of beech trees involving different plant compartments including leaves, the stem, and fine and coarse roots (Yang et al., 2016; Zavišić and Polle, 2018; Zavišić et al., 2018).

P can be recycled in the plant on two levels: on the cellular level, through the storage and flux of P into and out of the vacuole (Lee et al., 1990; Lee and Ratcliffe, 1992; Mimura et al., 1996); and on the whole plant level, from various organs including senescing leaves, roots, and the stem via vascular tissues. The storage, mobilization, and recycling of P at both levels follow seasonal patterns depending on the current status of the plant (i.e., intensive leaf growth, mid-season, senescence) (Raghothama, 1999; Lin et al., 2009, 2014; Brant and Chen, 2015; Netzer et al., 2017; Zavišić and Polle, 2018; Zavišić et al., 2018). The seasonally varying relationship between soil P availability and plant internal P cycling has been shown for P concentrations in various plant compartments, i.e., leaves, fine roots, coarse roots, and stem (Netzer et al., 2017; Zavišić and Polle, 2018), for P concentrations in the xylem and phloem (Yang et al., 2016; Netzer et al., 2017), and P resorption from older and senescent leaves (Hofmann et al., 2016; Netzer et al., 2017).

P acquisition from soil

In forest ecosystems, P is acquired by trees mostly from soil P sources of geogenic origin, as fertilization and atmospheric deposition are often limited. Although the total amount of P in the soil may be high, it is often present in forms unavailable for plants, such as organic P (e.g., nucleic acids, phospholipids, phytate), calcium (Ca) bound inorganic P, and iron (Fe) or aluminum (Al) bound inorganic P (Brady and Weil, 1999). The relative proportions and importance of these P forms depend on soil and site properties, e.g., geology, moisture, pH, texture, and vegetation. Forest soils contain as much as 80 to 95% organically bound P, mostly in organic topsoil horizons.

Plant roots only absorb P dissolved in the soil solution in the form of water-soluble inorganic P, mainly as $H_2PO_4^-$ or HPO_4^{2-} depending on pH, with dedicated phosphate transporter proteins. Through the uptake f available P, a plant root creates a P depletion zone

that leads to the transfer of P by slow diffusion from the most concentrated area (soil solid phase) to the least concentrated area (root surface) (Schachtman et al., 1998). To overcome this limitation, plants have developed several adaptations aiding soil exploration, efficient mobilization, and uptake of P from organic and inorganic P sources (Trolove et al., 2003; Vance et al., 2003). Due to slow diffusion of dissolved P in the soil, root traits allowing exploration of significant volumes of soil are especially important. Plant root architecture and morphology are essential for maximizing P uptake because root systems that have higher ratios of surface area to volume will more effectively explore a larger volume of soil (Lynch, 1995). Low soil P availability has been shown to influence multiple root traits (e.g., Williamson et al. ,2001; Hodge et al. ,2009; Weemstra et al., 2017), in particular through the inhibition of primary root growth, promotion of lateral root growth, enhancement of root hair development, and, in some families, cluster root formation (Drew, 1975; Lynch, 1995; Gahoonia et al., 1997; reviewed by Niu et al., 2013). Besides, low soil P availability can lead to physiological changes in the roots, such as upregulation of P membrane transport systems in response to P deficiency (Caldwell et al., 1992; Schachtman et al., 1998). Another strategy employed in particular by trees is the production of long-lived fine roots, often exhibiting a large diameter (Weemstra et al., 2016) and foraging strategies resulting in preferential root proliferation to and within P-enriched patches (e.g., Drew et al., 1975; Jackson et al., 1990; Hodge, 2004).

Furthermore, rhizodeposition (alteration of soil pH and exudation of organic acid anions and other P-solubilizing compounds), can potentially lead to an increase in P availability via a range of biogeochemical (e.g., ligand exchange, ligand- or proton-promoted dissolution of minerals) and biochemical (enzymatic hydrolysis of organic P compounds by phosphatases) processes in the rhizosphere (Hinsinger et al., 2011). Most of what we know about root exudation, particularly regarding its role in P mobilization, and about the role of plant nutritional status in governing root exudation comes from studies with crops, whereas much less is known for trees. Given the high organic P content in upper soil horizons of forest soils, the activity of hydrolyzing enzymes (phosphatases) involved in the release of phosphate from more complex compounds might play an essential role in P mobilization. The abundance of phosphatases in the soil is often linked to soil P availability (e.g., Hofmann et al., 2016; Marklein and Houlton, 2012), but also to plant P demand (Weintraub, 2011 and references

therein). The release of organic acid anions can likewise be induced by a low P nutritional status, as has been shown for crops (e.g., Hoffland et al., 1989; Hinsinger, 2001), but can also be a response to high Al concentrations in acidic soils (Richardson et al., 2009) or be part of the constitutive release of excess carbon (C) (Heim et al., 2001; Eldhuset et al., 2007). Although proton release by roots can be induced by P deficiency (e.g., Hoffland et al., 1989; Shen et al., 2004; Shahbaz et al., 2006), alteration of rhizosphere pH is often attributed to the form of mineral N taken up by the plant (Riley and Barber 1971; Marschner and Römheld 1983; Hinsinger 2001).

Phenotypic plasticity of beech trees

Plants are subjected to multiple abiotic and biotic stresses. Their immobility forces them to develop tolerance (acclimate) to changing environmental conditions during their lifetime in order to survive. Phenotypic plasticity, defined as rapid trait alterations during the lifetime of an organism (Sultan, 2004; Hodge, 2009), has been recognized as the primary mean of rapid acclimation to adverse environmental conditions, and it can concern morphological, anatomical, physiological, reproductive, and developmental traits. When the plasticity of traits correlates with fitness advantages under certain environmental conditions, this is termed adaptive phenotypic plasticity (Ghalambor et al., 2007). Tree species with greater phenotypic plasticity may be more likely to survive environmental changes caused by human activity because such changes typically occur too rapidly to allow for an evolutionary or migratory response (Matesanz et al., 2010; Nicotra et al., 2010; Vitasse et al., 2010). High phenotypic plasticity of beech has been observed in response to drought, increased temperature, and altered light intensity, with a focus on traits such as phenology (e.g., Kramer, 1995; Vitasse et al., 2009), biomass and root morphology (Curt et al., 2005; Weemstra et al., 2016), leaf anatomy (Stojnić et al., 2015a,b), stem anatomy (Stojnić et al., 2013; Diaconu et al., 2016), and mass allocation and growth rate (Rolo et al., 2015).

Role of the microbial community in plant P nutrition

The rhizosphere is defined as the soil in the root proximity that is influenced by the root (Marschner et al., 2004). Because root deposition increases C availability, the rhizosphere is characterized by a higher abundance of microorganisms that strongly influence nutrient uptake by plants by either enhancing or decreasing nutrient availability. Bacteria and saprophytic and mycorrhizal fungi have developed several mechanisms to solubilize P, such

as producing and exuding organic acid anions, protons, and siderophores (Richardson et al., 2009; Frossard et al. 2011; Oburger et al., 2011; Spohn and Kuzyakov, 2013), as well as releasing extracellular phosphatases (Plassard and Dell, 2010; Nannipieri et al., 2011). These compounds can potentially improve plant P nutrition by increasing the availability of P in the root proximity. On the other hand, microorganisms can decrease the availability of P to plants by immobilizing P in their biomass, decomposing P-mobilizing organic compounds released by roots, and by counteracting root-induced pH decreases through proton consumption during ammonification (Richardson et al., 2009; Marschner et al., 2011) (Figure 1.2).

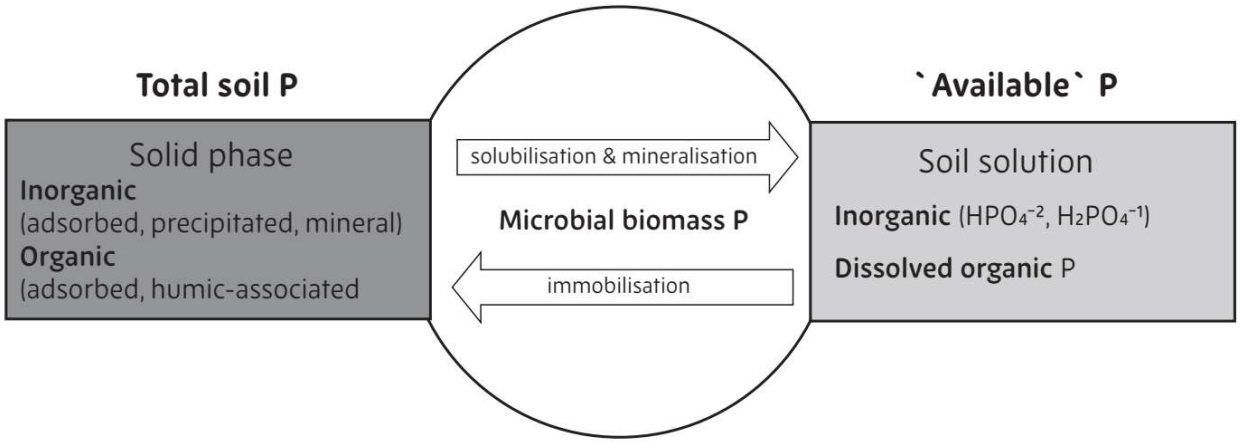

FIGURE 1.2 | Schematic representation of the importance of microorganisms to P availability in soil. Microbes and their interactions in soil play a critical role in mediating the distribution of P between available P sub-pool in soil solution and the solid phase P in total soil P through solubilization and mineralization reactions and immobilization of P into microbial biomass and/or formation of sparingly available forms of inorganic and organic soil P. Modified from Richardson & Simpson (2011).

Given that plant roots in soil are always colonized by microbes, and that both microbes and plant roots are capable of potentially P-mobilizing exudation, it is challenging to differentiate between processes driven by plants and by microbes (Plassard et al., 2011; Oburger et al., 2011). Therefore, little is known about the extent to which plants can influence the P mobilization potential of their rhizosphere, i.e., directly via root exudation of P-mobilizing compounds and/or indirectly via stimulating and shaping the soil microbial community through general carbon inputs or directed exudation of signaling molecules.

Mycorrhizal symbiosis has been proven to play a vital role in the P nutrition of forest trees in temperate zones (Plassard and Dell, 2010; Chen et al., 2016; Eissenstat et al., 2015).

Observations have demonstrated that mycorrhizal plants show growth improvement and increased total plant P content compared with non-mycorrhizal plants (Chalot et al., 2002; Smith and Read 2008). The most common and widespread mycorrhizae are the arbuscular mycorrhizae (AM), but ectomycorrhizae (ECM) is essential in temperate and boreal forests with highly organic topsoil horizons (Smith and Read, 2008; Zemunik et al., 2015). A fungal mantle entirely covers roots colonized by ECM; the absorbing hyphae in some species can extend far into the soil, allowing extensive soil exploration. Mycorrhizal hyphae enlarge the absorbing surface and the accessible soil volume, but also – due to their smaller diameter – penetrate smaller soil pores than roots (Plassard and Dell, 2010). ECM fungi rely on a supply of C derived from photosynthesis by the plant (Smith and Read, 2008). Still, the amount of P transferred to the plant varies depending on the genus and hyphal soil exploration type of the fungus and on environmental conditions (Plassard, 2011). Despite strong links between ECM colonization and P nutrition of trees, there is not a direct link between soil P availability and the level of mycorrhization (e.g., Yang et al., 2016, Spohn et al., 2018).

Spatial heterogeneity of the microbial community in the rhizosphere

The rhizosphere is a highly heterogeneous environment due to constant root growth and activity. In general, according to the model of root growth proposed by Marschner (2011), a root tip is pushed through the soil by cell division and the elongation of apical cells. Microbial colonization then begins just behind the meristematic tissue by chemotaxis or, as a legacy effect from the border cells, sloughed from the root cap. In the zone, immediately behind the root tip (elongation zone), we find root exudates such as sugars, organic acid anions, phenols, and amino acids (Hoffland et al., 1989; Roemheld, 1991; Marschner et al., 2011). The abundance of readily available carbon in the form of root exudates stimulates microbial growth and attracts more soil microorganisms to the root surface. In subsequent root zones (root hair zone, older root parts), root exudation is lower. In older root parts with little or no root exudation, the primary substrates for microbial growth include cellulose and other recalcitrant cell wall materials from sloughed-off root cortex tissue. Consequently, microbial growth rates and activity decrease with increasing distance from the elongation zone (Nguyen and Guckert, 2001). The differences in type and quantity of carbon available in different root zones not only influence microbial growth but could also create a distinct

rhizosphere community in the soil around the zones potentially affecting plant nutrition (Yang and Crowley, 2000; Baudoin et al., 2001; Marschner et al., 2001).

Research gaps and structure of the thesis

Multiple authors have reported a decreasing trend over time in the P concentration in the leaves of beech trees across Europe. However, the main problem with interpreting this trend has been a lack of understanding of its mechanism. Given the high plasticity of beech to multiple environmental factors, a reasonable hypothesis is that the observed decrease is the result of acclimation to change in pedoclimatic conditions. Due to a lack of experimental data on beech acclimation to soil P availability, there was a need for a comprehensive insight into the P nutrition of beech trees and their acclimation ability in terms of internal P allocation, strategies of P mobilization, and P acquisition from the soil, as well as the role of microbes in these processes.

We decided to tackle this problem from three angles. First, we assessed the plasticity of beech saplings in terms of P and mass allocation; second, we investigated beech plasticity regarding root architecture and rhizosphere chemistry; and third, we considered the relationship of beech with local bacterial and fungal communities.

Experimental approach

We carried out a two-year-long experiment (2015–2016) that enabled us to compare all these different aspects of tree acclimation directly. We set up rhizoboxes with saplings of beech (*Fagus sylvatica* L.) of similar size collected during their dormant period on the core research sites of the Priority Program 1685 “Ecosystem Nutrition” in Unterlüss (Lower Saxony, Germany (LUE); low soil P availability) and Bad Brückenau (northern Bavaria, Germany (BBR); high soil P availability; Figure 1.3).

site	Lüss (LUE)	Bad Brückenau (BBR)
geographic coordinates (Gauss-Krüger)		
easting	3585473	3566195
northing	5857057	5579975
m a.s.l. [m]	115	809
annual mean temperature [°C]	8.0	5.8
annual precipitation [mm]	779	1031
forest stand	beech	european beech - spruce
humus		
humus form	Mor-like Moder	*Mull-like Moder*
pH ($CaCl_2$)	2.9	3.6
soil		
parent material	sandy till	basalt
soil profile		
type of soil (WRB2014)	Hyperdystric Folic Cambisol (Arenic, Nechic, Protospodic)	Dystric Skeletic Cambisol (Hyperhumic, Loamic)
pH ($CaCl_2$) at 0 - 5 cm	3.0	3.2
pH ($CaCl_2$) at 55 cm	4.2	4.5

FIGURE 1.3| An overview of the research sites of Priority Program SPP 1685 "Ecosystem Nutrition" project of the German Research Foundation (DFG) from which soil and plants were collected. Modified from: https://www.ecosystem-nutrition.uni-freiburg.de/.

These sites were chosen because they sustain mature mono-specific beech stands but differ profoundly in soil P content. Soils were collected from the Bh horizon in LUE and the Bv horizon in BBR.

In April 2015, rhizoboxes were set up with beech saplings planted either in the soil from their site of origin or in the contrasting soil from the other site. The rhizoboxes had inner dimensions of 60 cm x 25 cm x 1.5 cm (Figure 1.4a). They consisted of PVC walls and a removable transparent lid. Rhizoboxes with trees were placed in a greenhouse with temperature control (22 ± 2 °C during the day / 18 ± 2 °C at night) and with natural light and shading from direct sunlight during the growing season and dark, cold room (4°C) in winter. The soil was kept dark. To stimulate the formation of a quasi-planar root system along the

transparent lid (Figure 1.4b,c), the rhizoboxes were inclined at an angle of about 30°. Soil water potential in the rhizoboxes was kept at approximately -8 kPa by using irrigation tubes, providing a P-free artificial rain solution based on the composition of natural precipitation.

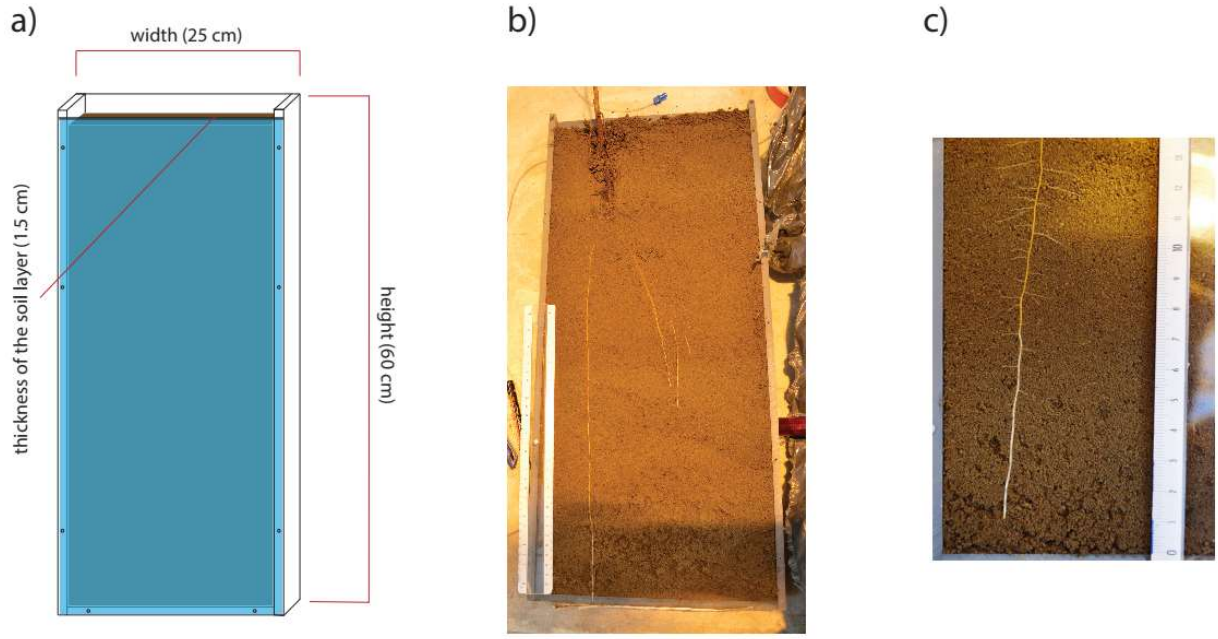

FIGURE 1.4| Schematic drawing of the rhizobox a) photograph of opened rhizobox with roots grown during 1st growing season in BBR soil b) a close up on the beech root growing in BBR soil c).

During the first growing season (2015), we sampled leaves from the plants to assess TCA-soluble P fraction (metabolic P; measured only in fully expanded leaves only; August) and nutrient contents (fully expanded leaves (August) and senescent leaves (shed leaves, December). After temporarily opening the front plate of the rhizoboxes, we measured rhizosphere properties: potential phosphatase activity with zymography, collected organic and inorganic anions with anion exchange membranes, and measured the pH around the root ends with optodes. We also collected small amounts of soil from around the root zones (root tip zone, root elongation zone, side root zone, old root zone) and in the bulk soil for DNA extraction and subsequent amplicon sequencing analysis of the microbial community along the root axis.

In August of the second growing season (2016), we repeated measurements of rhizosphere properties and sampled the rhizoboxes destructively. We scanned fine roots to assess their architecture, determined the mycorrhization, measured phloem, and xylem exudate P

concentrations, assessed the metabolic P in leaves, and determined plant age based on tree rings. All plant parts (leaves, stem and twigs, coarse roots, fine roots) were weighed, dried, and ground, and their nutrient content was measured. Microbial C, N, and P were assessed in bulk and rhizosphere soil.

We analyzed data in a two-factorial way, always considering the factors “current soil” defined as the soil in which plants were growing during the experiment (BBR or LUE) and “plant origin” representing plant P nutritional status implied by the soil of origin (four combinations BBR in BBR, BBR in LUE, LUE in BBR, LUE in LUE).

More detailed descriptions of the material and methods can be found in the respective sections of chapters 2, 3, and 4.

Summary of main objectives

In the context of exploring the acclimation of beech from high to low and from low to high soil P availability, we aimed to:

- identify and quantify the main responsive traits of *Fagus sylvatica* L. saplings, in terms of biomass and P allocation, contributing to acclimation,
- investigate root-soil interactions involved in the acclimation of beech in terms of root architecture and morphology, mycorrhization, and the occurrence of P-mobilizing compounds in the rhizosphere,
- determine the impact of beech on the rhizosphere microbiome and its potential role in plant P nutrition, by sampling at different zones along the root axis.

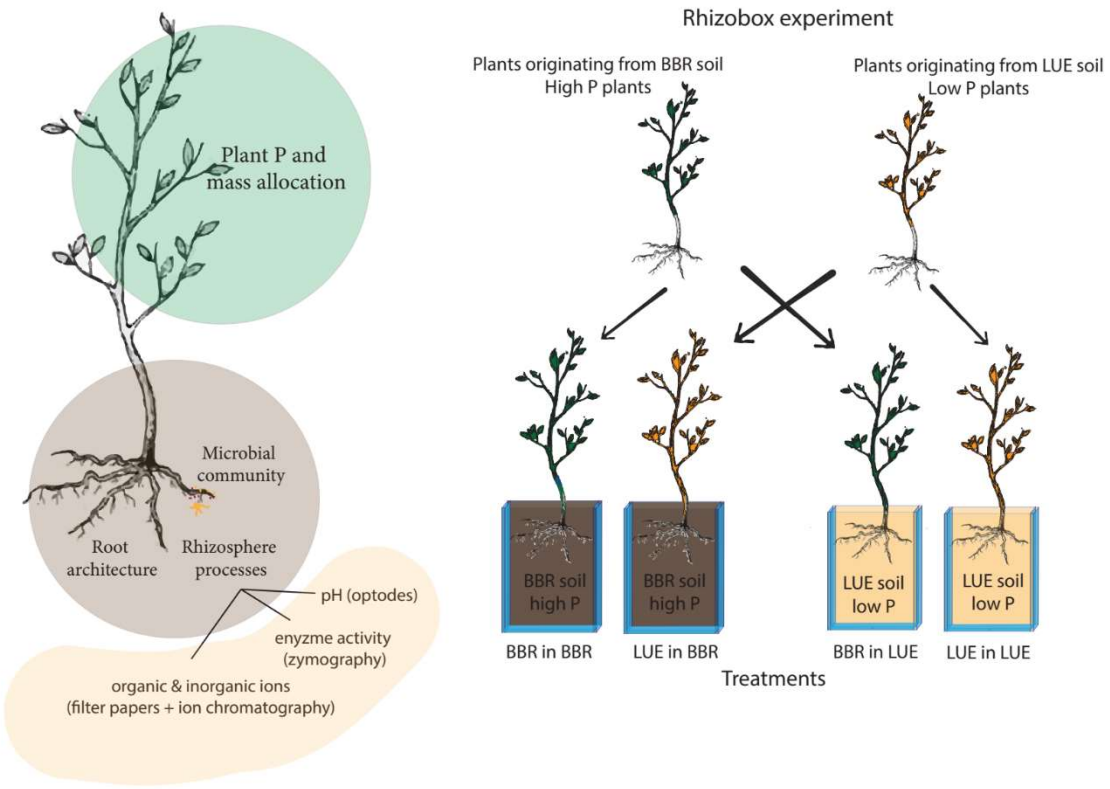

FIGURE 1.5| An overview of the PhD-Thesis. We grew two groups of 12–15-year-old beech saplings originating from sites with high and low soil P availability in mineral soil from their own site and soil from the other site in rhizoboxes for two years in the greenhouse. We assessed the plasticity of beech saplings in terms of P and mass allocation, root architecture, and rhizosphere chemistry, and we considered the relationship of beech with local bacterial and fungal communities.

This thesis was embedded in the 1st phase of the Priority Program SPP 1685 "Ecosystem Nutrition" project of the German Research Foundation (DFG) and the Swiss National Science Foundation (SNF). The objective of the 1st phase of SPP 1685 was to identify and investigate potential adaptation mechanisms of forest ecosystems to an inadequate P supply (https://www.ecosystem-nutrition.uni-freiburg.de).

CHAPTER 2

Phosphorus allocation to leaves of beech saplings reacts to soil phosphorus availability.

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Abstract

Decreasing phosphorus (P) concentrations in leaves of beech (*Fagus sylvatica* L.) across Europe raise the question about the implications for forest health. Considering the distribution of beech forests on soils encompassing a broad range of nutrient availability, we hypothesized that this tree species exhibits high phenotypic plasticity allowing it to alter mass, and nutrient allocation in response to local nutrient availability. To test this, we grew two groups of 12–15-year-old beech saplings originating from sites with high and low soil P availability for 2 years in mineral soil from their own site and in soil from the other site. After two growing seasons, P concentrations in leaves and stem, as well as mass allocation to leaves, and fine roots were affected by both soil and plant origin. By contrast, relative P allocation to leaves and fine roots, as well as P concentrations in fine roots, were determined almost entirely by the experimental soil. Independent of the P nutritional status defined as average concentration of P in the whole plant, which still clearly reflected the soil conditions at the site of plant origin, relative P allocation to leaves was a particularly good indicator of P availability in the experimental soil. Furthermore, a high plasticity of this plant trait was indicated by a large difference between plants growing in the two experimental soils. This suggests a strong ability of beech to alter resource allocation in response to specific soil conditions.

Keywords: acclimation, beech, Fagus sylvatica, forest health, phenotypic plasticity, phosphorus allocation, phosphorus nutritional status, soil phosphorus availability

Introduction

Forests dominated by beech (*Fagus sylvatica* L.) cover a large part of Europe (ca 14–15 Mha) from southern Norway to southern Italy and from northern Spain to northwest Turkey (Brunet et al., 2010; Durrant et al., 2016). Throughout this area, beech populations occur on a wide variety of soils realizing a broad ecological niche in terms of soil chemical properties including pH (3.2–7.3), base saturation (3–99%), and plant-available phosphorus (P) pools in the mineral topsoil (11–1287 mol P m−2 10 cm−1) (Leuschner et al., 2006; Batjes, 2011; Yang et al., 2013). Analysis of data from forest monitoring plots (ICP forest level II) across Europe between 1991 and 2010 revealed a significant decline in P concentrations and an increase of N/P ratios in beech leaves on the majority of plots (Jonard et al., 2015; Talkner et al., 2015) confirming findings of earlier regional studies (Flückiger and Braun, 1998; Duquesnay et al., 2000; Jonard et al., 2009; Braun et al., 2010). These changes in leaf nutrient status have been attributed to continuing high nitrogen (N) deposition and increasing atmospheric carbon dioxide concentrations. Both accelerated tree growth due to high N and carbon (C) input and negative effects of elevated N on fine root biomass, mycorrhization, and litter mineralization appear to have created a higher need for other nutrients which cannot be met by the supply from the soil (Kjøller et al., 2012; Peñuelas et al., 2013; Talkner et al., 2015). On 40% of the level II plots, the average leaf P concentrations indicated P deficiency (Jonard et al., 2015) and average N/P ratios on 60% of the plots were higher than “good for harmonious nutrition” (Talkner et al., 2015). The currently widely accepted critical values for nutrient concentrations and ratios in leaves (Mellert and Göttlein, 2012) are based on a large number of studies comparing leaf values with deficiency symptoms, growth or reaction to fertilization. However, results from surveys and experiments on the relation between P concentrations in plant tissues and soil P availability are partially conflicting. While concentrations of P in leaves and fine roots of mature trees across different forest sites were not well related to measures of P availability in the soil (Talkner et al., 2015; Lang et al., 2017), this relation was stronger for saplings from two acid forest sites and considering different plant compartments including leaves, stem, fine and coarse roots (Yang et al., 2016; Zavišić and Polle, 2018; Zavišić et al., 2018). Furthermore, Zavišić et al. (2018) observed a strong increase of P concentrations in all compartments upon fertilization. One reason for the conflicting results could be that often only total P concentrations in soil are available which not sufficiently reflect this elements bioavailability. Another explanation could be the strong seasonal and site dependent

variability of P concentrations in plant tissues (Yang et al., 2016; Netzer et al., 2017; Zavišić and Polle, 2018). This points to the importance of seasonal dynamics of nutrient uptake and plant internal nutrient allocation.

However, surprisingly little is known on how beech adapts its internal P allocation to different P availability in soil, and thus to what degree P concentrations in leaves directly reflect soil P availability or the ability of the tree to regulate its P content on the cellular and the whole-plant level (Marschner, 1996). On the cellular level, P homeostasis can be maintained by adjusting the flux of P into and out of the vacuole (Lee et al., 1990; Lee and Ratcliffe, 1992; Mimura et al., 1996). On the whole plant level, P redistribution is regulated via vascular tissues, and it follows seasonal patterns employing storage in and recycling from various organs including senescing leaves, roots, and stem (Raghothama, 1999; Lin et al., 2009, 2014; Brant and Chen, 2015; Netzer et al., 2017; Zavišić and Polle, 2018; Zavišić et al., 2018). The seasonally varying relations between soil P availability and internal P cycling have been shown for P concentrations in various plant compartments comprising leaves, fine roots, coarse roots and stem (Netzer et al., 2017; Zavišić and Polle, 2018), for P concentrations in xylem and phloem (Yang et al., 2016; Netzer et al., 2017), and for P resorption from older and senescent leaves (Hofmann et al., 2016; Netzer et al., 2017). Furthermore, growth rates of adult beech not always reflected soil P availability (Netzer et al., 2017) highlighting the potential role of high-efficiency internal cycling in trees growing on soils with low P availability.

Alteration of internal nutrient cycling can be part of the adaptive response (phenotypic plasticity) of plants to external factors. For many plants, phenotypic plasticity was shown to comprise rapid trait alterations during a lifetime of an organism (Sultan, 2004; Hodge, 2009), including morphological, anatomical, physiological, reproductive, and developmental traits. Considering the long-time scales of migration and natural selection, phenotypic plasticity is the most important asset of tree populations to cope with the ongoing environmental changes (Matesanz et al., 2010; Nicotra et al., 2010; Vitasse et al., 2010). The phenotypic plasticity of beech in response to drought or increased temperature has been well studied, comprising plant traits such as phenology (e.g., Kramer, 1995; Vitasse et al., 2009), biomass and root morphology (Curt et al., 2005; Weemstra et al., 2016), leaf anatomy (Stojnić et al., 2015a,b), stem anatomy (Stojnić et al., 2013; Diaconu et al., 2016), mass allocation, and

growth rate (Rolo et al., 2015). By contrast, little is known about the adaptive response of beech to changes in P availability. Our objective was therefore to identify the respective most responsive traits of this tree species. Considering the important role, the plant nutritional status plays in governing nutrient acquisition (Marschner, 1996), we performed experiments with beech saplings adapted to grow in soil with either high or low soil P availability, and assessed their response to contrasting soil conditions. We hypothesized that the response of a beech sapling with a given P nutritional status, as defined by its site of origin, to higher or lower soil P availability in terms of biomass and P allocation to different plant compartments is mainly driven by the plant striving to increase a low foliar P concentration as fast as possible or to maintain a high foliar P concentration as long as possible.

Materials and methods

Plant and soil materials

Plant and soil materials were collected on the core research sites of the priority program 1685 "Ecosystem nutrition" of the German and Swiss National Science Foundation in Unterlüss (LUE, Lower Saxony, Germany) with low P availability in the soil, and Bad Brückenau (BBR, northern Bavaria, Germany) with high P availability in the soil. The sites were chosen because they both sustain mature mono-specific beech stands but differ profoundly in soil P stocks and cycling. For details on the sites refer to Lang et al. (2017). Saplings of beech (*F. sylvatica*) of similar size (approx. 45 cm in height and approx. 8 mm base diameter) were gently dug out at the sites during their dormancy period in December 2014, and stored at 4°C with their roots embedded in soil from their own site until planting. Soil materials were collected from the Bh horizon in LUE and the Bv horizon in BBR, air-dried at 15°C, sieved to 4 mm, and homogenized. Plant residues were removed from the soils. Basic physical and chemical properties are listed in Table 2.1 and were obtained as follows. Soil texture was determined using the pipette method (Gee and Bauder, 1986). Soil pH was measured in a 1:2 slurry in 0.01 M $CaCl_2$ or water after 30 min. equilibration. Organic C (C_{org}) and total N (N_{tot}) contents of ground soil samples were measured using an elemental analyzer (NC 2500, CE Instruments Ltd, Hindley Green, Wigan, United Kingdom). Exchangeable cations (Al_{ex}, Ca_{ex}, Fe_{ex}, K_{ex}, Mg_{ex}, Mn_{ex}, and Zn_{ex}) were extracted with 1M NH4Cl for 1 h at 20°C and a soil:extractant ratio of 1:10. The filtered extracts were analyzed for total elemental concentrations by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 7300 DV; Perkin Elmer, Waltham, MA, United

TABLE 2.1| Physical and chemical properties of homogenized material from the Bv soil horizon at Bad Brückenau (BBR) and the Bh soil horizon at Unterlüss (LUE) used in the experiment; this includes grain size fractions, pH in two different extractants, exchangeable metal cations (M_{ex}), organic C (C_{org}), total N (N_{tot}), and the following P fractions obtained by sequential extraction: resin exchangeable inorganic P (P_{resin}), sum of inorganic P (extractable P_{inorg}) and organic P (extractable P_{org}) in various extracts (for details see text); all concentrations are given per mass dry soil; shown are means ± standard deviations of two technical replicates, except for sum parameters and element ratios.

		LUE	BBR
Sand	($g\ kg^{-1}$)	811 ± 3	287 ± 14
Clay	($g\ kg^{-1}$)	43 ± 4	253 ± 14
pH in H_2O/ 0.01 M $CaCl_2$		3.99 ± 0.01/ 3.31 ± 0.01	4.76 ± 0.04/ 3.99 ± 0.01
Al_{ex}	($mmol_c\ kg^{-1}$)	19.7 ± 0.2	40.6 ± 0.2
Ca_{ex}	($mmol_c\ kg^{-1}$)	0.56 ± 0.01	2.13 ± 0.04
Fe_{ex}	($mmol_c\ kg^{-1}$)	1.35 ± 0.02	0.04 ± 0.006
K_{ex}	($mmol_c\ kg^{-1}$)	0.49 ± 0.02	0.56 ± 0.04
Mg_{ex}	($mmol_c\ kg^{-1}$)	0.33 ± 0.003	0.62 ± 0.01
Mn_{ex}	($mmol_c\ kg^{-1}$)	0.10 ± 0.003	0.79 ± 0.01
Zn_{ex}	($mmol_c\ kg^{-1}$)	0.02 ± 0.001	0.03 ± 0.001
C_{org}	($g\ kg^{-1}$)	18.5 ± 0.04	41.9 ± 1.0
N_{tot}	($g\ kg^{-1}$)	0.75 ± 0.01	3.22 ± 0.01
P_{resin}	($mg\ kg^{-1}$)	0.44 ± 0.04	5.5 ± 1.3
Extractable P_{inorg}	($mg\ kg^{-1}$)	29	911
Extractable P_{org}	($mg\ kg^{-1}$)	89	1256
C_{org}/N_{tot}	($g\ g^{-1}$)	24.7	13.0
C_{org}/P_{org}	($g\ g^{-1}$)	208	33
N_{tot}/P_{org}	($g\ g^{-1}$)	8.4	2.6

States). Sequential P extraction was performed according to Hedley et al. (1982) as modified by Tiessen and Moir (2006). In Table 2.1, resin exchangeable inorganic P (P_{resin}), the sum of inorganic P (P_{inorg}) in various extracts (0.5 M NaHCO3, 0.1 M NaOH before and after sonication, 1 M HCl, concentrated HCl) and the sum of organic P (P_{org}) in the $NaHCO_3$ and NaOH extracts are shown. The soil from BBR exhibited much higher concentrations of both inorganic and organic extractable P than the LUE soil, but most importantly also resin exchangeable

inorganic P, a measure of inorganic P in soil solution, and loosely sorbed to soil particles and thus of available P (Tamburini et al., 2012), was much higher in the BBR soil.

Experimental setup

In April 2015, rhizoboxes were set up with beech saplings planted either in the soil from their site of origin or in the contrasting soil from the other site. In a completely randomized design, each treatment was replicated seven times. The rhizoboxes had inner dimensions of 60 cm × 25 cm × 1.5 cm. They consisted of PVC walls and a removable transparent lid made of polymethyl methacrylate. The soil was filled in at a bulk density of 1.2 kg/dm^3. After 1 week of soil conditioning under irrigation as described below, the saplings were planted. The roots of the saplings were washed with tap water to remove sticking soil, and approximately 2 cm of tap root were cut to stimulate new root formation. For each tree, the front plate of one rhizobox was opened, the roots pressed into the soil, and the front plate was closed again. At this time point, saplings possessed up to 10 cm long tap roots of 0.5–1.5 cm diameter but almost no fine roots, which presumably had died off during the storage. Rhizoboxes with trees were placed in a greenhouse with temperature control (22 ± 2 °C during the day/18 ± 2 °C at night), natural light and shading from the direct sun. Since shading with movable blinds was the only means for active cooling, at some days in summer temperatures higher than 22°C occurred for short periods. The soil was kept dark, and to stimulate the formation of a quasi-planar root system along the transparent lid, the rhizoboxes were inclined at an angle of about 30°. Soil water potential in the rhizoboxes was kept at approximately -8 kPa by using irrigation tubes ("Rhizon irrigators," Rhizosphere research products, Wageningen, Netherlands) providing P-free artificial rain solution based on the composition of natural precipitation [2.1 µM K_2SO_4, 3.7 µM Na_2SO_4, 3.0 µM $CaCl_2$, 4.4 µM $CaSO_4$, 1.9 µM $MgCl_2$, 26.4 µM NH_4NO_3, 2.0 µM $Ca(NO_3)_2$; Holzmann et al., 2016]. During summer, additional periodic irrigation from the top was needed to compensate for high evapotranspiration. At the end of the first growing season (end of September 2015), the rhizoboxes were placed outside of the greenhouse, but protected by a roof, to induce dormancy. In November 2015, they were moved to a dark cold room at 4°C and periodically irrigated with artificial rain from the top. End of March 2016, after the last frost, the rhizoboxes were moved first to the protected area outside of the greenhouse, and in May, after appearance of the first leaves, back into the greenhouse with temperature control set to the same conditions as in the year before.

Plant harvest and analyses

During the first growing season in August 2015, when plants reached the phenological stage of fully developed leaves in both soils (Yang et al., 2016), five fully expanded leaves per plant were collected. Senescent leaves were collected at the end of the season after natural leaf abscission (December 2015) into nets spread around the plants. In August 2016 of the second growing season, the whole plants were harvested. At that time point, six saplings each from BBR growing in soil from BBR and LUE, 7 saplings from LUE growing in soil from BBR, and 3 saplings from LUE growing in soil from LUE had survived. The plants were divided into leaves, stem, coarse roots, and fine roots (diameter ≤ 2 mm). The following analyses were performed on fresh tissue samples. The age of the saplings at final harvesting was determined by staining thin sections of the stem and subsequent tree-ring analysis (Gärtner and Schweingruber, 2013). According to this, the saplings from BBR were 11.7 ± 2.7 years old, and those from LUE 14.7 ± 1.6 years old. Subsamples of fully developed leaves were used to measure the trichloroacetic acid (TCA)- soluble P fraction (also called metabolic P; Wilcox et al., 2000). Approximately 200 mg of fresh leaves were frozen in liquid N_2 in a 15 ml reagent tube and crushed to a fine powder using metal beads and vortexing. Powdered leaves were extracted for 1 h at 4°C with 4 ml of 0.3 M TCA on a shaker. Extracts were filtered at 0.45 µm using glass fiber GF/F filters (Whatman International Ltd.). Inorganic P concentrations in TCA extracts were measured colorimetrically using malachite green (Van Veldhoven and Mannaerts, 1987). Bark and wood exudates were collected with the EDTA (Ethylene diamine tetra acetate) technique (Rennenberg et al., 1996; Gessler et al., 1998; Yang et al., 2016). Briefly, approximately 2 cm of the basal stem was collected fresh and separated into bark and wood. These parts were washed with deionized water and incubated in 2 ml of a solution 10 mM in Na_2EDTA (pH 7) and 15 µM in chloramphenicol for 5 h at room temperature. Then, inorganic P in the incubation solution was measured colorimetrically as described above. Plant parts not used for the analyses described above, were oven dried at 60°C for 48 h (leaves and fine roots) or 72 h (stems and coarse roots), weighed, and ground to fine powder using a ball mill (Retsch MM400 Mixer Mill, Retsch GmbH, RetschAllee 1–5, 42781 Haan, Germany) with receptacle and balls made of agate. Total carbon and nitrogen contents of the ground material were measured by combustion using an elemental analyzer (NC 2500, CE Instruments Ltd, Hindley Green, Wigan, United Kingdom). The contents of total P were determined by ICP-OES (Optima 7300 DV; Perkin Elmer, Waltham, MA, United States) of digests obtained with a

solution 8.3 M in HNO_3 and 0.6M in HF using a microwave digestion unit (MW ultraCLAV, MLS, Milestone Inc., Shelton, CT, United States).

Mass and P allocation parameters

Biomass allocation to a specific plant compartment was calculated as mass fraction (g) of the compartment in percent of the total dry mass of the whole plant (g) (Poorter and Sack, 2012). Phosphorus allocation to a specific plant compartment was calculated as the mass fraction of P (g) in the plant compartment in percent of total plant P (g). Resorption efficiency (RE) of nutrient elements X (X = P, N) from senescent leaves collected in December 15 was estimated by Eq. 1.

$$\mathrm{RE(X)} = \left(1 - \left(\frac{(1 - 0.21) \times Xs}{Xf}\right)\right) \times 100 \qquad (1)$$

Here Xs and Xf stand for nutrient concentrations in senescent and full season leaves, respectively. As no specific data on mass loss during senescence for beech was available, we used the average value of mass loss (21%) based on a multiple species' analysis by Van Heerwaarden et al. (2003). A similar average mass loss of 21.6% was reported for "deciduous angiosperms" by Vergutz et al. (2012). Considering the crucial role that xylem plays for P recycling in beech (Netzer et al., 2017), the P sink strengths of leaves (S_{Leaves}), and fine roots ($S_{fine\ roots}$) were calculated as total P concentration in leaves and fine roots, respectively, divided by concentrations of inorganic P in wood exudates.

Plasticity indices

We adapted the concept of plasticity indices (Valladares et al., 2006) to quantify the change of a given plant trait during the response of a beech sapling with a given P nutritional status to changes in soil P availability. For more on rationalizing the following equations see Respective Section "Discussion." In a first step, we calculated the average potential span for a given trait (ΔTr) as the arithmetic mean of pairwise differences between trait values of the m and n replicates, respectively, within the two treatments with beech saplings from BBR ($TR_{BBRinBBR}$) and LUE ($TR_{LUEinLUE}$) growing in soil from their own site using Eq. 2.

$$\Delta Tr = \frac{|\sum_{i=1..n,\, j=1..m}(Tr_{BBR\ in\ BBR}(i) - Tr_{LUE\ in\ LUE}(j))|}{m * n} \qquad (2)$$

Only traits with significant differences between the mean trait values for the two treatments were taken into account. In a second step, we calculated average plasticity indices for the response of saplings from LUE to soil with high P availability ($PL_{LUEplant}$) and for the response of saplings from BBR to soil with low P availability ($PL_{BBRplant}$). The plasticity indices were calculated as the arithmetic mean of pairwise differences between trait values of the m and n replicates, respectively, within two treatments with beech saplings from a given site growing in soil from their own site and in soil from the other site, divided by the trait span (Eqs 3 and 4).

$$PL_{LUE\ plant} = \left(\frac{|\sum_{i=1..n,\, j=1..m} (Tr_{LUE\ in\ BBR}(i) - Tr_{LUE\ in\ LUE}(j))|}{m * n} \right) / \Delta Tr \qquad (3)$$

$$PL_{BBR\ plant} = \left(\frac{|\sum_{i=1..n,\, j=1..m} (Tr_{BBR\ in\ BBR}(i) - Tr_{BBR\ in\ LUE}(j))|}{m * n} \right) / \Delta Tr \qquad (4)$$

Statistical analysis

Using analysis of variance (ANOVA), we assessed to what extent current soil (the soil in which the beech saplings were growing during the experiment) on one hand, and plant origin (forest site where the beech saplings were collected) on the other hand, influenced the measured plant traits in the first and second growing season. All ANOVA analyses were performed in R, version 3.1.2 (R Core Team, 2014) with marginal type II test (Anova, package: “car”) in order to account for unequal group sizes. Prior to ANOVA, data was subjected to Levene’s test (leveneTest, package: “stats”) for homogeneity of variance inside the groups and to the Shapiro-Wilk normality test (shapiro.test, package: “stats”) for normality of residuals. Statistical significances indicated with letters in figures and tables are the result of a one-factorial ANOVA (with treatment as explanatory variable) with a Tukey post hoc test (HSD.test, package: “agricolae”). Average plasticity indices for plants from BBR and LUE were compared using multiple t-tests. Each pair was analyzed individually, without assuming a consistent standard deviation, using GraphPad Prism 7.02 Software. The standard error of the

mean for the plasticity indices was computed for the true sample size using Gaussian error propagation and assumption of no error in ΔTr.

Results

Foliar element concentrations and nutrient resorption in the first growing season

During the first growing season, both total and metabolic P concentrations in leaves were slightly higher for beech saplings originating from BBR, the site with high soil P availability, than for those from LUE, and the site with low soil P availability (Table 2.2). According to ANOVA, both concentrations were significantly affected only by the factor "plant origin" (Table 2.3). The first measured reaction of the saplings to the soil they were growing in during the experiment was a higher P resorption from senescent leaves for all plants growing in LUE soil than for those growing in BBR soil, irrespective of plant origin (Figure 2.1). ANOVA revealed "current soil" as sole significant factor (Table 2.3). On the other hand, N resorption from senescent leaves did not differ among the treatments and was not affected neither by plant origin nor by current soil conditions (Figure 2.1 and Table 2.3).

TABLE 2.2 | Concentrations of P (total P, P_{tot}; metabolic P, $P_{metabolic}$) and total N (N_{tot}) in full season and senescent leaves of beech (Fagus sylvatica L.) saplings, measured in the first growing season of the experiment; saplings originated from the sites Bad Brückenau (BBR) with high soil P availability and Unterlüss (LUE) with low soil P availability, and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE; data represent mean concentrations per unit mass dry weight ± SE; different letters indicate significant differences between means according to the Tukey post hoc test.

			BBR in BBR	**BBR in LUE**	**LUE in BBR**	**LUE in LUE**
Full season	P_{tot}	(mg g^{-1})	1.23±0.09 ab	1.42±0.14 a	0.94±0.06 b	1.04±0.13 ab
	$P_{metabolic}$	(mg g^{-1})	0.35±0.07 ab	0.38± 0.05 a	0.12±0.01 c	0.17±0.05 bc
	N_{tot}	(mg g^{-1})	21.5±0.9 a	21.0±0.9 a	23.3±0.4 a	24.2±1.5 a
Senescent	P_{tot}	(mg g^{-1})	1.35±0.23 a	0.68±0.05b	0.83±0.05 b	0.54±0.13 b
	N_{tot}	(mg g^{-1})	14.2±0.7 a	13.5±0.7 a	15.3±0.4 a	16.0±1.7a

TABLE 2.3 | Analysis of variance for different traits of beech (Fagus sylvatica L.) saplings as determined in the first growing season of a rhizobox experiment; traits include concentrations of P (total P, P_{tot}; metabolic P, $P_{metabolic}$) and total N (N_{tot}) in full season and senescent leaves as well resorption efficiency for P and N; saplings originated from the sites Bad Brückenau (BBR) with high soil P availability and Unterlüss (LUE) with low soil P availability (factor plant origin), and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE (factor current soil); shown are F values for the factors and their interactions; statistical significance is indicated as ∗∗∗P < 0.001, ∗∗P < 0.01, ∗P < 0.05, ns P > 0.05.

		Source of variation		
		Current soil	**Plant origin**	**Current soil × plant origin**
Full season leaves	P_{tot}	1.97 ns	9.93**	0.15 ns
	$P_{metabolic}$	1.71 ns	33.0***	0.30 ns
	N_{tot}	1.15 ns	18.6**	0.05 ns
Senescent leaves	P_{tot}	24.0***	10.8**	0.67 ns
	N_{tot}	0.01 ns	4.17 ns	0.50 ns
Resorption efficiency	P_{tot}	26.4***	0.03 ns	0.29 ns
	N_{tot}	0.27 ns	0.82 ns	0.86 ns

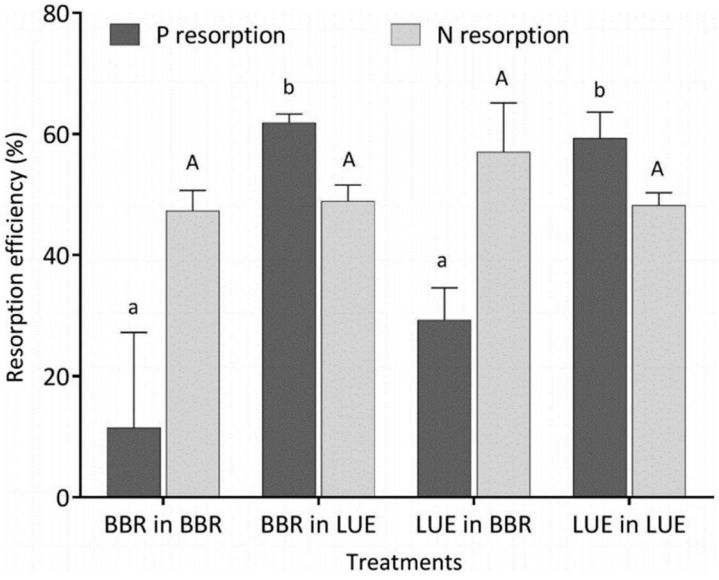

FIGURE 2.1| Resorption efficiency for P (black bars) and N (gray bars) of beech (*Fagus sylvatica* L.) saplings in the first growing season of the experiment; saplings originated from the sites Bad Brückenau (BBR) with high soil P availability and Unterlüss (LUE) with low soil P availability, and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE; data represent mean values ± SE for the different treatments; different letters indicate significant differences between means according to the Tukey post hoc test (lowercase letters, P resorption; uppercase letters, N resorption).

TABLE 2.4 | Concentrations of P (total P, P_{tot}; metabolic P, $P_{metabolic}$; inorganic P in bark exudates, $P_{Bark\_Exudates}$; inorganic P in wood exudates, $P_{wood\_exudates}$) in and biomass of different compartments of beech (Fagus sylvatica L.) saplings, measured in the second growing season of a rhizobox experiment; saplings originated from the sites Bad Brückenau (BBR) with high soil P availability and Unterlüss (LUE) with low soil P availability, and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE; data represent mean concentrations per unit mass dry weight (concentrations) or g dry weight (biomass) ± SE; different letters indicate significant differences between means according to the Tukey post hoc test

			BBR in BBR	BBR in LUE	LUE in BBR	LUE in LUE
Leaves (full season)	P_{tot}	(mg g^{-1})	1.53 ± 0.12 a	0.93 ± 0.09 b	0.92 ± 0.07 b	0.72 ± 0.27 b
	$P_{metabolic}$	(mg g^{-1})	0.67 ± 0.07 a	0.18 ± 0.02 b	0.19 ± 0.04 b	0.20 ± 0.11 b
	Biomass	(g)	1.62 ± 0.13 a	1.03 ± 0.16 b	1.18 ± 0.09 ab	0.37 ± 0.16 c
Stem	P_{tot}	(mg g^{-1})	0.81 ± 0.03 a	0.66 ± 0.04 b	0.36 ± 0.03 c	0.28 ± 0.07 c
	$P_{Bark\_exudates}$	(mg g^{-1})	0.134 ± 0.007 a	0.092 ± 0.006 b	0.071 ± 0.006 bc	0.052 ± 0.006 c
	$P_{Wood\_exudates}$	(mg g^{-1})	0.016 ± 0.001 a	0.009 ± 0.001 b	0.007 ± 0.0004 b	0.004 ± 0.001 c
	Biomass	(g)	4.8 ± 0.4 a	4.0 ± 0.6 a	5.8 ± 1.0 a	4.5 ± 0.4 a
Coarse roots	P_{tot}	(mg g^{-1})	0.76 ± 0.07 a	0.66 ± 0.11 a	0.31 ± 0.02 b	0.22 ± 0.05 b
	Biomass	(g)	8.8 ± 0.5 a	8.1 ± 0.4 a	8.2 ± 0.3 a	6.3 ± 0.1 b
Fine roots	P_{tot}	(mg g^{-1})	0.95 ± 0.04 a	0.66 ± 0.02 b	0.88 ± 0.07 a	0.48 ± 0.04 b
	Biomass	(g)	2.57 ± 0.35 a	1.49 ± 0.40 ab	1.86 ± 0.23 ab	0.62 ± 0.18 b
Whole plant	P_{tot}	(mg g^{-1})	0.88 ± 0.05 a	0.69 ± 0.07 b	0.43 ± 0.03 c	0.27 ± 0.05 c
	Biomass	(g)	17.7 ± 1.0 a	14.6 ± 1.5 ab	17.2 ± 1.3 a	11.7 ± 0.5 b

TABLE 2.5 | Analysis of variance for different traits of beech (Fagus sylvatica L.) saplings as determined in the second growing season of a rhizobox experiment; traits include, for different plant compartments, concentrations of P (total P, P_{tot}; metabolic P, $P_{metabolic}$; inorganic P in bark exudates, $P_{Bark\_Exudates}$; inorganic P in wood exudates, $P_{wood\_exudates}$), biomass, sink strength, and relative allocation of total P and biomass; saplings originated from the sites Bad Brückenau (BBR) with high soil P availability and Unterlüss (LUE) with low soil P availability (factor plant origin), and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE (factor current soil); shown are F values for the factors and their interactions; statistical significance is indicated as ***P < 0.001, **P < 0.01, *P < 0.05, ns P > 0.05.

		Source of variation for P concentrations, biomass, or sink strength			Source of variation for P or mass allocation		
		Current soil	Plant origin	Current soil × plant origin	Current soil	Plant origin	Current soil × plant origin
Leaves	P_{tot}	13.8**	12.6**	3.08 ns	41.8***	2.73 ns	0.24 ns
(Full season)	$P_{metabolic}$	17.6**	20.0***	8.92**			
	Biomass	29.0***	17.8**	0.80 ns	33.6***	35.3***	2.39 ns
	Sink strength	1.24 ns	10.1**	2.07 ns			
Stem	P_{tot}	10.6**	126***	1.04 ns	3.68 ns	5.78*	2.83 ns
	$P_{Bark\_exudates}$	23.8***	67.6***	2.68 ns			
	$P_{Wood\_exudates}$	43.8***	77.7***	4.38 ns			
	Biomass	3.05 ns	1.94 ns	0.02 ns	0.36 ns	19.2***	1.06 ns
Coarse roots	P_{tot}	4.01 ns	54.6***	0.49 ns	7.00*	6.63*	0.00 ns
	Biomass	10.9**	8.04*	2.79 ns	6.77*	0.57 ns	0.12 ns
Fine roots	P_{tot}	45.8***	5.38*	1.16 ns	10.1**	2.34 ns	1.95 ns
	Biomass	13.5**	6.25*	0.07 ns	12.4**	7.78*	0.12 ns
	Sink strength	2.69 ns	34.4***	0.09 ns			
Whole plant	P_{tot}	11.9**	72.4***	0.09 ns			
	Biomass	11.0**	1.36 ns	0.91 ns			

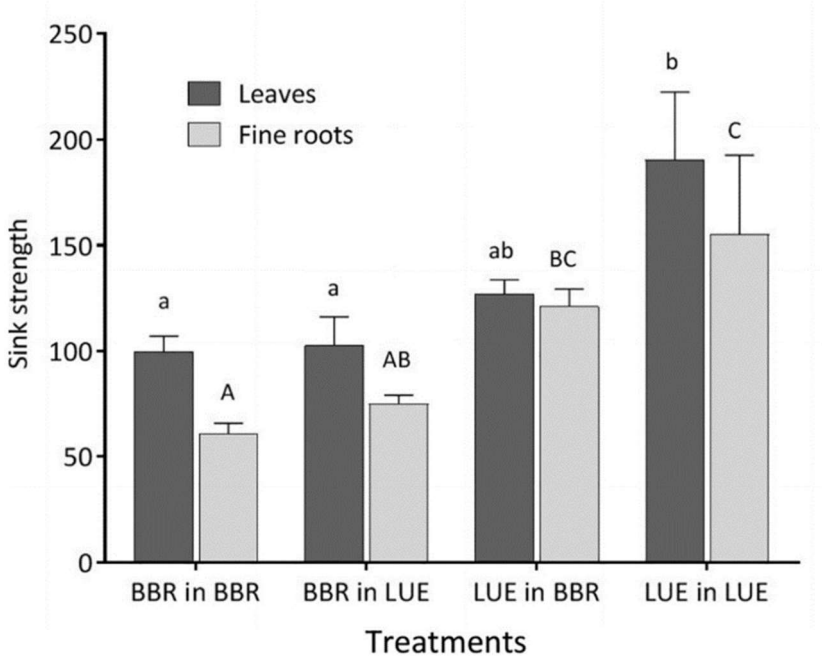

FIGURE 2.2 | Sink strength of leaves (black bars) and fine roots (gray bars) of beech (*Fagus sylvatica* L.) saplings in the second growing season of the experiment; saplings originated from the sites Bad Brückenau (BBR) with high soil P availability and Unterlüss (LUE) with low soil P availability, and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE; data represent mean values ± SE for the different treatments; different letters indicate significant differences between means according to the Tukey post hoc test (lowercase letters, leaves; uppercase letters, fine roots).

Phosphorus concentrations in different plant compartments in the second growing season

In the second growing season, total P concentrations in stem and coarse roots, and average concentrations in the whole plant still reflected the soil P availability at the site of plant origin with higher values for the saplings from BBR than for those from LUE (Table 2.4). Plant origin dominated as factor in the ANOVA (Table 2.5). By contrast, the P concentrations in fine roots were significantly affected mainly by the factor current soil (Table 2.5) and were higher for saplings growing in the BBR soil (Table 2.4). Total and metabolic P concentrations in leaves as well as inorganic P concentrations in bark and wood exudates were similarly affected by both plant origin and current soil (Table 2.5), which led to higher values for the saplings from BBR growing in mineral soil from their own site than for all other treatments (Table 2.4). The sink

strengths of leaves and roots were still clearly dominated by the factor plant origin (Table 2.5) and were higher for the saplings from LUE (Figure 2.2).

Biomass and P allocation to leaves and roots in the second growing season

Total plant biomass and biomass of leaves and roots were mainly and significantly affected by the factor current soil (Table 2.5), with the smallest values for saplings from LUE growing in soil from their own site (Table 2.4). By contrast, stem biomass did not differ among the treatments. Irrespective of the treatment, the largest percentage of biomass and P was allocated to coarse roots (Figure 2.3D). Of all measured plant traits, relative allocation of P to leaves most clearly reflected the factor current soil (Table 2.5) with significantly higher values for beech saplings growing in the BBR soil, irrespective of their site of origin (Figure 2.3A). Also, P allocation to fine roots exhibited a significant influence of current soil (Table 2.5), with values tending to be higher for saplings growing in BBR soil (Figure 2.3C). Noteworthy was the clearly highest value of all treatments for saplings from LUE growing in soil from BBR. On the other hand, allocation of biomass to the stem was still mainly determined by plant origin (Table 2.5) with a tendency to higher values for saplings from LUE (Figure 2.3B). A significant but similarly strong influence of both current soil and plant origin was detected for the allocation of biomass to leaves and fine roots (Table 2.5), with the highest and lowest values for the saplings from BBR and LUE, respectively, growing in their own soil (Figures 2.3A,C).

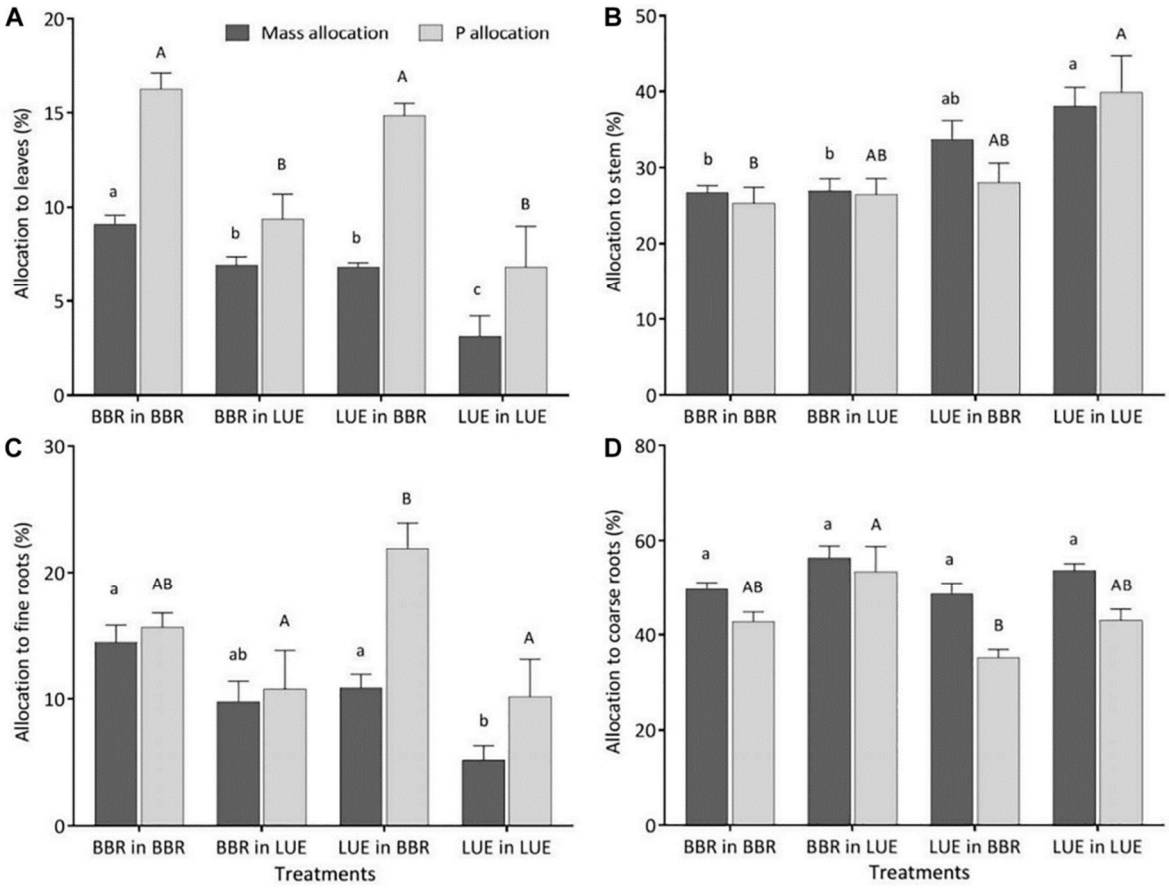

FIGURE 2.3| Relative allocation of biomass (black bars) and P (gray bars) to different compartments of beech (Fagus sylvatica L.) saplings (A: leaves; B: stem; C: fine roots; D: coarse roots) in the second growing season of the experiment; saplings originated from the sites Bad Brückenau (BBR) with high soil P availability and Unterlüss (LUE) with low soil P availability, and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE; data represent mean values ± SE for the different treatments; different letters indicate significant differences between means according to the Tukey post hoc test (lowercase letters, mass allocation; uppercase letters, P allocation).

Plasticity Indices

Figure 2.4 shows the plasticity indices for beech saplings from LUE and BBR considering all plant traits for which the precondition of a significant difference between the two treatments with the saplings growing in soil from their own site was fulfilled. Total and metabolic P concentrations in full season leaves, total P concentrations in senescent leaves, and P resorption were more plastic for beech saplings from BBR responding to soil with low P availability. On the other hand, P and mass allocation to stem were more reactive for beech saplings from LUE acclimating to soil with high P availability. For all other traits, plasticity was similar for beech saplings from both origins, and thus independent on the direction of acclimation. However, values differed strongly with particularly high indices for P allocation

to leaves and P concentration in fine roots and particularly low indices for P concentrations in stem and coarse roots.

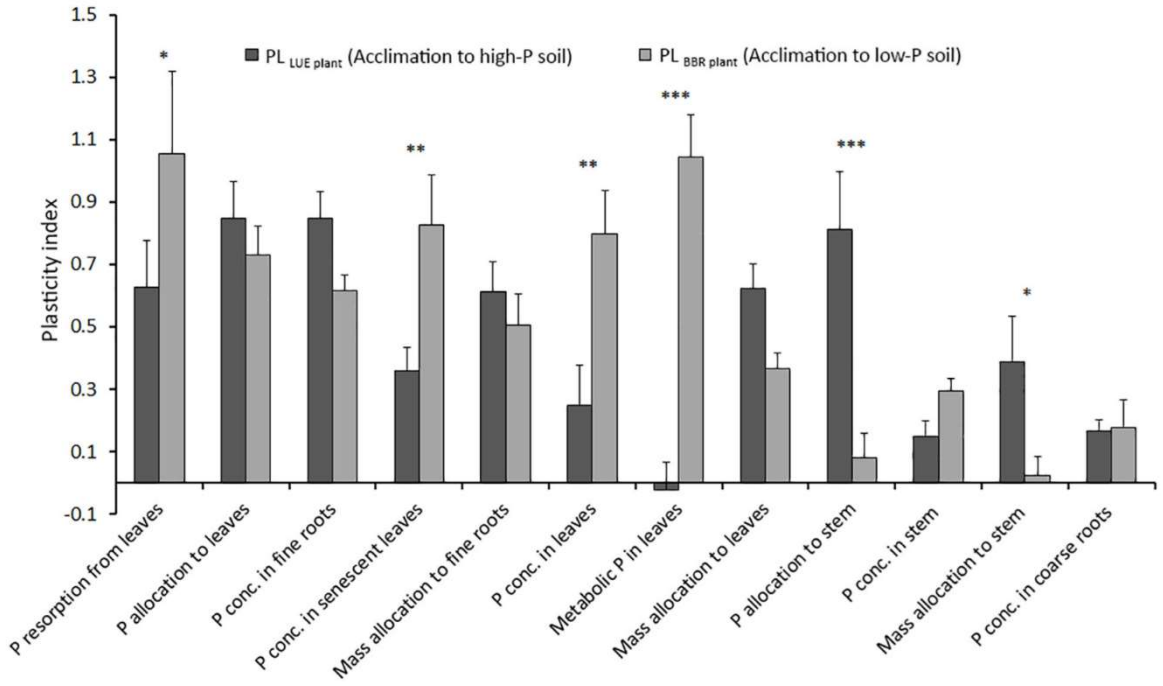

FIGURE 2.4 | Average plasticity indices for the response of beech (*Fagus sylvatica* L.) saplings from the site Unterlüss (LUE) with low P nutritional status to mineral soil from Bad Brückenau (BBR) with high P availability (black bars) and for saplings from the site BBR with high P nutritional status to mineral soil from LUE with low P availability (gray bars); data represent mean values ± SE of all data pairs; statistical significance is indicated as $***P < 0.001$, $**P < 0.01$, $*P < 0.05$.

Discussion

Our experimental model systems employed beech saplings from forest sites strongly differing in soil P availability in terms of both measures of available P such as P_{resin} and total P stocks (Lang et al., 2017), BBR with high, and LUE with low P availability. At the end of the experiment, the saplings from the two sites still exhibited a significantly different P nutritional status in terms of average P concentration in the whole plant. Using material from mineral soil horizons from the two sites as model soils, while providing homogeneous material for the experimental replicates, possibly changed soil P availability compared to the natural situation, in particular in the case of the LUE site, where 50% of the P stock is stored in the organic surface layer (Lang et al., 2017). Nevertheless, in the second growing season total P concentrations in all plant compartments where similar to beech saplings collected at the

same sites in the same year as our saplings, but were excavated together with an undisturbed soil core, and further grown this way for one season in the greenhouse (Zavišić et al., 2018). The results of our experiment thus allow to discuss the influence of P nutritional status on the plant internal allocation of P and biomass for beech saplings growing in soil with different P availability. We consider the treatments with saplings growing in the soil from their own site to represent the situation of plants adapted to a given soil situation. We furthermore assume that the results from the treatments with saplings growing in the soil from the other site provide clues on the plastic response of beech that allows it to acclimate to changes in soil nutrient conditions.

Foliar P concentrations in beech depend on both soil p availability and P nutritional status of the plant

As pointed out in the introduction, P concentrations in tissues of trees can strongly vary with the season (Eschrich et al., 1988; Netzer et al., 2017; Zavišić and Polle, 2018) which indicates site dependent dynamics of nutrient uptake and plant internal nutrient allocation. Some studies even suggest that P utilization in beech can be decoupled from P uptake, i.e., the growth of young leaves may strongly depend on the transport of nutrients stored in the previous season in organs such as stem and coarse roots, but also from older leaves during a growing season (Schachtman et al., 1998; Güsewell, 2004; Zavišić et al., 2018). This could well explain our observations, that during the first growing season, total and metabolic P in leaves reflected the P availability of the soil at the site of plant origin rather than the soil in which the beech saplings were growing during the experiment. The results of the second growing season point to combined effects of current soil and plant origin. On one hand, the low P concentrations in leaves and roots of the beech saplings growing in the LUE soil, irrespective of their site of origin and thus their growing in LUE soil, these results point to the ability of beech to deal with different soil P availability optimally at a given P nutritional status of the plant by internal allocation of P and mass. It suggests a priority to alleviate the primary light limitation by producing as much photosynthetic organs as possible, while vacuolar P concentrations are kept low when soil P availability and/or the P nutritional status of the whole plant is low. The latter has been shown to be a successful mechanism in other plants (Lee et al., 1990; Lee and Ratcliffe, 1992; Mimura, 1995; Mimura et al., 1996). Comparing the leaf P concentrations in the second growing season with the threshold values for young beech trees published by Göttlein (2015) suggests the following. While a P concentration within the

normal range (1.1–2.1 mg/g DW) indicates both a high P nutritional status of the plant and high soil P availability, a P concentration below the lower threshold of 1.1 mg/g DW may indicate that either the P nutritional status of the plant or the soil P availability or both are low. The same interpretations seem to apply to N/P ratios which, according to Mellert and Göttlein (2012) were in the normal range for BBR plants growing in BBR soil and above in all other cases.

P resorption from senescent leaves is the first reaction to current soil conditions

Phosphorus resorption from senescent leaves is a nutrient conservation mechanism, common for several plant species including beech, which reduces losses, and decreases the nutrient uptake demand for the next year (Aerts, 1996; Brant and Chen, 2015). As a consequence, there is less input of P with litterfall diminishing a recycling pool in the ecosystem (Lang et al., 2017). The higher P resorption efficiency of plants grown in the LUE soil than for those grown in the BBR soil, irrespective of their P nutritional status, are in general agreement with findings from recent chronosequence studies by Richardson et al. (2004) and Hayes et al. (2014). The sensitive reaction of this parameter to the current soil in the first growing season, when the concentrations in mature green leaves still reflected the nutrient situation at the site of plant origin, confirms the findings of Hofmann et al. (2016) who showed that fertilization of beech saplings growing in soil low in P led to a decrease in P resorption efficiency. By contrast, the similar values of N resorption efficiency, measured in our experiment for all treatments, indicate a similar degree of N availability. The observed level of about 50% falls within the average range compiled by Aerts (1996) for deciduous trees and shrubs.

P allocation to leaves is a sensitive indicator of soil P availability

In this section we discuss in more detail the patterns of relative P and mass allocation to various plant compartments in the second growing season. While the link between nutrient allocation and a number of site factors such as precipitation, forest type, latitude, and plant age has been well studied (Sardans and Peñuelas, 2013), little is known about how soil P availability affects P allocation (Yang et al., 2016). Let us first compare the saplings from LUE and BBR when growing in the soil from their site of origin. The lower allocation of mass and P to leaves and fine roots and higher allocation to the stem for the beech saplings from LUE are consistent with a conservative strategy reducing growth under nutrient limitation. Yang et al. (2016) also observed a slower growth of beech saplings from LUE than from BBR when

growing in undisturbed soil cores from their own site of origin which represents a more natural situation than our experiment. A smaller root system in soil of low P availability is in line with the resource economics hypothesis (Grime, 1977; Craine, 2009) and the general characteristic that plant ecotypes from nutrient-limited environments grow slower than ecotypes from fertile soils. At the same time a tendency to a higher ratio between allocation of biomass to fine roots and leaves for the LUE saplings (mean ± SE: 2.0 ± 0.5) than for BBR saplings (mean ± SE: 1.6 ± 0.2) is in accordance with the notion that under nutrient limited conditions plants should allocate proportionally more resources to roots (George et al., 2011). We do not know to what degree the difference in the size of the root system between these two treatments translates into volume of soil explored. In both treatments about 50% of the root tips were mycorrhized (mean ± SE: BBR in BBR, 49 ± 3%; LUE in LUE, 51 ± 10%), but extension of the hyphal system was not assessed. When considering the two treatments with beech saplings growing in soil from the other site, the most striking result is that P allocation to leaves was the same as for the saplings adapted to the respective site, while mass allocation was intermediate in both cases. This indicates that P allocation to leaves might be a particularly good indicator of soil P availability, irrespective of P nutritional status of the plant. It further may be a key trait that the plant adjusts by balancing leaf biomass production against the transfer of nutrients from the soil or from/to internal storage organs such as stem or coarse roots. Our results for the BBR plants growing in LUE soil indicate that the response of beech saplings with a high P nutritional status to low soil P availability is to decrease leaf P concentration, but to still produce as much leaf biomass as possible under the new low P flux into the roots. At this stage of plastic response, the substantial amount of P stored in coarse roots, and stem allows to partly compensate for the low P uptake. This compensation is also indicated by intermediate P concentrations in bark and wood exudates, maintaining the sink strength of leaves, and roots at a low value similar to BBR plants growing in their own soil. From the results for the LUE plants growing in BBR soil it appears that a beech sapling with low P nutritional status in response to high soil P availability increases leaf biomass production as much as possible under the new high P influx, while it keeps P concentrations in leaves low. Phosphorus concentrations in fine roots as high as for the saplings from BBR adapted to this soil, and the highest P allocation to fine roots of all treatments might be explained by inefficient P translocation to the aboveground plant compartments or by P recycling from stem and old leaves to support fine root growth (Marschner et al., 1996; Netzer et al., 2017).

The higher sink strength of fine roots for LUE than BBR plants when growing in BBR soil argue rather in favor of inefficient translocation. The significant difference in P allocation to leaves between beech saplings growing in BBR and LUE soil was not found for saplings growing in undisturbed soil cores from their own site (Zavišić and Polle, 2018; Zavišić et al., 2018). For a sampling time in July/August P allocation in saplings from both sites was similar to the one we measured for saplings growing in BBR soil, but also varied strongly during the growing season. This discrepancy may be explained by the presence of the organic surface layer in the undisturbed soil cores which could have increased the effective availability of soil P in the case of the LUE plants. The importance of the organic surface layer for the nutrition of young beech trees from soil low in P was clearly demonstrated by Hauenstein et al. (2018) who showed that the presence of the organic surface layer improved P nutrition and growth of beech seedlings in the LUE soil but had no effect in the BBR soil. The effect of the organic surface layer at LUE was attributed on one hand to a particularly high microbial activity promoted by a high root density, but also to a high water retention capacity minimizing loss of added or mobilized P to the mineral soil (Hauenstein et al., 2018). In particular, the high water holding capacity of the surface layer at LUE might have led to a similar effective P availability in the LUE soil as in the BBR soil during the well watered experiments with undisturbed soil cores. On the other hand, at on average drier conditions in the field one would expect a smaller effect, being consistent with the lower P nutritional status of beech saplings from LUE than from BBR. Furthermore, we cannot exclude that stress induced by the transplantation process (accidental cutting of some roots during sampling, dying-off of fine roots during storage between sampling, and planting) may have affected P allocation even in the second growing season. In particular, “recovery” of a mycorrhized root system in the new soil could have led to a temporary elevated biomass and P allocation to the fine roots. A comparison with the more natural situation in the studies of Zavišić and Polle (2018) and Zavišić et al. (2018) revealed that biomass and P allocation were twice as high in our rhizoboxes than in the undisturbed soil columns of the mentioned studies for saplings from BBR but similar for saplings from LUE. Considering (i) the high variability among individual saplings, (ii) the differences in environmental conditions between the rhizobox and column studies, and (iii) that the larger differences occurred for the more fertile soil from BBR, this comparison does not indicate a large effect of transplantation in our rhizoboxes.

Phosphorus allocation to leaves and P concentrations in fine roots represent best the plastic response of beech to changes in soil P availability

In this section we discuss the differences in various plant traits among the experimental treatments in terms of a plastic response of beech saplings to changes in soil P availability. For this, we assume that the trait values of saplings growing in the soil from their site of origin represent the extremes and thus define the potential trait span. We further assume, that the higher the degree to which the value of this trait changes relative to the trait span for a sapling exposed to soil from the other site, the higher is the plastic response of this trait. We did neither include P allocation to fine roots nor P and mass allocation to coarse roots, because there was no significant trait span according to the definition. We interpret the differences between the trait plasticity for BBR plants exposed to low P soil and for LUE plants exposed to high P soil as differences between the acclimation processes from high to low and from low to high soil P availability. Considering all our plasticity indices, they indicate a rather high plasticity compared with other studies employing various plasticity quantification methods (Valladares et al., 2002; Stojnić et al., 2013, 2015a). The direction of acclimation affected the plasticity index for some traits but was unimportant for others. While the plasticity of P resorption and P concentration in leaves was higher for the beech saplings with a high P nutritional status acclimating to low P soil, P, and mass allocation to the stem were more reactive for saplings with a low P nutritional status acclimating to soil with high P availability. The asymmetry in size of the reaction of the mentioned traits emphasizes the importance of taking into account the initial nutritional status of a plant when assessing plasticity. On the other hand, the strong responses of P allocation to leaves and P concentration in fine roots, which were similar for both directions of acclimation, suggest that these plant traits are most suitable to assess plasticity of beech in response to soil P availability.

Conclusion

A two-year cross-growth experiment with beech saplings and mineral soil from two forest sites differing strongly in soil P availability demonstrated a high plasticity of juvenile *F. sylvatica* to differences in soil P availability. Some influence of recovery from stress implicated by the transplantation on these results, can however, not be excluded. Relative P allocation to leaves appears to be a particularly good indicator of soil P availability, irrespective of the P nutritional status of the plant. In contrast to P allocation to leaves, foliar P concentrations were not a clear indicator of soil P availability, which may partly explain the

lack of relation between leaf P concentrations and soil P availability in studies comparing forest sites on the European or regional scale (Talkner et al., 2015; Lang et al., 2017). In particular, the ambiguity with respect to the interpretation of foliar P concentrations below threshold values for normal growth implies that the observation of such low values for beech on monitoring sites (Jonard et al., 2015; Talkner et al., 2015) not necessarily indicates P deficiency and respective growth reduction, but rather a tree with still good P nutritional status but reacting to a decrease in soil P availability. Overall, the results clearly rebut our working hypothesis, that adaptation of beech saplings to new soil conditions is driven by the plant striving to achieve or maintain high foliar P concentrations. By contrast, they point to a sensitive signaling network that allows the plant to produce as much biomass as possible under given soil conditions by regulating mass and nutrient allocation accordingly.

Data availability

The datasets generated for this study are available on request to the corresponding author.

Author contributions

All authors designed the experiment and contributed significantly to the final version of the manuscript. SM and JL collected the plant and soil materials used in the study. SM set-up and carried out the experiment, performed most of the chemical analyses, analyzed the data, and wrote the first version of the manuscript.

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and chemical laboratories provided various technical support. Andrea Polle (University of Göttingen) provided data from their experiments with undisturbed soil cores for comparison.

CHAPTER 3

Plant nutritional status explains the modifying effect of provenance on the response of beech sapling root traits to differences in soil nutrient supply.

Abstract

Forests dominated by beech (*Fagus sylvatica* L.) cover large parts of Europe where they occupy a broad ecological niche in terms of soil fertility. This indicates a large potential to adapt to different soil conditions over long time periods. Recent changes in tree mineral nutrition across Europe raise the question to what degree beech can acclimate to changing soil conditions in the short term. In this study, we aimed at assessing the plasticity of root traits and rhizosphere properties of young beech trees from populations, that are adapted to either high or low nutrient supply, when growing in soils differing in their fertility.

We sampled beech saplings from two forest sites differing in nutrient supply, most distinctly in phosphorus. We grew them for two years in rhizoboxes in mineral soil either from their own site or from the other site. We assessed the influence of the factors „plant origin“ and „current soil“ on root traits and rhizosphere properties. Fine root traits related to growth (biomass, length), architecture (branching) and morphology (diameter) responded strongly to the properties of the soil. This response was modified by an effect of provenance, that was consistent with an influence of the plant status in those nutrients, which were not in sufficient supply in the soil. An additional genotypic difference in the sensitivity of the beech saplings to different soil nutrient supply could not be excluded. Fine root parameters normalized for length, mass or volume (root tip density and frequency, specific root length and area, root tissue density) did not differ among the treatments. Differences in mycorrhizal colonization of root tips and rhizosphere parameters related to phosphorus mobilization potential (pH, abundance of organic acid anions, phosphatase activity) were small and mainly determined by the soil. Provenance had only a minor modifying effect, possibly due to differences in the ability to transfer carbon compounds from the shoot to the root and the fungal partner.

Our results indicate a high plasticity of young beech trees to adapt root growth, architecture and morphology to different soil nutrient supply, thereby also taking into account internal nutrient reserves.

Introduction

Forests dominated by European beech (*Fagus sylvatica* L.) cover large parts of Europe where climatic conditions are suitable (Durrant et al., 2016). They occupy a broad ecological niche in terms of soil chemical properties including pH (3.2-7.3), base saturation (3–99%), C:N ratios

(15-34 mol mol^{-1}) and plant-available P pools in the mineral topsoil (11–1287 mol P m^{-2} 10 cm^{-1}) (Leuschner et al., 2006). This indicates a large potential of beech to adapt to different soil conditions over long periods of time. Considering the recent changes in tree mineral nutrition across Europe (Jonard et al., 2015), the question arises to what degree beech can acclimate to changing soil conditions in the short term. Of particular concern is phosphorus (P), since plant-available P occurs at only low concentrations in the soil solution, while most P is present in unavailable forms adsorbed to reactive surfaces of the soil solid phase or is bound in minerals or soil organic matter (Hinsinger 2001).

Root-soil interactions have been shown to play a major role in adaptation to given soil conditions in relation to nutrient acquisition. Such interactions can include alterations of root growth, architecture and morphology, formation of mycorrhizae, and root exudation affecting nutrient availability in the rhizosphere (Richardson et al., 2009).

Root growth, architecture and morphology can be highly plastic in response to soil nutrient availability (Hodge et al., 2009). In particular, alterations in reaction to low soil availability of P and major nutrient cations (Mg, K, Ca), that have been found across a large range of plant species, include inhibition of primary root growth and promotion of lateral root growth (Gruber et al., 2013; Niu et al., 2013). Relations are not so clear in the case of N, which is demonstrated by maximum root length and branching of the model plant *Arabidopsis* at intermediate N limitation (Kiba et al., 2016), and effects on branching appear in addition to depend on the chemical form of N (nitrate or ammonium). While these mechanisms have been well established for crops, evidence for trees is scarce, and assessing effects of nutrient availability in the field is often made difficult by interaction with other soil properties such as texture (Weemstra et al., 2017), environmental factors such as the availability of water (Hertel et al., 2013) or light (Minotta and Pinzauiti, 1996), or stand age (Finér et al., 2007). Preferential root proliferation in nutrient-enriched patches and layers has been observed frequently (Hodge, 2006, Chen et al., 2016). Particularly important for trees growing in nutrient-poor soils in temperate forests is preferential exploration of the topsoil, including the organic surface layer (Borken et al., 2007; Hauenstein et al., 2018).

Uptake via the mycorrhizal pathway is of major importance for N and P nutrition of trees in temperate zones (Plassard and Dell, 2010; Chalot and Plassard, 2011). While fertilization with

P often decreases mycorrhization in inoculation experiments (Garbaye and Wilhelm, 1985; Kazantseva et al., 2009), under field conditions relationships between soil P availability and measures of mycorrhization or mycorrhizal P uptake are less clear and might differ seasonally (Yang et al., 2016; Spohn et al., 2018). In most studies on the effect of N availability, mycorrhizal colonization increased with decreasing N availability (e.g. Brunner et al., 2001, Sun et al., 2010), however under natural concentration gradients also higher colonization was found at lower C:N ratios (Hawkins et al., 2015). Under field conditions, also the effects of both N and P supply on mycorrhization have to be considered (e.g. Bahr et al., 2013). Studies assessing host effects on ectomycorrhizal fungi provide a variable picture on the degree to which trees can actively shape the rhizosphere fungal community (Ishida et al., 2007; Lang et al., 2017; Spohn et al., 2018). There can also be competition between fungal partner and host plant, leading to limited nutrient transfer to the host (e.g. Simon et al. 2017).

On a small scale in the rhizosphere, root exudation can lead to an increase in the abundance of compounds that potentially increase the bioavailability of P, including protons, low molecular-weight organic acid anions, and phosphatases (Hinsinger et al., 2011). However, under field conditions it is often difficult to differentiate between the different sources of these compounds. Organic acid anions and phosphatases can be produced and released to the soil by roots, mycorrhizal hyphae, and free-living microorganisms (Gianfreda and Ruggiero, 2006; Oburger et al., 2011; Plassard et al., 2011). Therefore, also little is known to which degree plants are able to influence the P mobilization potential in their rhizosphere directly via root exudation, and/or indirectly via stimulating microbial activity or shaping the soil microbial community. For example, root exudates can stimulate P mineralization by heterotrophic bacteria in the rhizosphere (Spohn et al., 2013). Phosphatase activity in soil is often linked to soil P availability (Marklein and Houlton, 2012; Hofmann et al., 2016) but this relation can be masked, e.g. by the generally strong correlation with soil organic matter content (Nannipieri et al., 2011). The root exudation of organic acid anions may be induced by a low P nutritional status of the plant, as has been shown for crops (Hinsinger, 2001). However, it can also be a reaction to other conditions such as high Al concentrations in acid soils (Richardson et al., 2009), or be part of constitutive release of excess carbon (Heim et al., 2001; Eldhuset et al., 2007). Although proton exudation by roots can be induced by P

deficiency (e.g. Shahbaz et al., 2006), alteration of rhizosphere pH often depends on the form of mineral nitrogen taken up by the plant (Riley and Barber, 1971; Hinsinger, 2001).

To our best knowledge, there are no studies related to the ability of beech populations, adapted to sites differing in resource availability, to grow in soils with different nutrient supply. Such a provenance effect could be related to differences in plant nutritional status, considering that signaling of this status is involved in controlling root development, initiating mycorrhizal symbiosis, and producing and exuding mobilising substances (George et al., 2011; Chalot and Plassard, 2011; Niu et al. 2013; Xuan et al., 2017). However, also genotypic differences related to adaptation to specific site conditions could be involved. Beech populations across central Europe have been shown to be genetically closely related in terms of neutral markers, such as microsatellite loci, but to differ in genes related to adaptive traits (e.g. Buiteveld et al., 2007). Nevertheless, genotypic diversity has often been found to be larger within than among populations, including also adaptive traits (e.g., Cuervo-Alarcon et al., 2018). In contrast to the lack of studies on acclimation to changes in nutrient supply, the ability of beech populations from sites with different climatic conditions to acclimate in the short term to increased drought frequency has recently received much attention (Meier and Leuschner, 2008; Cuervo-Alarcon et al., 2018). Specifically, Meier and Leuschner (2008) found that while root traits such as relative fine root growth and turnover of beech populations from sites differing in precipitation responded generally strongly to drought treatment, the effect of provenance was small. Above-ground adaptive traits related to resource acquisition such as photosynthetic activity have been considered in the so-called “resource economics” framework. This differentiates between “acquisitive” and “conservative resource strategies” exhibited by plants growing at resource rich and resource poor sites, respectively (Craine, 2009). However, Weemstra et al. (2016) concluded from their review that there is little evidence for root physiological and morphological traits being indicative of specific nutrient acquisition strategies. Specifically, fine root diameter had often been found to correlate with root longevity and therefore been considered a respective potential below-ground indicator. Taking together the information on genotypic relations among beech populations, their acclimation to drought, and relation between below-ground plant traits and nutrient acquisition strategies, we do not expect strong genotypic provenance effects on root traits during acclimation to a different soil nutrient supply.

In this study, we aimed at assessing the plasticity of root traits and rhizosphere properties of young beech trees from populations, that are adapted to either high or low nutrient supply, when growing in soils differing in their fertility. To this end, we sampled beech saplings from two forest sites differing in nutrient supply, most distinctly in P. We grew the saplings in mineral soil either from their own site or from the other site. In all four experimental treatments, we assessed the influence of the factors „plant origin“ and „current soil“ on root growth, architecture and morphology, mycorrhization and the occurrence of P mobilizing compounds in the rhizosphere. In this “cross-exchange” approach, the factor “current soil” was considered to reflect not only differences in physicochemical soil properties but also in microbial communities adapted to these properties.

We hypothesized, first, that the assessed root traits and rhizosphere parameters are determined mainly by the factor “current soil”. We hypothesized, second, that the factor „plant origin“ modifies the effects of the soils, and that the modifying effect can be attributed mainly to differences in the plant nutritional status.

Materials and methods

Plant and soil materials

Plant and soil materials were collected at the core research sites of the priority programme 1685 “Ecosystem nutrition” of the German Science Foundation (DFG) (http://www.ecosystem-nutrition.uni-freiburg.de/) in Unterlüss (Lower Saxony, Germany, LUE) and Bad Brückenau (northern Bavaria, Germany, BBR). The sites both sustain mature mono-specific beech stands, but differ in environmental conditions and soil properties (Lang et al., 2017), as summarized in Supplementary Table 3.S1. The site LUE has a drier climate than the site BBR. The soil at BBR contains more N and P than the one at LUE in terms of total element stocks, as well as concentrations in organic surface layer and mineral soil. In particular, the P concentration in the mineral soil is much higher at BBR, whereas the organic surface layer is an important source of N and P at LUE. Both organic surface layer and mineral soil at LUE are more acidic and exhibit a lower base saturation than their BBR counterparts. Furthermore, the mineral soil at BBR has a loamy texture with a higher cation exchange capacity than the sandy mineral soil at LUE. Mature beeches are of similar age and height at both sites; however, their average diameter is much smaller at LUE.

Saplings of beech (*Fagus sylvatica* L.) of similar size were collected during their dormancy period in December 2014 and stored at 4°C with their roots embedded in soil until planting. Based on tree-ring counting, they were between 12 and 15 years old at the end of our experiment (Meller et al., 2019). Total nutrient contents in various plant compartments were analysed as described by Meller et al. (2019), with the method for P also applying to Mg, K, and Ca.

Soil materials were taken from the Bh horizon in LUE and the uppermost part of the Bv horizon in BBR. This choice represented a compromise between root density within the soil profile – and thus potential importance for nutrient uptake – and organic matter content of the material being sufficiently low to not interfere with the assessment of rhizosphere properties. Soils were air-dried at 15°C, sieved to 4 mm, and homogenized. Plant residues were removed. Selected physical and chemical properties of the soils are summarized in Table 3.1 and were mostly determined as described by Meller et al. (2019). Sequential P extraction was performed according to Hedley et al. (1982) as modified by Tiessen and Moir (2006). In Table 3.1, resin exchangeable inorganic P (P_{resin}), inorganic P (P_i) in various extracts (0.5 M $NaHCO_3$, 0.1 M NaOH before and after sonication, 1 M HCl, concentrated HCl) and organic P (P_{org}) in the $NaHCO_3$ and NaOH extracts are shown. The soil material from BBR exhibited a finer texture, a higher pH, a higher content in exchangeable nutrient cations, a higher organic carbon content, lower C_{org}/N_{tot}, C_{org}/P_{org} and N_{tot}/P_{org} ratios and much higher concentrations of all inorganic and organic P fractions than the material from LUE. On the other hand, the LUE soil exhibited a higher proportion of P_{org} than the BBR soil, and the base cation to Al ratio was similar in both soils.

TABLE 3.1 | Properties of the soil materials from the forest sites Bad Brückenau (BBR, Bv horizon) and Unterlüss (LUE, Bh horizon); BC / Al refers to the ratio between the sum of exchangeable base cations (Mg, K, Ca) and exchangeable Al; inorganic P (P_i) and organic P (P_{org}) used in element ratios refer to the respective total extractable fractions; data are from Meller et al. (2019) except for the concentration of P fractions.

		BBR Bv	LUE Bh
General soil properties			
Sand	(g kg^{-1})	287	811
Clay	(g kg^{-1})	253	43
pH in H_2O		4.8	4.0
Sum of exchangeable Mg, K, Ca	($mmol_c$ kg^{-1})	3.3	1.4
BC / Al	(mol_c mol_c^{-1})	0.08	0.07
C_{org}	(g kg^{-1})	41.2	18.5
N_{tot}	(g kg^{-1})	3.2	0.7
Inorganic P (P_i)			
resin P_i	(mg kg^{-1})	5.3	0.4
$NaHCO_3$ extractable P_i	(mg kg^{-1})	88.2	1.9
NaOH extractable P_i	(mg kg^{-1})	334.9	5.9
NaOH extractable sonic P_i	(mg kg^{-1})	52	1.2
1M HCl extractable P_i	(mg kg^{-1})	240	1.6
HCl_{conc} extractable P_i	(mg kg^{-1})	195.9	18.1
Total extractable P_i (without residual P)	(mg kg^{-1})	916.3	29.1
Organically bound P (P_{org})			
$NaHCO_3$ extractable P_{org}	(mg kg^{-1})	78.9	12.3
NaOH extractable P_{org}	(mg kg^{-1})	1036.2	42.5
NaOH extractable sonic P_{org}	(mg kg^{-1})	140.9	34.3
Total extractable P_{org} (without residual P)	(mg kg^{-1})	1256	89.1
P_{org} / P_i	(g g^{-1})	1.37	3.07
Stoichiometric ratios			
C_{org} / N_{tot}	(g g^{-1})	12.8	24.7
C_{org} / P_{org}	(g g^{-1})	33	208
N_{tot} / P_{org}	(g g^{-1})	2.6	8.4

Experimental setup

In April 2015, rhizoboxes were set up with beech saplings planted either in the soil from their site of origin or in the contrasting soil from the other site. In a completely randomized design, each treatment was replicated six times. The rhizoboxes had inner dimensions of 60 cm x 25 cm x 1.5 cm. They consisted of PVC walls and a removable transparent front plate made of polymethyl methacrylate. The soil was filled into the boxes at a bulk density of 1.2 kg/dm^3. After one week of soil conditioning under irrigation as described below, the saplings were planted. At this time point, saplings possessed up to 10 cm long tap roots of 0.5 to 1.5 cm diameter but almost no fine roots, which presumably had died off during storage. The roots were washed with tap water to remove sticking soil, and approximately 2 cm of tap root were cut to stimulate new root formation. For each tree, the front plate of one rhizobox was opened, the roots pressed into the soil, and the front plate closed again. Rhizoboxes were placed in a greenhouse with temperature control (day 22 ± 2 °C; night 18 ± 2°C), natural light and shading from the direct sun. Since shading with movable blinds was the only means for active cooling, at some days in summer temperatures higher than 22°C occurred for short periods. The soil was kept dark by covering the rhizoboxes with black plastic foil, and to stimulate the formation of a quasi-planar root system along the front plate, the rhizoboxes were inclined at an angle of about 30°. Soil water potential in the rhizoboxes was kept at approximately -8 kPa by using irrigation tubes ("Rhizon irrigators", Rhizosphere research products, Wageningen, The Netherlands) providing P-free artificial rain solution based on the composition of natural precipitation (2.1µM K_2SO_4, 3.7µM Na_2SO_4, 3.0µM $CaCl_2$, 4.4µM $CaSO_4$, 1.9µM $MgCl_2$, 26.4µM NH_4NO_3 , 2.0µM $Ca(NO_3)_2$; Holzmann et al., 2016). During summer, additional periodic irrigation from the top was needed to compensate for high evapotranspiration. At the end of the first growing season (end of September 2015), the rhizoboxes were placed outside of the greenhouse, but protected by a roof, to induce dormancy. In November 2015, they were moved to a dark cold room at 4°C and periodically irrigated with artificial rain from the top. End of March 2016, after the last frost, the rhizoboxes were moved first to the protected area outside of the greenhouse, and in May, after appearance of the first leaves, back into the greenhouse with temperature control set to the same conditions as in the year before.

Measurement of rhizosphere parameters

In August 2015 and 2016, non-destructive and minimally invasive membrane-based methods were applied to the surface exposed roots after carefully removing the front plate. For each rhizobox, all measurements, as described in detail in the following paragraphs, were performed on the same day in the following order: pH (8 to 9 am), exchangeable anions (10 am to 1 pm), potential phosphatase activity (2 to 4 pm). For this, the rhizoboxes were laying horizontally on their back side. Two or three rhizoboxes were assessed per day within two weeks, and the order of replicates among the four treatments was selected randomly. Five replicates per plant/soil combination were selected. Since some of the LUE saplings growing in LUE soil died during the first months after planting, replication was only 3 in this case.

pH distribution

The pH in the rhizosphere was mapped using prototypes of planar optodes with an optimal measurement range between pH 3.5 and 5.0 and signal detection by a VisiSens camera (PreSens GmbH, Regensburg, Germany). For principles of measurement and calibration, refer to Blossfeld and Gansert (2007). Optodes of about 2x2 cm were applied to the terminal part of at least three newly grown roots per rhizobox including the surrounding soil and left to equilibrate for 15 min, protected with a small piece of clear acrylic glass. Then, signals were measured using a cylindric aluminum spacer of 6 cm length between camera and acrylic glass. In order to cover its whole area, several overlapping partial areas of each optode were measured, and photos were subsequently merged into one image using Adobe Photoshop. After use, optodes were rinsed with deionized water and stored in a buffer of pH 4 in a dark plastic bag at 4°C. After overlaying the pH maps with a mask for the location of the roots, using Adobe Photoshop, two zones were defined: root surface (values in the middle of the root) and bulk soil (>2mm from root edge). Averaged values of bulk soil and root surface per rhizobox were used in the statistical analysis.

Nutrients and organic acid anions

Nutrients and organic acid anions (nitrate, phosphate, sulfate, oxalate, citrate) were collected from the rhizosphere using anion exchange membranes (AEM; Shi et al. 2011). Strips of AEM (2cm x 0.5cm; No. 55164 2S, BDH Laboratory Supplies, England) were soaked in deionized water for 24 h, and then converted into HCO_3^- form by equilibration with 2.2 ml of various agents per cm^2 as follows: (i) for 10 min with 0.5 M HCl, (ii) twice for 1 hour with 0.5 M NaCl,

(iii) three times for 30 Min. with 0.5 M $NaHCO_3$. In between and at the end the AEMs were rinsed with deionized water and stored in deionized water at 4° C until use. The membranes were applied to at least three newly-grown roots including tip, elongation zone, side roots and the respective rhizosphere per rhizobox for 3 hours, covered with a plastic sheet to keep them moist during this time. After collection, the AEMs were rinsed with deionized water to remove sticking soil and extracted for 3 hours with 0.3 ml of 1.75 M HCl in 2 ml Eppendorf tubes (opened periodically to release an excess of CO_2 produced) using an end over end shaker at room temperature. The extracts were measured with ion chromatography (Thermo Scientific DIONEX ICS-3000 with InGuard Ag and Na Column 9*24 mm, an Ultratrace Anion Concentrator Column and a conductivity detector). Data from all membranes per rhizobox were averaged.

Potential phosphatase activity

Spatial distribution of potential phosphatase activity (PA) in the rhizosphere and bulk soil was mapped using zymography as developed by Spohn and Kuzyakov (2013) with slight modifications. Polyamide membranes (pore size 0.45um, Sartorius Stedim Biotech GmbH, Goettingen, Germany) were coated with 4-methylumbelliferyl phosphate (MUF-P, Sigma-Aldrich) by soaking in a 12 mM solution of this substrate in 10^{-4} M HCl (unbuffered solution with pH similar to soil pH) directly before application. Membranes of approximately 150 cm^2 and varying shapes - as to match the roots - were applied to newly grown long roots including the surrounding soil with a 1 mm protective layer of agarose gel (in 10^{-4} M HCl) between membrane and soil for 20 minutes. After incubation, the membrane was exposed to UV light (366 nm) in a dark chamber to visualize the fluorescence of the reaction product (4-Methylumbelliferone, MUF). Images (RGB) of the membrane were taken from a fixed distance of 28 cm using a Nikon D3200 Camera with an AF-S Nikkor 18-55 mm lens employing a series of fixed exposure settings. The images were quantified for PA by comparison with a standard curve prepared by soaking small pieces of the membrane in solutions of increasing MUF concentrations. The amount of MUF per unit area was calculated based on the amount of solution taken up and the surface of the membrane. For reliable quantification, the images required further processing. First, we selected a green channel from the RGB image least affected by light reflected from the UV lamp, using ImageJ (version 1.18; Schneider et al., 2012). Second, we wrote a custom R (version 3.1.2 (2014-10-31)) code to correct for unequal

illumination of the membrane resulting in a gradient in light intensity, decreasing linearly from the center to the peripheries of the image. Then we overlayed the corrected zymograms with masks representing the root distribution. This was achieved by aligning photos of the roots with photos of the membrane applied on the rhizobox and of the membrane under UV light. Gray values assigned to pixels on the root surface and in the bulk soil (areas >2mm from the edge of a root mask) were ranked and calibrated using a standard curve obtained as described above. For each rhizobox value, median values of PA for root and bulk soil of all individual membranes applied to this box were averaged.

Root morphology

The whole root systems of the saplings were excavated at the end of the experiment (August 2016), rinsed with tap water, and then scanned and analyzed for morphological characteristics using the WinRHIZO software employing 0.1mm steps for root diameter. Specific root length (m g^{-1}) was calculated as length of fine roots (diameter ≤ 2 mm) divided by their dry mass (M), specific root area (m^2 kg^{-1}) as surface of fine roots divided by M, and root tissue density (kg m^{-3}) as M divided by volume of fine roots. Root tip density and frequency are expressed as number of root tips per unit length and per unit dry mass of fine roots, respectively. Branching was calculated as the number of forks divided by the total length of fine roots. Mycorrhization of root tips was quantified visually under the binocular on a representative subsample.

Carbon, nitrogen, and phosphorus in soil microbial biomass

At the end of the experiment, bulk soil and rhizosphere soil - defined as soil sticking to the roots after gentle shaking - were collected from the rhizoboxes. Microbial biomass C and N (C_{mic}, N_{mic}) were determined using the chloroform fumigation-extraction method (Voroney et al. 2006). Organic C, and total N in the K_2SO_4 extracts were measured using a TOC/TN analyzer (Shimadzu TOC-V). The measured values for C_{mic} and N_{mic} were used without factors accounting for soil specific recovery. Microbial biomass P (P_{mic}) was determined using the hexanol fumigation method employing AEMs in bicarbonate form (Bünemann et al. 2004; Kouno et al. 1995; for preparation see above). The re-sorption of P released by hexanol fumigation, was accounted for by employing a suitable P spike as described by Bünemann et al. (2004).

Statistical analysis

All analyses were performed in R (R version 3.1.2 (2014-10-31)). Differences among individual treatments were assessed by using analysis of variance (ANOVA) followed by a Tukey post-hoc test. In addition, results were tested for an influence of the factors „plant origin" and „current soil" as well as their interactions using two-way ANOVA. The following variables were log transformed to meet the requirements of the ANOVA: concentrations of exchangeable phosphate in the first and second season, exchangeable nitrate in the first season, foliar N/Ca ratios in the first and second season, foliar K concentrations and N/P ratios in the second season. Marginal type II test (ANOVA, package: "car") was performed due to unequal group sizes.

Results

Nutritional status of the beech saplings

The N, P, and Mg concentrations and respective concentration ratios in full-season leaves of beech saplings in the first growing season were significantly determined by the factor „plant origin" (Table 3.2). Leaves of LUE plants exhibited a higher supply with N, and a lower supply with P and Mg than the leaves of BBR plants. On the other hand, foliar K concentrations were higher for LUE plants growing in LUE soil than in all other treatments, and N/K ratios were lower for saplings growing in the LUE soil.

TABLE 3.2 | Nutrient concentrations and ratios in full season leaves, as well as average nutrient concentrations in the whole plant for beech (*Fagus sylvatica* L.) saplings originating from the sites Bad Brückenau (BBR) and Unterlüss (LUE), respectively. The saplings were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE. The first four data columns show mean values ± standard error of replicate plants; different letters indicate significant differences between means according to the Tukey post hoc test. The last three columns show results of the two-way analysis of variance on the factors "„current soil" and "„plant origin"; shown are *F* values for the factors and their interactions; statistical significance is indicated as $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, ns not significant. P data are taken from Meller et al. (2019).

						Source of variation		
		BBR in BBR	BBR in LUE	LUE in BBR	LUE in LUE	current soil	plant origin	current soil x plant origin
Leaves season 1								
N	(mg g^{-1})	21.5±0.9a	21.0±0.9a	23.3±0.4a	24.2±1.5a	1.2 ns	7.2 *	0.6 ns
P	(mg g^{-1})	1.2±0.1ab	1.4±0.1a	0.9±0.1b	1.0±0.1ab	2.0 ns	9.9 **	0.2 ns
Mg	(mg g^{-1})	2.5±0.2a	2.0±0.2ab	1.3±0.2b	1.2±0.1b	3.9 ns	28 ***	1.1 ns
K	(mg g^{-1})	4.4±0.3b	4.9±0.3b	4.7±0.3b	7.1±0.6a	16.2 **	9.4 **	8.3 *
Ca	(mg g^{-1})	7.0±0.3a	5.7±0.7a	7.8±0.5a	6.4±1.6a	3.5 ns	1.2 ns	0.01 ns
N/P	(g g^{-1})	18±1bc	15±2c	25±2a	24±3ab	1.1 ns	19 **	0.1 ns
N/Mg	(g g^{-1})	9±1b	11±1b	19±2a	21±2a	1.7 ns	33 ***	0.0 ns
N/K	(g g^{-1})	4.9±0.2a	4.3±0.1ab	5.1±0.3a	3.4±0.1b	18 ***	0.9 ns	4.1 ns
N/Ca	(g g^{-1})	3.1±0.2a	3.9±0.5a	3.1±0.2a	4.3±1.0a	4.4 ns	0.04 ns	0.2 ns
Leaves season 2								
N	(mg g^{-1})	16.1±1.3c	22.0±1.5b	17.6±0.9bc	28.4±1.0a	38 ***	7.3 *	3.5 ns
P	(mg g^{-1})	1.6±0.1a	0.9±0.1b	0.9±0.1b	0.7±0.3b	14 **	13 **	3.1 ns
Mg	(mg g^{-1})	2.8±0.3a	1.5±0.2b	2.4±0.2a	1.1±0.5b	28 ***	2.6 ns	0.04 ns
K	(mg g^{-1})	5.9±0.6b	9.4±1.0a	5.5±0.2b	11.3±0.4a	36 ***	0.3 ns	1.9 ns
Ca	(mg g^{-1})	6.9±0.4ab	4.1±0.2c	8.3±0.5a	5.0±1.1bc	32 ***	5.3 *	0.3 ns
N/P	(g g^{-1})	11±1c	25±2ab	20±2b	51±15a	41 ***	20 ***	0.0 ns
N/Mg	(g g^{-1})	6±0.3b	16±2b	7±1b	39±12a	29 ***	7.8 *	9.2 **
N/K	(g g^{-1})	2.9±0.4a	2.4±0.2a	3.2±0.1a	2.5±0.02a	4.2 ns	0.8 ns	0.2 ns
N/Ca	(g g^{-1})	2.4±0.2b	5.4±0.4a	2.2±0.1b	6.3±1.4a	81 ***	0.0 ns	1.2 ns
Whole plant								
N	(mg g^{-1})	5.8±0.3b	8.2±0.6a	5.8±0.3b	9.5±0.5a	47 ***	1.6 ns	2.3 ns
P	(mg g^{-1})	0.9±0.1a	0.7±0.1b	0.4±0.03c	0.3±0.1c	12 **	72 ***	0.1 ns
Mg	(mg g^{-1})	1.5±0.1a	1.1±0.1b	1.3±0.1ab	0.7±0.1c	43 ***	14 **	2.7 ns
K	(mg g^{-1})	2.5±0.1b	3.2±0.1a	2.1±0.1b	3.3±0.3a	54 ***	1.8 ns	2.8 ns
Ca	(mg g^{-1})	3.5±0.2ab	3.1±0.1b	4.1±0.2a	4.1±0.3a	1.5 ns	15 **	1.8 ns

In the second growing season, the situation had changed and the foliar concentrations of most nutrients were now mainly and significantly determined by „current soil“ (Table 3.2). The leaves of saplings growing in the BBR soil showed a lower supply with N and K but a higher supply with Mg and Ca than of those growing in the LUE soil. By contrast, foliar P concentrations were still strongly co-determined by „plant origin“, and were higher for BBR plants growing in BBR soil than in all other treatments. For N/P ratios the soil effect dominated with higher values for plants growing in the LUE soil. The average concentrations of N, Mg, and K in the whole plant reflected the situation in the leaves and were also determined mostly by „current soil“. On the other hand, average P concentrations in the whole plant were still mainly determined by „plant origin“ with distinctly lower concentrations in the saplings from LUE.

Growth, architectural and morphological traits of fine roots

Two-way ANOVA revealed „current soil“, as the dominant and highly significant factor determining size, branching, and diameter fractions of fine roots of beech saplings after two growing seasons, but „plant origin“ played an additional significant role (Table 3.4). When growing in the BBR soil, saplings exhibited a larger root system – in terms of mass, length, and number of root tips - with a higher proportion of roots of the smallest diameter categories <0.2 mm than the saplings growing in the LUE soil (Table 3.3, Figure 3.1). “Plant origin” had a smaller influence on fine root traits for saplings growing in the BBR than the LUE soil, in particular on branching and diameter. In the LUE soil, the fine roots of the saplings from BBR were longer, more branched and thinner than those of the LUE saplings.

TABLE 3.3| Morphological traits of fine roots (<2mm diameter), as measured during the second growing season in a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings originating from the sites Bad Brückenau (BBR) and Unterlüss (LUE), respectively. The saplings were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE. Mass based parameters are as or related to dry weight. Data represent mean values ± standard error of replicate plants; different letters indicate significant differences between means according to the Tukey post hoc test.

		BBR in BBR	BBR in LUE	LUE in BBR	LUE in LUE
Total mass	(g)	2.6±0.4a	1.5±0.4ab	1.9±0.2ab	0.6±0.2b
Total length	(m)	9.1±0.6a	5.6±1.0b	7.6±0.8ab	1.7±0.4c
Branching	(cm^{-1})	14.8±0.7a	12.6±0.6a	15.1±0.9a	7.4±0.2b
Number of root tips	(no.)	29’213±2’674a	19’449±2’525a	25’2497±3’868a	4’804±869b
Root tip density	(cm^{-1})	3.9±0.3a	4.1±0.1a	3.9±0.4a	3.7±0.4a
Specific root length	($m\ g^{-1}$)	37±4a	41±4a	44±6a	28±4a
Specific root area	($m^2\ kg^{-1}$)	35±3a	39±4a	40±5a	30±4a
Root tissue density	($kg\ m^{-3}$)	201±15a	198±21a	190±20a	265±36a
Root tip frequency	(mg^{-1})	12.1±1.8a	14.7±1.9a	14.3±2.3a	8.4±1.3a
Mycorrhization	(%)	49±3ab	66±7a	42±2b	51±8ab

TABLE 3.4 | Two-way analysis of variance for different fine root traits of beech (Fagus *sylvatica* L.) saplings, as measured in the second growing season of a rhizobox experiment; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) (factor „plant origin“), and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE (factor „current soil“); shown are *F* values for the factors and their interactions; statistical significance is indicated as $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, ns not significant.

	Source of variation		
	current soil	plant origin	current soil x plant origin
Total mass	13.4 **	6.4 *	0.0 ns
Total length	35.4 ***	10.3 **	2.3 ns
Branching	37.8 ***	6.5 *	13.3 **
Number of root tips	23.2 ***	7.7 *	3.2 ns
Root tip density	1.9 ns	0.9 ns	3.3 ns
Specific root length	0.8 ns	0.0 ns	3.7 ns
Specific root area	0.1 ns	0.0 ns	2.8 ns
Root tissue density	1.9 ns	0.9 ns	3.3 ns
Root tip frequency	0.2 ns	0.3 ns	4.6 *
Fractions of root diameter categories:			
0.0 - 0.1 mm	17.4 ***	5.0*	14.4 **
0.1 – 0.2 mm	59.9 ***	5.0*	3.4 ns
0.2 – 0.3 mm	17.9 ***	1.0 ns	0.7 ns
0.3 – 0.4 mm	2.5 ns	0.1 ns	0.0 ns
Mycorrhization	7.7 *	3.9 ns	0.7 ns

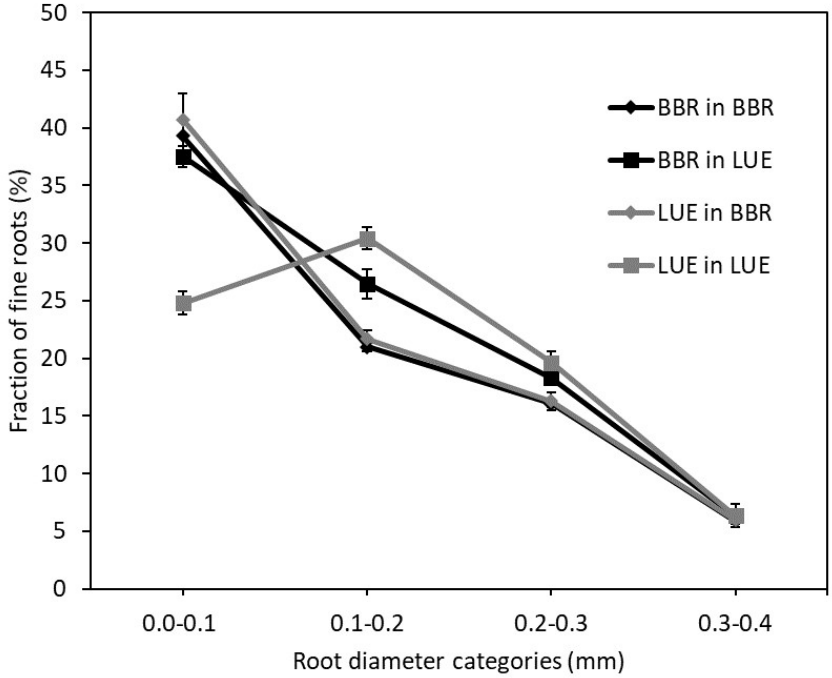

FIGURE 3.1| Fractions of fine roots in different diameter categories in (%) of total length of fine roots with diameter ≤ 2mm, as measured in August of the second growing season in a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings originating from the sites Bad Brückenau (BBR) and Unterlüss (LUE). The saplings were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE. Data represent mean values ± SE for replicate saplings (n=5, except n=3 for LUE in LUE); lines serve as visual aid only.

There was a significant interaction between the factors "current soil" and "plant origin" for branching and the proportion of roots < 0.1 mm (Table 3.4). These traits were almost equal for BBR saplings, irrespective of the soil they were growing in, while they differed more strongly for saplings from LUE when growing in the two soils.

Fine root parameters normalized for length, mass or volume (root tip density and frequency, specific root length and area, root tissue density) were equal for all four plant-soil combinations (Table 3.3).

Mycorrhization of fine roots

After two growing seasons, there was a weakly significant effect of „current soil" on mycorrhization of fine roots with slightly higher colonization in LUE soil when comparing treatments with saplings from the same site (Tables 3.3 and 3.4). There was an additional weak effect (F = 3.9; *P* = 0.06) of "plant origin" with a tendency to higher colonization of saplings from BBR when comparing treatments in the same soil.

Microbial biomass in the bulk soil and the rhizosphere

Microbial biomass C, N, and P in bulk soil and rhizosphere were strongly determined by the factor „current soil“ with higher values in the BBR than in the LUE soil (Tables 3.5 and 3.6). Considering both bulk soil and rhizosphere data for all plant-soil combinations, there was a weakly significant rhizosphere effect for C_{mic} (P = 0.02) and N_{mic} (P = 0.008) with slightly higher concentrations in the rhizosphere.

TABLE 3.5 | Soil microbial biomass C, N, and P concentrations (per dry weight) in the bulk soil (bulk) and the rhizosphere (RS), as well as ratios in the rhizosphere, as measured during the second growing season in a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings originating from the sites Bad Brückenau (BBR) and Unterlüss (LUE). The saplings were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE. Data represent mean values ± standard error of replicate rhizoboxes; different letters indicate significant differences between means according to the Tukey post hoc test.

		BBR in BBR	BBR in LUE	LUE in BBR	LUE in LUE
C_{mic} bulk	(mg kg^{-1})	224±13a	51±8b	219±17a	43±16b
C_{mic} RS	(mg kg^{-1})	259±13a	65±7b	251±15a	48±11b
N_{mic} bulk	(mg kg^{-1})	31.6±0.9a	5.0±0.6b	31.6±1.7a	4.7±0.9b
N_{mic} RS	(mg kg^{-1})	35.7±0.8a	8.0±0.7b	32.8±0.8a	4.4±1.4b
P_{mic} bulk	(mg kg^{-1})	6.2±1.5a	1.9±0.2ab	4.4±1.2ab	1.2±0.4b
P_{mic} RS	(mg kg^{-1})	5.9±1.1a	2.4±0.4a	5.2±1.2a	2.3±0.3a
C_{mic}/N_{mic} RS	(g g^{-1})	7.3±0.4b	8.0±0.5ab	7.6±0.4b	11.6±2.4a
C_{mic}/P_{mic} RS	(g g^{-1})	58±19a	29±6a	86±39a	26±2a
N_{mic}/P_{mic} RS	(g g^{-1})	5.2±0.4ab	3.5±0.9ab	6.3±1.3a	2.3±0.6b

TABLE 3.6 |Analysis of variance for microbial biomass C, N, and P (C_{mic}, N_{mic}, P_{mic}) in bulk soil (bulk) and rhizosphere (RS), the difference between pH on the root and in the bulk soil (ΔpH), exchangeable anions (nitrate, phosphate, oxalate, citrate) in the rhizosphere, and phosphatase activity (PA) on the root and in the bulk soil, as measured in the first and/or second growing season of a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) (factor „plant origin"), and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE (factor „current soil"); shown are *F* values for the factors and their interactions; statistical significance is indicated as $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, ns not significant.

	Source of variation		
	current soil	plant origin	current soil x plant origin
C_{mic} bulk (season 2)	138.73 ***	0.16 ns	0.017 ns
C_{mic} RS (season 2)	221.70 ***	0.78 ns	0.10 ns
N_{mic} bulk (season 2)	525.93 ***	0.04 ns	0.03 ns
N_{mic} RS (season 2)	922.03 ***	12.17 **	0.13 ns
P_{mic} bulk (season 2)	11.60 **	1.86 ns	0.03 ns
P_{mic} RS (season 2)	5.06 *	0.24 ns	0.05 ns
ΔpH (season 2)	0.22 ns	1.27 ns	0.00 ns
Nitrate (season 1)	19.54 ***	4.13 ns	0.49 ns
Nitrate (season 2)	3.62 ns	0.05 ns	1.01 ns
Phosphate (season 1)	14.57 **	0.04 ns	1.20 ns
Phosphate (season 2)	1.29 ns	0.08 ns	0.43 ns
Oxalate (season 1)	1.51 ns	10.96 **	1.21 ns
Oxalate (season 2)	4.55 *	0.15 ns	0.58 ns
Citrate (season 1)	0.15 ns	3.50 ns	0.49 ns
Citrate (season 2)	0.14 ns	1.71 ns	0.14 ns
PA root (season 1)	9.15 **	0.18 ns	0.01 ns
PA root (season 2)	6.20 *	3.24 ns	0.97 ns
PA bulk soil (season 1)	2.69 ns	0.92 ns	0.93 ns
PA bulk soil (season 2)	11.89 **	8.05 *	3.43 ns
PA/ P_{mic} root (season 2)	1.53 ns	1.28 ns	0.33 ns
PA/ P_{mic} bulk (season 2)	3.94 ns	2.30 ns	1.12ns

pH changes in the rhizosphere

In the second growing season, we observed small pH increases from bulk soil to the roots for all plant soil combinations (Figure 3.2). However, effects of neither „plant origin“ nor „current soil“ were significant (Table 3.6). Unfortunately, measurements performed in the first growing season provided no reliable data.

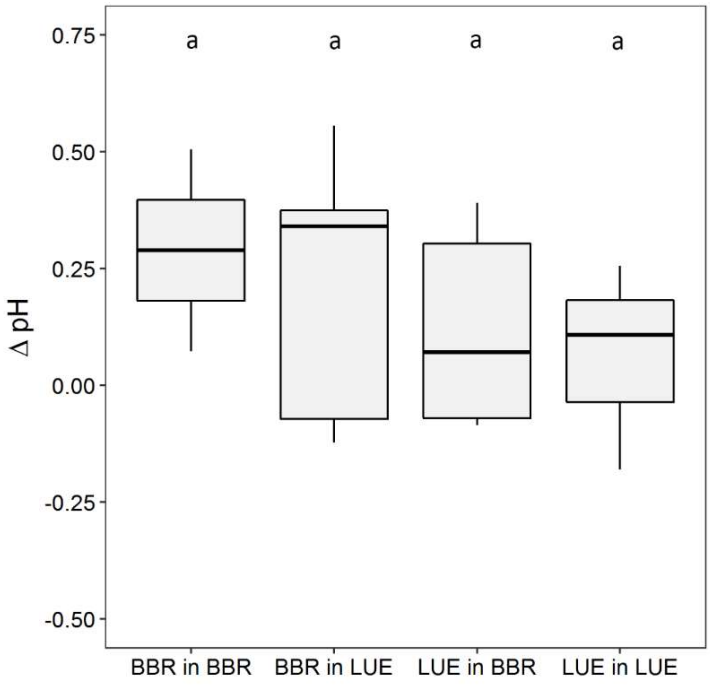

FIGURE 3.2| Difference between pH measured with optodes on the surface of roots and in the bulk soil (ΔpH) in August of the second growing season in a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings originating from the sites Bad Brückenau (BBR) and Unterlüss (LUE). The saplings were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE. Shown are box plots based on values for replicate rhizoboxes in the second growing season (n=5, except n=3 for LUE in LUE and n=2 for BBR in BBR); different letters indicate significant differences between means according to the Tukey post hoc test.

Resin extractable anions in the rhizosphere

Resin extractable nitrate in the rhizosphere exhibited different patterns in the two growing seasons. The factor „current soil” was significant in the first year (Table 3.6), with nitrate being higher in the BBR soil than in the LUE soil, while there was a tendency to the opposite in the second growing season (F=3.6; *P*=0.08) (Figure 3.3a).

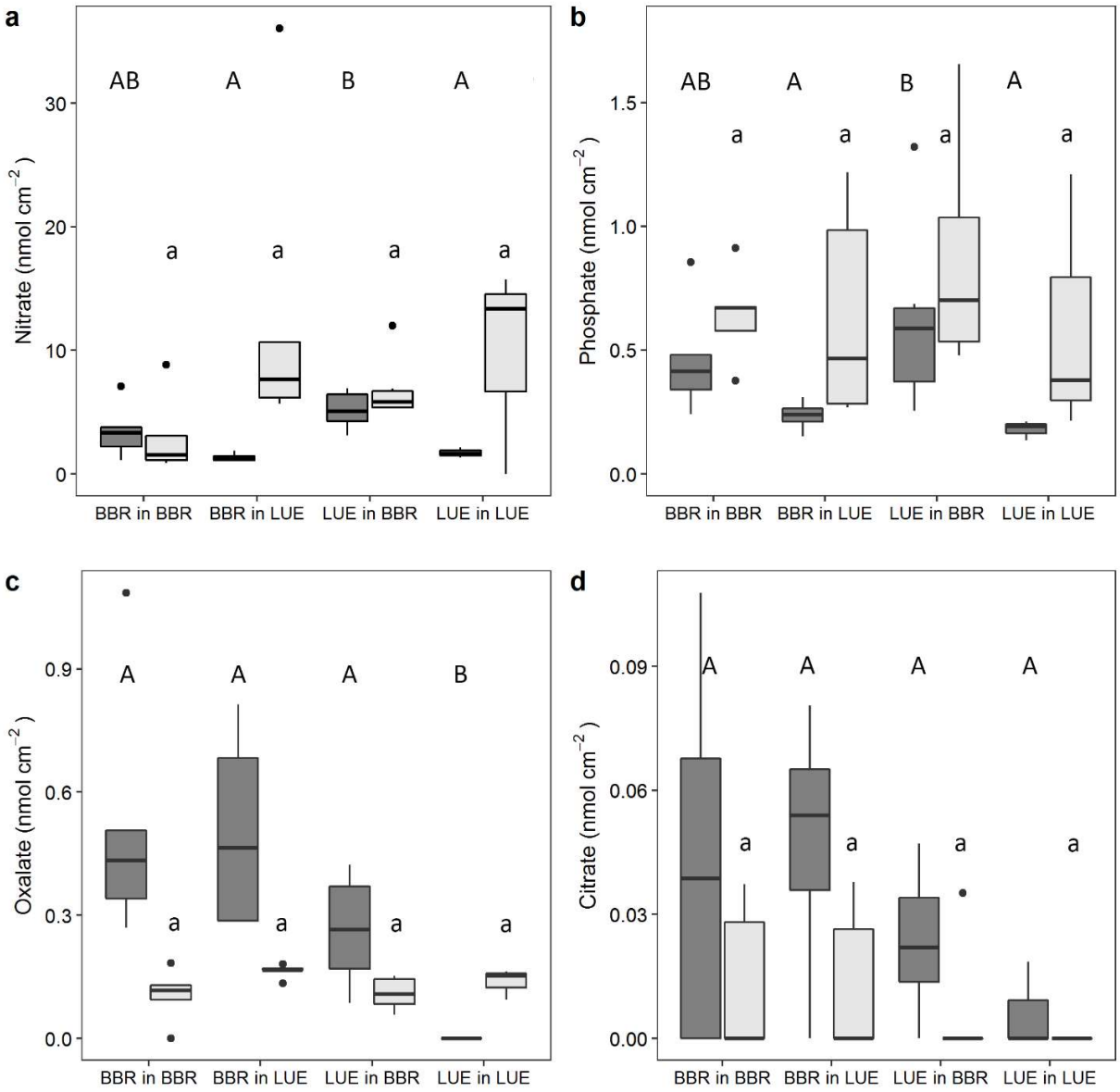

FIGURE 3.3| Nitrate (a), phosphate (b), oxalate (c), and citrate (d) collected with anion exchange membranes in the rhizosphere in a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings originating from the sites Bad Brückenau (BBR) and Unterlüss (LUE). The saplings were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE. Shown are box plots based on values for replicate rhizoboxes in August of the first (dark gray) and second (light gray) season (n=5, except n=3 for LUE in LUE); different letters indicate significant differences between means according to the Tukey post hoc test (uppercase letters, first growing season; lowercase letters, second growing season).

During the first growing season, also resin extractable phosphate was significantly determined by the factor „current soil" with higher concentrations in the BBR soil (Figure 3.3b, Table 3.6). In the second growing season, the pattern remained the same as a trend and concentrations were generally higher than in the first growing season.

Amounts of resin extractable organic acid anions were generally low and often below the detection limit, in particular citrate, which occurred in about ten times lower amounts than oxalate (Figure 3.3c,d). Both oxalate and citrate were higher during the first growing season than in the second growing season, in particular in the rhizosphere of the beech saplings from BBR. Higher amounts in the rhizosphere of the BBR than the LUE plants led to a significant effect of „plant origin“ in the first growing season for both oxalate and citrate (Table 3.6). In the second growing season, the few cases of detectable amounts of citrate suggest a similar, however not significant, effect of „plant origin“. For oxalate, however, there was no plant effect anymore, but a weakly significant effect of „current soil“ with slightly higher amounts in the LUE soil.

Potential phosphatase activity in the bulk soil and on the root surface

In general, potential phosphatase activity (PA) was highest on the main roots, intermediate on the side roots, and lowest in the bulk soil (see example in Supplementary Figure 3.S1). Enzyme activities observed in the first growing season were generally higher than in the second growing season, both on the roots and in the bulk soil (Figure 3.4a). During the first growing season, PA tended to be higher in the BBR than in the LUE soil, irrespective of the origin of the beech saplings. This effect of „current soil” was significant for PA on roots but not for the bulk soil (Table 3.6). In the second growing season, the effect of „current soil“ was significant for PA on roots and in the bulk soil, but in both cases overlaid by a weak effect of „plant origin“ with a tendency to higher values for saplings from BBR (Figure 3.4a; Table 3.6). In contrast to PA, PA per unit microbial P (PA/P_{mic}) exhibited a tendency towards higher values in the LUE soil, both on the root and in the bulk soil (Figure 3.4b).

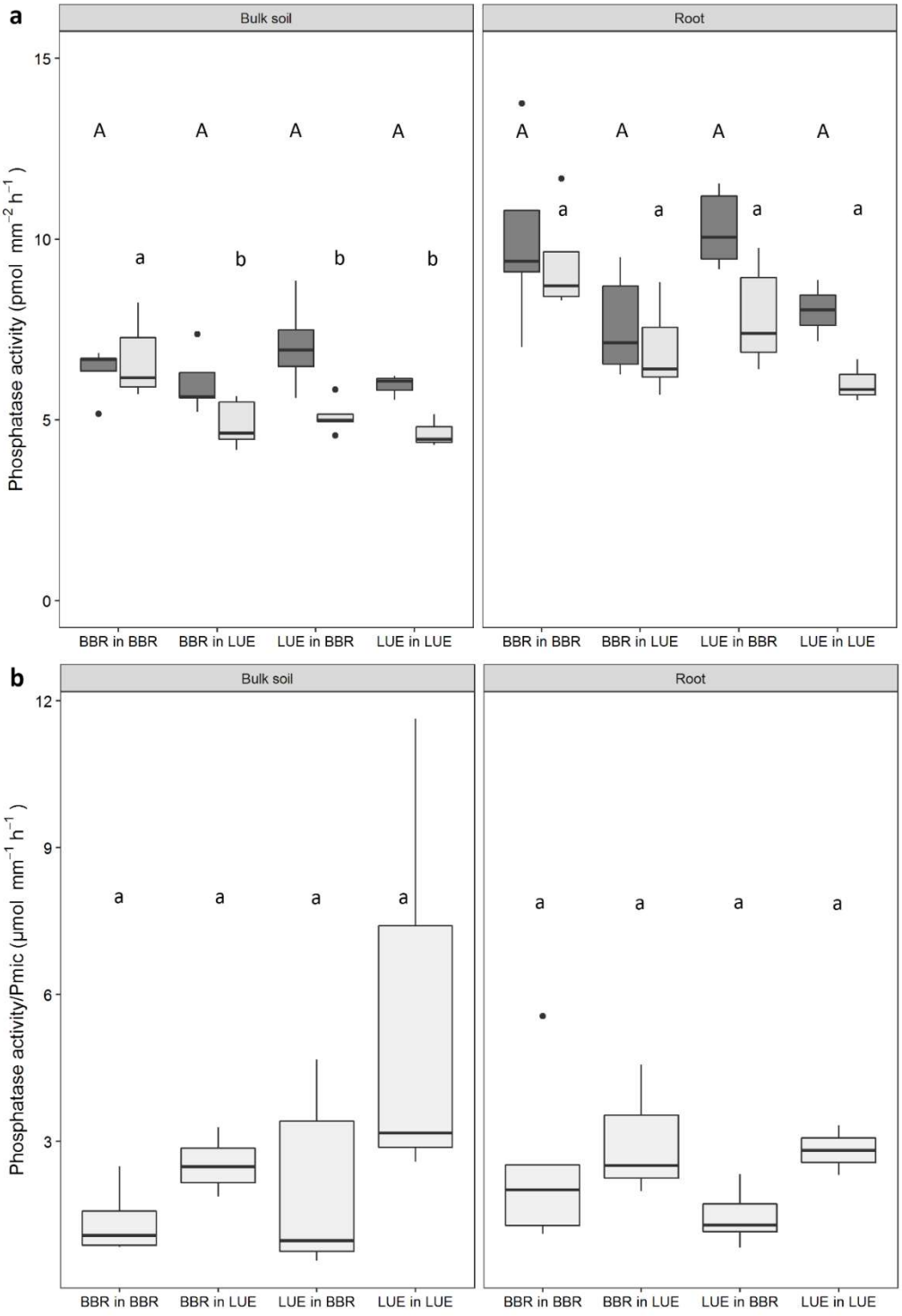

FIGURE 3.4| Potential phosphatase activity (PA) measured in the bulk soil and on the surface of the roots using zymography (a) and the ratio between potential PA and microbial P (P_{mic}) in bulk soil and in the rhizosphere (PA on the root divided by P_{mic} in the rhizosphere) (b) in a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings originating from the sites Bad Brückenau (BBR) and Unterlüss (LUE). The saplings were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE. Shown are box plots based on values for replicate rhizoboxes in August of the first (dark gray) and second (light gray) season (n=5, except n=3 for LUE in LUE); different letters indicate significant differences between means according to the Tukey post hoc test (uppercase letters, first growing season; lowercase letters, second growing season).

Discussion

Availability of nutrients in the soil and plant nutritional status

Our results show that after two growing seasons the nutrient concentrations in different compartments of the beech saplings, irrespective of their provenance, had become largely determined by the experimental soil, with the notable exception of P. In this section we discuss these results by first reflecting on the soil conditions and plant characteristics at the sampling sites, and then evaluating the plant nutritional status in our experiment as basis for the discussion of root and rhizosphere data in the following sections.

The comparison of the climatic conditions and soil properties at the two sites indicates more favorable conditions for plant growth at BBR than LUE both in terms of water and nutrients. An overall adaptation of the ecosystem at LUE to the low fertility of the mineral soil is the recycling of nutrients via a thick organic surface layer (Bünemann et al., 2016; Lang et al., 2017). The two soil materials from the Bv horizon at BBR and from the Bh horizon at LUE, which we used in the experiment, reflect well the general differences between the mineral soils at the two sites in terms of texture and nutrient concentrations. The more fertile conditions in the soil material from BBR than from LUE are also reflected by the differences in microbial C, N, and P concentrations between the two soils at the end of the experiment.

While the beech population at BBR is putatively autochthonous, this is not certain for the one at LUE (H.-P. Dietrich and H. Meesenburg, personal communications). Nevertheless, considering (i) the generally close relationship between beech populations from different sites (see introduction), (ii) the age of the stands, and (iii) the generally strong selection of trees at the juvenile stage, which allows for fast adaptation (e.g. Kremer et al., 2012), trait differences between beeches at the two sites are likely to a large degree due to adaptation to the specific site conditions. These adaptations are expressed in a slower growth of the mature beeches at LUE than at BBR, as is indicated by a smaller diameter. When grown under identical climatic conditions in undisturbed soil cores from their own site, saplings from natural rejuvenation at LUE produced smaller leaves with a lower photosynthetic activity and stomatal conductance than saplings from natural rejuvenation at BBR (Yang et al., 2016; Zavišić et al., 2018). In particular, the difference in photosynthetic activity can be considered an indication of a more conservative resource strategy of the beech saplings at LUE (Craine,

2009; Weemstra et al., 2016). Comparing foliar nutrient concentrations and nutrient ratios with threshold values by Mellert and Göttlein (2012; ratios) and Göttlein et al. (2015; concentrations in mature trees), the mature beeches at BBR exhibit a balanced nutrition except for a latent deficiency in K and N/K ratios above the normal range, while the trees at LUE show a latent deficiency in P and N/P, N/Mg and N/Ca ratios above the normal range. A lower P nutritional status of juvenile beech trees from LUE than from BBR was documented by Zavišić et al. (2018). They found a respective difference in P concentration for all plant compartments.

The beech saplings from natural rejuvenation used in our experiment were of similar age as those investigated by Yang et al. (2016) and by Zavišić et al. (2018). In contrast to the strong influence of „current soil“ on the N, Mg, and K concentrations in the second year, P concentrations in different plant compartments were still similar to P concentrations of saplings from the two sites when grown in their natural soil (Zavišić et al. 2018). In the following, we assess the relative nutritional status of the saplings and its change during the experiment (i) by evaluating the foliar nutrient concentrations based on threshold values for juvenile beech trees by Göttlein et al. (2015) and foliar N to nutrient ratios based on threshold values by Mellert and Göttlein (2012), and (ii) by comparing the average concentrations in the whole plant among the treatments. Figure 3.5 summarizes the results of this assessment for the second year. The figure also includes an assessment of the effective nutrient availability in the soil. This is based on the influence of the factor “current soil” on the concentrations in the plant as it should reflect what the plant effectively took up from the soil.

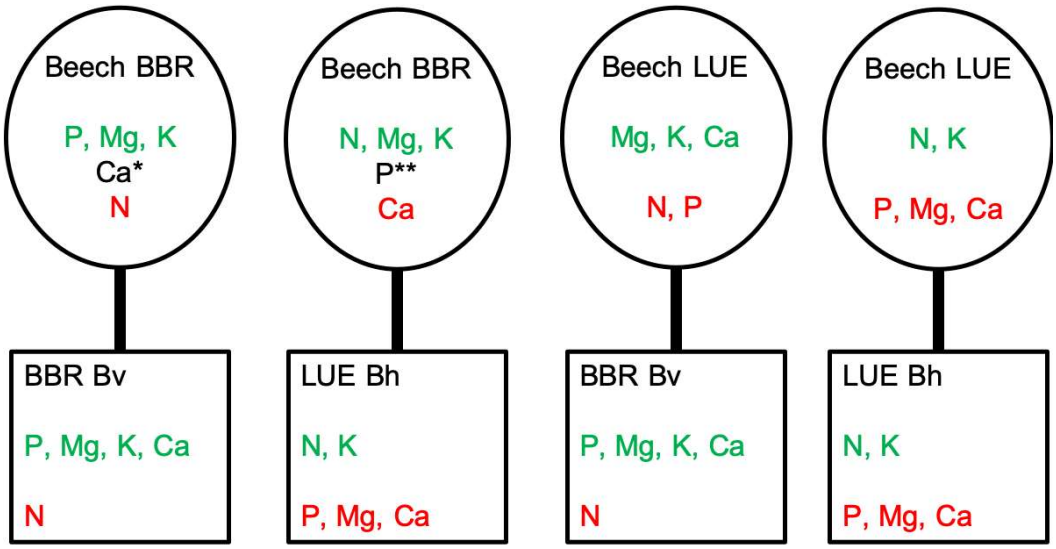

FIGURE 3.5|Evaluation of plant nutritional status and effective nutrient availability in the soil in a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings originating from the sites Bad Brückenau (BBR) and Unterlüss (LUE). The saplings were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE. For the plant nutritional status (i) the comparison of foliar nutrient concentrations and ratios (Table 3.2) with published threshold values (Mellert and Göttlein, 2012; Göttlein et al., 2015; green normal or surplus; red deficient or above/below normal), and (ii) the average nutrient concentrations in the whole plant (green low, red high) are considered; the assessments agree except for *: foliar Ca in deficient range, N/Ca in normal range; **: foliar P in deficient range, N/P above normal range, whole plant P high; effective nutrient availability in the soil (green sufficient, red not sufficient) is estimated from the influence of the factor "current soil" on the plant nutritional status in the second year of the experiment.

According to this assessment, saplings from LUE growing in the soil from LUE exhibited in both years a low P, Mg, and Ca status and thus reflected well the nutritional status at the provenance site throughout the experiment. The same saplings, when growing in the soil from BBR, also maintained a low P status throughout the experiment, but were low in K instead of Mg and Ca in the first year, and became low in N in the second year (although N/P was still above normal). In the first year, and irrespective of the „current soil", the saplings from BBR were low in K, as were the mature trees at this site. In the second year, the K status had improved, but N was low when growing in the soil from BBR, and Ca was low when growing in the soil from LUE. In the latter case, the P status was low in terms of foliar concentrations, however still high when considering the concentrations in the whole plant. The reason for the low N status in the second year of saplings growing in the BBR soil, becomes apparent neither from the chemical properties of our experimental soils, nor when inspecting the microbial C, N and P concentrations and related nutrient ratios in the second year. A possible explanation

is competition between beech saplings and mycorrhizal partner expressed as limited transfer of N to the plant (Simon et al., 2017). While ammonium has been shown to be the preferred N form taken up by mycorrhizal roots of beech (Gessler et al. 2005), nitrate appears to be much better transferred to the plant (Leberecht et al., 2016). This explanation is thus consistent with the tendency to lower exchangeable nitrate in the BBR than the LUE soil in the second year. In summary, based on the assessment of the plant nutritional status at the end of the experiment, the experimental treatments represent the growth of beech saplings differing in P and Mg status in a soil which does not provide sufficient P, Mg, and Ca, and the growth of beech saplings differing in P status in soil that does not provide sufficient N (Figure 3.5).

Root growth and morphology

The dominant effect of "current soil" on growth, architectural and morphological root traits demonstrates a high plasticity of the root system of juvenile beech trees when growing in soils differing in nutrient supply. The additional effect of "plant origin" points to a modifying effect of provenance that was expressed as a stronger reaction of the beech saplings from LUE than BBR to the differences in soil nutrient supply. We cannot rule out that this may be due to a genotypic difference in sensitivity. However, taking into account the assessment of plant nutritional status and effective soil nutrient availability at the end of our experiment from the previous section, the provenance effect is also consistent with an influence of the plant status in those nutrients, which are not in sufficient supply in the soil. Specifically, when grown in the LUE soil, which did not provide sufficient P, Mg and Ca, the beech saplings from the two sites differed in their P and Mg status and their fine roots strongly differed in growth, branching and diameter. By contrast, when grown in the BBR soil, the saplings from the two sites exhibited a very similar root system, in particular in terms of branching and fine root diameter. In this case, the saplings differed mainly in their nutritional status in P, which was supplied sufficiently by the soil, but not in their status in N, which was not supplied sufficiently by the soil. Furthermore, when accepting the modifying role of the plant nutritional status, the results suggest that the observed differences in root traits can be mainly attributed to differences in the supply of P and/or Mg rather than N. To further examine this, we compare in the following our findings on specific root traits with the results from earlier studies on the effects of P, Mg, or N supply.

The results on biomass and length of fine roots in our experiment are consistent with either inhibition of root growth by low P and/or Mg supply, or stimulation by low N supply. They are thus in agreement with the general finding that P and Mg starvation inhibit primary root growth (Gruber et al., 2013; Niu et al., 2013). Effects of differences in N supply on root growth are less clear, since results for the model plant *Arabidopsis* show, that mild N deficiency increases and strong N deficiency decreases root growth (Gruber et al., 2013). Independent on the considered nutrient, findings are more variable under natural than under controlled laboratory conditions. For beech under natural conditions, only small effects of soil fertility on root growth have been found (Leuschner et al., 2004). This can be attributed partly to interaction with other factors, that can affect root growth, such as soil texture (Hertel et al., 2013; Weemstra et al., 2017), water availability (Leuschner et al., 2004; Hertel et al., 2013), stand age (Finér et al., 2007) or light (Minotta and Pinzauti, 1996; Yang et al., 2016). Soil texture cannot be excluded as a factor explaining differences in root growth between our two experimental soils. However, such an effect would be opposite to results of earlier studies that found higher fine root growth of beech in sandy than loamy or clayey soils (Hertel et al., 2013; Weemstra et al., 2017).

The lower root branching of the saplings from LUE growing in LUE soil, than of the ones in all other treatments, is mainly consistent with inhibition by a low combined P and/or Mg supply from soil and plant reserves. This is in contrast to the findings of many studies across different plant species, including trees, that P starvation promotes lateral root growth (Niu et al., 2013; Zhou et al., 2018). However, a lower branching at low P availability was observed earlier for pine (Theodorou and Bowen, 1993).

Our results on root diameter are consistent with thicker fine roots a low P and/or Mg supply, or with finer fine roots at low N. There are conflicting reports on how fine root diameter depends on soil P availability. Considering only trees, Yan et al. (2019) found thicker fine roots at low P supply for various tree species, but Razaq et al. (2017) found the opposite for maple. Reports on the effect of different N supply on root diameter are more coherent. Mostly thinner roots at low N supply have been reported for trees (e.g., Razaq et al., 2017; Yan et al., 2017). There are little indications that the differences in fine root diameter in our experiment are affected by genotypic differences in plant “resource strategy” (Weemstra et al., 2016; see also introduction). On one hand, the relatively thick fine roots of saplings from LUE when

growing in the soil from LUE could reflect a “conservative resource strategy” as expressed by these saplings at their site of origin. On the other hand, when the same saplings were grown in the BBR soil, they exhibited thin roots of the same diameter as those of saplings from BBR, which are rather indicative of an “acquisitive resource strategy”. Vice-versa, the BBR saplings formed significantly thicker roots when growing in the soil from LUE.

In contrast to growth, branching and diameter, fine root traits normalized for length, mass or volume were insensitive to differences in nutrient supply in our experiment. Generally, these traits appear not to be affected strongly or uniformly by soil P availability. Again, considering only trees, specific root length (SRL) was found to be smaller at lower P supply, e.g. for spruce (Clemensson-Lindell and Asp, 1995), while the opposite for found e.g. for pine (Zhang et al., 2013). The few reports on root tissue density (RTD) show rather higher values at lower P supply (e.g. Zhang et al., 2013). There are more studies that investigated these root traits in dependence on N supply, but also for this nutrient the results are variable. A higher SRL at higher N supply was observed, e.g. for larch (Liu et al., 2009) and the opposite, e.g. for maple (Razaq et al., 2017). A higher specific root area at low N supply was reported, e.g. for spruce (Gong et al., 2019). Root tissue density was higher at low N availability, e.g. for spruce (Gong et al., 2019), while the opposite was observed e.g. for poplar (Yan et al., 2019).

In summary, the comparison with the literature shows that attributing the differences in root traits mainly to differences in the combined supply from soil and plant reserves in P and/or Mg, is consistent with earlier findings on root growth. On the other hand, N supply may have played an additional role determining fine root diameter.

Mycorrhizal colonization

Compared to root traits, mycorrhizal colonization of root tips appeared to be relatively insensitive to the treatments. Nevertheless, as for root traits, the results indicate soil properties as the main and plant provenance as a modifying factor. Taking into account our assessment of plant nutritional status and effective nutrient availability, there are two potential explanations for higher mycorrhization of beech saplings from the same site when growing in the LUE than the BBR soil. This behavior could be related either to the lower P availability in the LUE soil, or to a reaction of the plants to the limited transfer of N from the fungal partners in the BBR soil, as postulated above.

Although a weak effect, the somewhat higher mycorrhization of beech saplings from BBR than LUE, when considering growth in the same soil, may point to a higher ability of the saplings with an overall higher nutritional status to provide the fungal partner with carbon compounds. A similar enhancing effect of high plant nutritional status was observed for stimulation of root growth of *Helianthus* in reaction to reduced N supply (Bowsher et al. 2016).

Phosphorus mobilization potential in the rhizosphere

As for mycorrhization, the results from the second year of our experiment indicate a less sensitive reaction of rhizosphere properties related to P mobilization to the treatments than root traits. Among these properties, the potential to mineralize organic P appeared to be most strongly affected with a main influence of the factor "current soil".

Higher resin extractable rhizosphere concentrations of phosphate in the BBR than LUE soil, irrespective of plant origin, as observed in the first year, reflect the better P availability in the former soil. The general increase in exchangeable phosphate concentrations between the first and the second year points to a respective difference in the balance between P mobilization and uptake by organisms. A similar reasoning may apply for the increase in nitrate from the first to the second year in the LUE soil. The importance of nitrate for the N nutrition of our beech saplings was also indicated by the general increase of pH in the rhizosphere. Root exudation of OH^- in exchange for NO_3^- is well known (e.g. Marschner et al., 2012). As a side effect, in acid soils a pH increase in the rhizosphere could contribute to a better P solubility due to a decrease of the positive soil surface charge density and thus weaker sorption of phosphate (Hinsinger, 2001).

As reviewed in the introduction, the source of organic acid anions in the rhizosphere can be root exudation, exudation by mycorrhizal hyphae or release by free-living microorganisms, but it is difficult to distinguish between the different sources. Following a similar argument as for exchangeable phosphate and nitrate, the much larger oxalate and citrate concentrations in the first than the second year, in particular in the BBR soil, point to a difference in the balance between exudation/release to and microbial degradation in the rhizosphere. The significance of microbial degradation for the effectiveness of low-molecular weight organic acid anions in mobilization of sorbed or mineral bound inorganic P was emphasized earlier

(e.g. Hinsinger, 2001). The higher concentration of organic acid anions in the rhizosphere of the BBR than the LUE saplings in the first year is likely related to a higher constitutive root exudation by the saplings from BBR, which increased the organic acid anion concentrations either directly or via stimulation of the production and release by free living microorganisms. Studies on trees have so far provided no evidence for organic acid anion exudation by roots being induced by specific soil conditions (Heim et al., 2001; Eldhuset et al., 2007). The weak effect of „current soil" in the second growing season with slightly higher abundance of oxalate in the rhizosphere of saplings growing in LUE than BBR soil was thus more likely due to lower microbial degradation than a reaction to lower soil P availability.

The higher phosphatase activity (PA) in the BBR than the LUE soil in both years is in good agreement with results for mineral bulk soil from the same sites (Bünemann et al., 2016; Spohn et al., 2018), and with the often observed good correlation between PA and soil organic matter content (Nannipieri et al., 2011). However, PA per unit microbial P, representing the relative investment of the microorganisms in phosphatases, tended to be higher in the LUE than the BBR soil and suggests a reaction to the differences in P supply. The lower PA in the second than the first growing season in all treatments, both on the roots and in the soil, may be explained as feedback to an increased P availability in the rhizosphere as indicated by the resin-extractable phosphate concentrations. Soil PA has been shown to react sensitively to increased P availability (Marklein and Houlton, 2012; Hofmann et al., 2016). The stronger reaction in the treatments with saplings from LUE is in good agreement with the results by Hofmann et al. (2016) who found a significant fertilization effect only in the rhizosphere of LUE saplings and not in the one of BBR saplings when growing in soil from their own site.

As organic acid anions, phosphatases can be produced and released by roots, mycorrhizal hyphae and free-living microorganisms (see introduction). The higher PA on the roots than in the bulk soil in our experiment can then be attributed either to production and release by the roots, or to stimulation of microbial enzyme production in the rhizosphere by root exudation of easily-degradable carbon compounds. Hofmann et al. (2016) argued that root exudation of such compounds could in addition alleviate the main C limitation of the microorganisms, as found for mineral soils from BBR and LUE by Heuck et al. (2015), and as a consequence induce P deficiency and further stimulate production of phosphatases. Similarly, the significant plant effect in the second growing season with slightly higher PA in the treatments with saplings

from BBR could be explained by the higher constitutive root exudation by these saplings, as discussed above.

Conclusions

From our cross-exchange experiment with beech saplings and mineral soil material from two acid forest sites differing in nutrient supply, we draw the following conclusions.

Beech saplings exhibited a high plasticity in adapting their root system to soils, differing in their nutrient supply, in terms of root traits related to growth, architecture and morphology. The results confirm our first hypothesis that the plastic reactions were determined mainly by the soil properties. Confirming our second hypothesis, plant provenance had a modifying effect, that was consistent with an influence of the plant status in those nutrients, which were not in sufficient supply in the soil. However, we cannot completely rule out an additional genotypic difference between the beech saplings from LUE and BBR in their sensitivity to differences in soil nutrient supply.

Compared to root traits, differences among treatments in mycorrhizal inoculation and rhizosphere parameters related to P mobilization were small and mainly determined by the soil properties. Plant origin had only a minor modifying effect, possibly due to differences in the ability to transfer carbon compounds from the shoot to the root and the fungal partner.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

JL, EF, and SM designed the experiment. SM and JL collected the plant and soil materials used in the study. SM set-up and carried out the experiment, performed most of the chemical analyses, analysed the data, and wrote the first version of the manuscript. MS provided the expertise in measuring phosphatase activity. All authors contributed significantly to the final version of the manuscript.

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Supplementary Material

TABLE 3.S1| Site conditions, soil properties and beech traits at the sampling sites Bad Brückenau (BBR) and Unterlüss (LUE); concentrations in organic surface layer refer to the Oe horizon at BBR and weighted average of Oe and Oa horizons at LUE; CEC: cation exchange capacity; BS: base saturation; DBH: diameter at breast heigh; P_{cit} refers to citrate extractable P; data are taken from Lang et al. (2017), except for K stocks (provided by J. Prietzel, TU Munich, Freising, Germany), element stocks in the organic surface layer, proportions of organic surface horizons (compiled and provided by J. Krüher, University of Freiburg i.B., Germany), base saturation (provided by K. Kaiser, Martin-Luther University Halle-Wittenberg, Germany) foliar nutrient concentrations at BBR (provided by H.-P. Dietrich, Bayerische Landesanstalt für Wald- und Forstwirschaft LWF, Freising, Germany) and LUE (provided by H. Meesenburg, Nordwestdeutsche Forstliche Versuchsanstalt NW-FVA, Göttingen, Germany); all methods are described in Lang et al. (2017).

	BBR	LUE
Altitude (m a.s.l.); mean annual prec. (mm)	809; 1031	115; 779
Element stocks soil		
N_{tot}; P_{tot}; K_{tot} whole profile (kg m^{-2})	1.3; 0.90; 3.4	0.71; 0.16; 8.7
proportion in organic surface layer (%)	2.8; 0.3; 0.1	30; 5.6; 0.4
Organic surface layer		
Turnover ($years^{-1}$)	1/5	1/39
P_{tot}; P_{cit} (mg kg^{-1})	1571; 390	713; 109
C_{org}/N_{tot}; C_{org}/P_{org}; N_{tot}/P_{org} (g g^{-1})	23; 634; 27	26; 831; 32
pH (H_2O); CEC ($mmol_c$ kg^{-1}); BS (%)	5.3; 543; 79	3.4; 590; 19
Mineral topsoil (0-5cm)		
Sand; clay (%)	8; 37	75; 6
C_{org} (g kg^{-1})	175	95
P_{tot}; P_{cit} (mg kg^{-1})	2966; 162	196; 15
C_{org}/N_{tot}; C_{org}/P_{org}; N_{tot}/P_{org} (g g^{-1})	16; 110; 7.0	26; 984; 38
pH (H_2O); CEC ($mmol_c$ kg^{-1}); BS (%)	3.8; 371; 21	3.5; 108; 8
Beeches		
Age (years); height (m); DBH (cm)	137; 27; 37	132; 27; 28
Leaf conc. N; P; Mg; K; Ca (mg g^{-1})	24; 1.4; 2.1; 5.5; 7.2	23; 1.2; 0.9; 8.5; 6.1
Leaf N:P; N:Mg; N:K; N:Ca	17; 13; 4.5; 3.5	19; 26; 2.7; 3.8

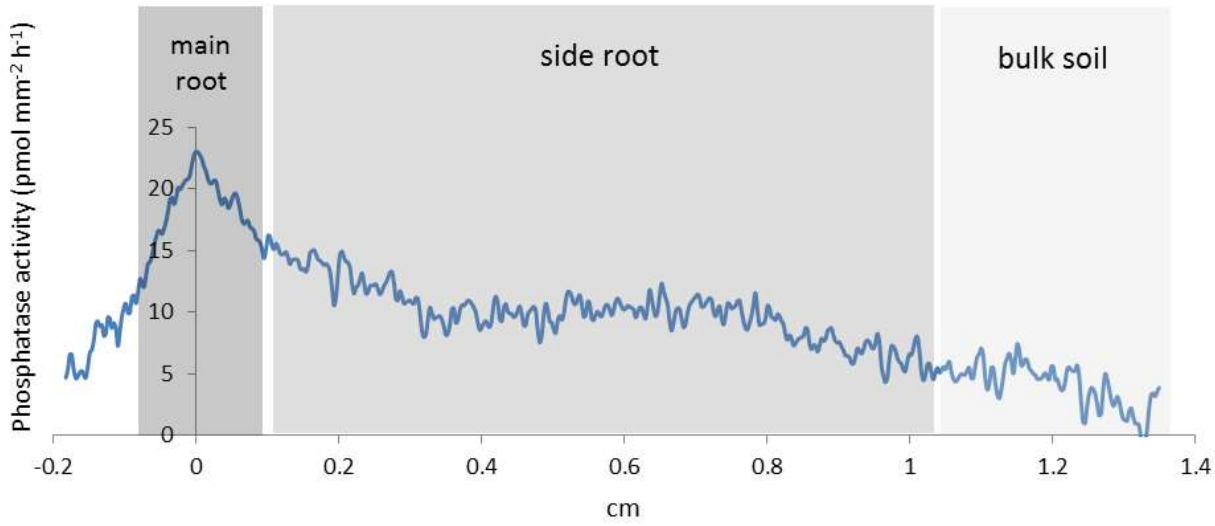


FIGURE 3.S1| Site Example of potential phosphatase activity along a transect from bulk soil, over the surface of a side root to the surface of a main root of a beech (*Fagus sylvatica* L.) sapling originating from the site Bad Brückenau growing in a rhizobox in material from the Bv horizon from this site, as measured using zymography in August of the first growing season.

CHAPTER 4

The microbial community composition in soil along the root axis of *Fagus sylvatica* L. saplings depends on the soil properties and the root zone.

Abstract

Decreasing foliar phosphorus (P) concentrations of beech (*Fagus sylvatica* L.) across Europe have raised concerns about forest health. Recently it was shown that beech saplings can exhibit high phenotypic plasticity in terms of internal nutrient allocation and root architecture, allowing for fast acclimation to altered soil P conditions and that the plant nutritional status influenced the relative changes of plant traits during the process. Considering the potential importance of rhizosphere microorganisms for plant nutrition, the objective of the present study was to assess to which degree beech saplings with a different P nutritional status can shape their rhizosphere microbial community to assist with acclimation to altered conditions.

We grew beech saplings collected from two forest sites with acid soil differing in P availability. In rhizoboxes, we grew these plants in mineral soil from either their site or from the other site. After one growing season, we determined the community composition and relative abundance of bacteria and fungi in bulk soil and different zones of the rhizosphere of freshly grown roots defined by root segments potentially differing in their functionality (root tip, elongation zone, side root, old roots). For this, we used Illumina MiSeq v2 sequencing of 16S rDNA of bacteria and ITS of fungi.

Irrespective of plant origin and zone, and correlating well with soil microbial biomass, overall OTU numbers of bacteria and fungi were higher in the soil from Bad Brückenau (BBR) with high P availability than in the soil from Unterlüss (LUE) with low P availability. The soil was also the main factor determining microbial diversity and composition. Alpha diversity of bacteria expressed as Shannon index was higher in the soil from BBR, while the fungal diversity was higher in the soil from LUE. Taxonomically, soils differed in the relative abundance of all main bacterial phyla, with a higher fraction of Chloroflexi and Verrucomicrobia in the BBR soil, and more Acidobacteria, Proteobacteria, and Planctomycetes in the LUE soil. In terms of fungal abundance, the BBR soil had a higher fraction of Basidiomycota, whereas Ascomycota, Mucoromycota, and Mortierellomycota dominated the LUE soil. Functional analysis of fungal genera in both soils highlighted the dominance of symbiotrophs in the BBR soil in contrast to a high fraction of saprotrophs in the LUE soil. In all treatments, and based on beta diversity, bulk soil samples were significantly different from rhizosphere samples.

Furthermore, based on the relative abundance of OTUs, the bacterial and fungal communities in the different rhizosphere zones grouped distinctly indicating a succession from bulk soil to older roots via root tip, elongation zone, and side root zone. This is thus the first study demonstrating the often-postulated gradual development of rhizosphere microbial communities along the root axis. Within the rhizosphere zones, there was a significant effect of plant nutritional status on bacterial beta diversity in the elongation zone and on fungal diversity in the elongation and root tip zones. Furthermore, microbial alpha diversity in the bulk soil and active rhizosphere zones was higher in treatments with beech saplings acclimating to altered soil P availability than in treatments with plants adapted to the respective conditions. The only indication of a plant effect potentially offering an advantage concerning P nutrition, was a higher relative abundance of the bacterial order Solibacterales, potentially important for the mobilization of inorganically bound P, in some zones in treatments with beech saplings exhibiting a low P nutritional status.

We conclude that overall, the P nutritional status of beech saplings had only a small influence on the soil microbial community in the rhizosphere. However, the related plant effects were strongest in the rhizosphere of the elongation zone, i.e. the potentially most active root segment in terms of root exudation.

Introduction

The European beech (*Fagus sylvatica* L.) is a dominant tree species in temperate forests across Europe. Its wide distribution has often been attributed to its broad ecological niche and plasticity, which allows beech to adapt to various pedoclimatic conditions, including low soil pH and widely differing nutrient availability (Leuschner et al., 2006; Batjes, 2011; Yang et al., 2013). Recently, Meller et al. (2019) have shown that young beech trees can rapidly respond to a change in soil P bioavailability by expressing high plasticity in internal P and biomass allocation. Thereby, allocation patterns were influenced by both the plant P nutritional status and soil P availability (Meller et al. 2019). As shown in Chapter 3 of this thesis, also changes in root morphology and ectomycorrhizal formation were involved in acclimation to altered soil P availability. On the other hand, it appeared that the P mobilization potential in the rhizosphere could be partially attributed to the soil microbial community, and the influence of the plant nutritional status was restricted to higher constitutive root exudation of organic

compounds by saplings with a higher nutritional status. The question remains to what degree root exudation by saplings differing in nutritional status can affect the rhizosphere microbiome to aid with nutrient mobilization and uptake at given soil P availability.

Major factors shaping the abundance and community structure of microorganisms in soils comprise the availability of carbon and major nutrients such as N and P, but also soil pH. Complex plant-soil interactions in the rhizosphere, driven by physicochemical soil properties on the one hand and plant physiological reactions, on the other hand, can lead, apart from alterations of root morphology (see, e.g., Chapter 3), to specific root exudation patterns. The latter, in turn, can strongly affect the composition of the rhizosphere microbial community (Berg and Smalla 2009; Colin et al. 2017; Fierer and Jackson 2006; Koranda et al. 2011; Nicolitch et al. 2016). Nevertheless, differences in the taxonomic composition of microbial communities do not necessarily imply different functionality. For example, Nicolitch et al. (2016) showed, a high functional redundancy among taxonomically diverse bacterial communities across a beech forest toposequence, with genes related to nutrient cycling being significantly enriched in the rhizosphere.

When investigating plant effects on the soil microbial community, it is essential to consider then the inhomogeneous distribution of the microorganisms in the rhizosphere. This is due to differences in root type (primary and secondary), root segment (apical zone incl. root tip, elongation zone, root hair zone, side roots, lignified older roots), and root movement through the soil. As different root segments vary in their exudation rate, age, and function (Marschner et al. 2011), it is likely that the rhizosphere zones next to these segments also harbor different microbial communities. Apices of primary and lateral roots, the elongation zone, and the root hair zone have been identified as the key sites of rhizodeposition for different plant species (Van Egeraat, 1975; Schilling et al., 1998; Jaeger et al., 1999; Massalha 2017). Folman (2001) demonstrated differences in the proportion of fast-growing and slow-growing bacteria between root tips and root base (approximately 0.5–1 cm beneath the stem base), which could reflect the succession of organisms in response to differences in root-derived carbon (Zelenev, 2005). Of the various root exudates, organic acid anions have been recognized to induce bacterial chemotaxis from bulk soil to the rhizosphere (Baudoin et al., 2002; Jones et al., 2003; Marschner et al., 2004). The implication of exudation controlled microbial

rhizosphere colonization could be that only specific rhizosphere zones next to root segments with elevated exudation rates would be under immediate plant control (Marschner, 2004).

To study a potential interactive effect of soil P availability and P nutritional status of beech on the rhizosphere microbiome, we performed a two-factorial experiment involving two levels of soil P availability and two types of beech saplings differing in their nutritional status. Specifically, we wanted to know (i) how the plant P nutritional status modifies the community structure of the rhizosphere microbiome in a given soil with a specific P availability, (ii) how the plant effect changes in the rhizosphere in different zones along the root axis, and (iii) whether these effects have the potential to improve the soil nutrient conditions for the plant.

We hypothesize that (i) the soil with its physicochemical properties is the main factor defining the microbial pool from which microorganisms are recruited into the rhizosphere by beech roots, (ii) the rhizosphere microbiome differs among zones related to root segments differing in their functionality, (iii) the plant nutritional status exerts the most considerable modifying effect on the rhizosphere microbiome in the elongation zone which is expected to be a hot spot of root exudation, and (iv) in the rhizosphere of beech saplings with a low P nutritional status microbial groups are fostered that have the potential to improve P mobilization.

Materials and methods

Plant and soil materials

Plant and soil materials were collected on the core research sites of the priority program 1685 "Ecosystem nutrition" of the German Science Foundation (DFG) (http://www.ecosystem-nutrition.uni-freiburg.de/) in Unterlüss (Lower Saxony, Germany, LUE) and Bad Brückenau (northern Bavaria, Germany, BBR). Both sites are dominated by European beech (*Fagus sylvatica* L.). The soil samples were collected from the Bh horizon in LUE and the Bv horizon in BBR, air-dried at 15°C, sieved to 4 mm, and homogenized. Plant residues were removed from the soils. The basic physical and chemical properties of the soils are summarized in Table 4.1 and were determined as described by Meller et al. (2019) (Chapter 2).

TABLE 4.1 | Physical and chemical properties of homogenized material from the Bv soil horizon at Bad Brückenau (BBR) and the Bh soil horizon at Unterlüss (LUE) used in the experiment; this includes grain size fractions, pH in two different extractants, organic C (C_{org}), total N (N_{tot}), and the following P fractions obtained by sequential extraction: resin exchangeable inorganic P (P_{resin}), sum of inorganic P (extractable P_{inorg}) and organic P (extractable P_{org}); all concentrations are given per mass dry soil; shown are means ± standard deviations of two technical replicates, except for sum parameters and element ratios.

		LUE	BBR
Sand	(g kg^{-1})	811±2	287±10
Clay	(g kg^{-1})	43±3	253±10
pH in H_2O / 0.01 M $CaCl_2$		3.99±0.01 / 3.31±0.01	4.76±0.03 / 3.99±0.01
C_{org}	(g kg^{-1})	18.5±0.03	41.9±0.7
N_{tot}	(g kg^{-1})	0.75±0.01	3.22±0.01
P_{resin}	(mg kg^{-1})	0.44±0.03	5.52±0.89
Extractable P_{inorg}	(mg kg^{-1})	29	911
Extractable P_{org}	(mg kg^{-1})	89	1256
C_{org}/N_{tot}	(g g^{-1})	24.7	13.0
C_{org}/P_{org}	(g g^{-1})	208	33
N_{tot}/P_{org}	(g g^{-1})	8.4	2.6

The two soil materials differed sharply in texture (BBR Bv: loam; LUE Bh: loamy sand; IUSS Working Group WRB. 2014). Besides, the LUE soil was more acidic and contained less organic carbon (2x) and total nitrogen (4.6x) then the BBR soil. Most importantly, the soil from BBR exhibited much higher concentrations of all inorganic and organic P fractions than the LUE soil, including resin exchangeable P (P_{resin}), a measure of directly plant-available P (Tamburini et al., 2012). On the other hand, the LUE soil exhibited a higher proportion of organic P (P_{org}) than the BBR soil. The overall lower nutrient availability of the LUE soil is also emphasized by the higher C_{org}/N_{tot}, C_{org}/P_{org} and N_{tot}/P_{org} ratios.

Beech saplings of similar size were collected at both sites during their dormancy period in December 2014 and stored at 4°C until planting in the following spring.

Experimental setup

In April 2015, rhizoboxes were set up with saplings from BBR or LUE planted either in the soil from their site of origin or in the contrasting soil from the other site. Each of the four treatments was replicated seven times in a completely randomized manner. The rhizoboxes had inner dimensions of 60 cm x 25 cm x 1.5 cm. They consisted of PVC walls and a removable

transparent lid made of polymethyl methacrylate. The soil was filled in at a bulk density of about 1.2 kg/dm^3, watered, and one week later, the trees were planted. Before planting, the roots of the saplings were washed with tap water to remove sticking soil, and approximately 2 cm of taproot was cut to stimulate new root formation. For each tree, the front plate of one rhizobox was opened, the roots pressed into the soil, and the front plate closed again. At planting, saplings from BBR were 11.7 ± 2.7 years old, and those from LUE 14.7 ± 1.6 years old (according to tree ring counting, Meller et al., 2019, Chapter 2), had developed a taproot of up to 10 cm length and a diameter of 0.5 to 1.5 cm and had almost no fine roots. Rhizoboxes with trees were placed in a greenhouse with temperature control (22 ± 2 °C during the day / 18 ± 2°C at night), natural light and shading from the direct sun. The soil was kept dark, and to stimulate the formation of a quasi-planar root system along the transparent lid, the rhizoboxes were inclined at an angle of about 30°. Rhizoboxes were kept at a constant water potential of -8 kPa by using irrigation tubes ("Rhizon irrigators," Rhizosphere research products, Wageningen, Netherlands) providing P-free artificial rain solution based on the composition of natural precipitation [2.1 µM K_2SO_4, 3.7 µM Na_2SO_4, 3.0 µM $CaCl_2$, 4.4 µM $CaSO_4$, 1.9 µM $MgCl_2$, 26.4 µM NH_4NO_3, 2.0 µM $Ca(NO_3)_2$; Holzmann et al., 2016]. During summer, additional periodic irrigation from the top was needed to compensate for high evapotranspiration.

Collection and molecular analysis of bulk soil and rhizosphere samples

We picked saplings with well-developed visible root zones as follows: five saplings each from BBR growing in soil from BBR and LUE, 5 saplings from LUE growing in soil from BBR, and 3 saplings from LUE growing in soil from LUE (due to mortality, only 3 saplings survived in LUE in LUE treatment). In August 2015, 200 soil samples were collected for DNA analysis (196 bacteria and 197 fungi samples were used in data analysis), from the bulk soil and different zones in the rhizosphere of selected newly grown fine roots. For this, a rhizobox was layed on a table with the transparent lid on the top. After removal of the lid, for each sample, approximately 0.1 g were scraped off the surface with a steel spatula pre-rinsed with distilled water and 70% ethanol. Bulk soil samples were collected in the upper, middle, and lower parts of the rhizobox without visible roots. Rhizosphere soil was collected in zones of about 0.5cm around the following specific root segments: root tip, elongation zone (1-2 cm from the root tip), short side roots (side roots), brown lignified roots (old roots). Soil samples were shock

frozen with liquid nitrogen and stored at -80°C. Subsequently, total genomic DNA was extracted with a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The obtained DNA was quantified by PicoGreen (Invitrogen, Carlsbad, CA, USA) and stored at -20°C. In each sample, the concentration of DNA was adjusted to 2.67 ng DNA per µL H_2O.

The targeted region of the prokaryotic (bacterial and archaeal) small-subunit (16S) rRNA gene was amplified with the primers 341F (CCTAYGGGDBGCWSCAG) and 806R (GGACTACNVGGGTHTCTAAT) according to Frey et al. (2016). The internal transcribed spacer region 2 (ITS2) of the eukaryotic (fungal) ribosomal operon was amplified with degenerate versions of the primers ITS3 (CAHCGATGAAGAACGYRG) and ITS4 (TCCTSCGCTTATTGATATGC) (Tedersoo *et al.* 2014, Frey et al. 2016). The 5` ends of the primers were tagged with the CS1 (forward) and CS2 (reverse) adapters required for multiplexing samples using the Fluidigm Access Array System (Fluidigm, South San Francisco, CA, USA). PCR amplification was performed with 10 ng soil DNA and the GoTaq G2 Hot Start Taq amplification kit (Promega, Dübendorf, Switzerland) in a final volume of 25 ul per sample (16S: 341F–806R: 2 min at 95 °C/36 cycles: 40 s at 94 °C, 40 s at 58 °C, 1 min at 72 °C/10 min at 72 °C; ITS2: 2 min at 95 °C/38 cycles: 40 s at 94 °C, 40 s at 58 °C, 1 min at 72 °C/10 min at 72 °C). Each sample was amplified in triplicates and pooled before purification with Agencourt AMPure XP beads (Beckman Coulter, Berea, CA) and quantification with the Qubit R 2.0 fluorometric system (Life Technologies, Paisley, UK). Amplicon pools were sent to the Génome Québec Innovation Center at McGill University (Montréal, Canada) for barcoding using the Fluidigm Access Array technology (Fluidigm, South San Francisco, CA, USA) and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA).

Illumina MiSeq data analysis

Quality control of bacterial and fungal reads was performed using a customized pipeline as described in Frey et al. (2016). Briefly, paired-end reads were first matched with USEARCH (Edgar and Flyvbjerg 2015) and substitution error was corrected for using Bayeshammer (Nikolenko et al. 2013). PCR primers were detected and trimmed (allowing for 1 mismatch, read length>300 bp for 16S and>200 bp for ITS primers) using Cutadapt (Martin 2011). Subsequently, sequences were dereplicated, and singletons were removed. Clustering was performed using USEARCH into OTUs at 97% identity, including an "on-the-fly" chimera

detection algorithm (Edgar 2013). Taxonomic assignments of the OTUs were obtained using Bayesian classifiers (Wang et al., 2007) with minimum bootstrap support of 60% implemented in MOTHUR (Schloss et al., 2009) by querying the bacterial reads against the SILVA database (Release 132) (Quast et al., 2013) and fungal reads against UNITE database (2019). Fungal taxonomy was additionally subjected to the FUNGuild tool (Nguyen et al., 2015) to gain more insights into life strategies of fungi by assigning them to one of the main categories: pathotrophs (gaining nutrients by harming host cells), symbiotrophs (gaining nutrients by exchanging some benefit with host cells), and saprotrophs (gaining nutrients by breaking down dead host cells).

Statistical analysis

Using analysis of variance (ANOVA), we assessed to what extent current soil (the soil in which the beech saplings were growing during the experiment), plant origin (forest site where the beech saplings were collected) and zone (bulk soil or rhizosphere next to a specific root segment) influenced alpha-diversity expressed as Shannon diversity index ($H' = -\sum pi \ln pi$, where p is the probability of the species i) and relative abundance of phyla (bacteria and fungi) and orders (bacteria only). Shannon diversity index estimation (estimate_richness function in package: "phyloseq") and all ANOVA analyses were performed in R, version 3.5.0 (R Core Team R, 2014) with marginal type II test (Anova, package: "car") in order to account for unequal group sizes. Before ANOVA, data were log-transformed or square rooted when necessary to meet the requirements of ANOVA. Subsequent Tukey post hoc test, where indicated, was performed with HSD.Tukey (package: "agricolae"). The beta-diversity analysis was based on Bray–Curtis similarity matrices generated from the square rooted OTU abundance tables and was performed in Primer 6 (version 6.1.13) and Permanova+ (version 1.0.3) software with 999 permutations (permutational analysis of variance, PERMANOVA). The overall variability in bacterial and fungal community structures was examined by Principal Coordinate Analysis (PCO). The zone effect (bulk soil, rhizosphere zones) was assessed with pair-wise PERMANOVA using subsequent pairwise comparison between zones and Canonical Analysis of Principal Coordinates (CAP) with the constrained factor "zone". The effect of plant origin in individual zones was tested with PERMANOVA on zone subsets.

Results

Alpha- and beta-diversity in the two soils

The analysis of the 5 139 278 16S rRNA and 8 066 427 ITS reads generated a total of 4409 bacterial and 1736 fungal OTUs. Coverages were between 88% to 95% (average 91%) for bacteria and 94% to 98% (average 96%) for fungi within the samples based on Good`s coverage estimation. Generally, coverage was better in LUE soil than in BBR soil.

Considering all samples from the four plant-soil combinations and all bulk soil and rhizosphere zones together, the soil type dominated alpha- and beta-diversity of the microbial community. Species richness of both bacteria and fungi was higher in samples from the BBR soil with high P availability than in samples from the LUE soil with low P availability (Table 4.2).

TABLE 4.2 | Bacterial and fungal alpha-diversity in the two soils from a rhizobox experiment with beech (Fagus *sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively, and were grown in soil from the Bv horizon at BBR or from the Bh horizon at LUE; shown are values for the bulk soil zone (mean ± SE). Richness is the number of OTUs.

	Richness	Shannon index (H`)
	Bacteria	
BBR soil	1202.9±7	5.5±0.0
LUE soil	820.7±7	5.3±0.0
	Fungi	
BBR soil	323±3	3.0±0.0
LUE soil	235±3	3.5±0.0

This was also the case for the Shannon diversity of bacteria, whereas fungal Shannon diversity was higher for LUE soil samples. The dominant influence of the soil type is emphasized on the one hand by a strong separation between the two soils in a principal component analysis (PCO) of the relative abundances of bacteria and fungi in the two soils (Figure 4.1), on the other hand by the highly significant effects of the factor „current soil" in an analysis of

variance (ANOVA) of Shannon diversity (Table 4.3) and permutational ANOVA (PERMANOVA) of beta diversity (Table 4.4).

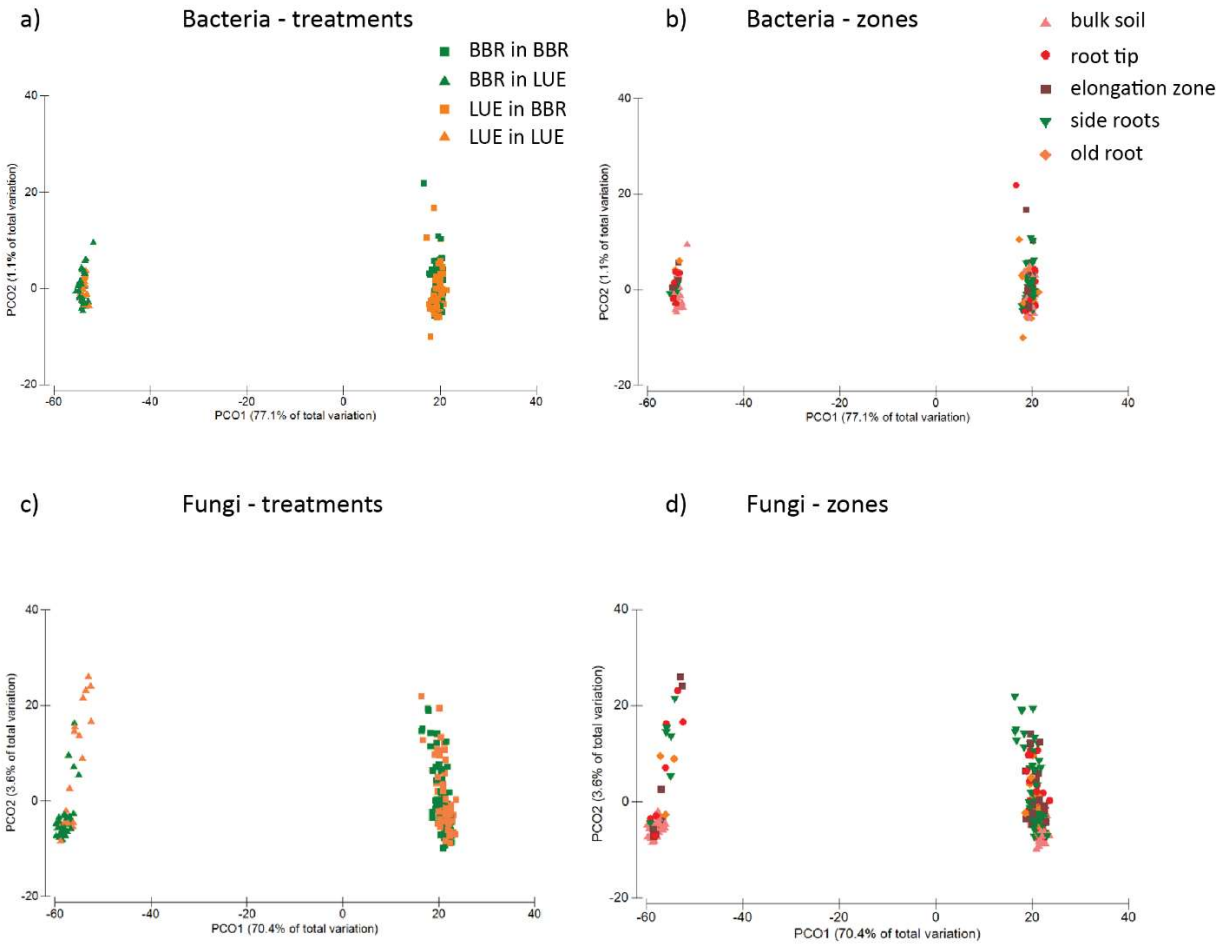


FIGURE 4.1| PCO analysis of bacterial (a,b) and fungal (c,d) microbial community structures in soil sampled in a rhizobox experiment with beech (Fagus *sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively and were grown in soil from the Bv horizon at BBR or from the Bh horizon at LUE. Within the two separated soil groups, different symbols highlight different treatments (a,c) or zone (b,d).

TABLE 4.3| Analysis of variance of Shannon (H`) diversity index of the bacterial and fungal community (OTU level) sampled in different zones (bulk soil and rhizosphere along various root segments; factor zone) of beech (Fagus *sylvatica* L.) saplings in a rhizobox experiment; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively (factor plant origin), and were grown in soil from the Bv horizon at BBR or from the Bh horizon at LUE (factor current soil); the analysis was performed either on the whole data set or on soil subsets; shown are *Chi-square* values for the factors and their interactions; statistical significance is indicated as $***P < 0.001$, $**P < 0.01$, $*P < 0.05$, ns = not significant.

	Bacteria		**Fungi**	
Whole data set				
	Chi-sq		Chi-sq	
Current soil	104.5	***	156.5	***
Plant origin	2.9	ns	2.7	ns
Zone	17.3	**	2.3	ns
Soil:plant	0.05	ns	0.3	ns
Soil:zone	9.9	*	3.4	ns
Plant:zone	4.6	ns	0.5	ns
Soil:plant:zone	2.6	ns	1.9	ns
BBR soil subset				
Plant origin	2.8	ns	0.67	ns
Zone	20.9	***	2.9	ns
Plant:zone	7.2	ns	0.26	ns
LUE soil subset				
Plant origin	1.2	ns	11.9	***
Zone	8.1	ns	8.7	ns
Plant:zone	1	ns	7.2	ns

TABLE 4.4| PERMANOVA analysis of bacterial and fungal relative abundance based square-root Bray–Curtis dissimilarities of soil sampled in different zones (bulk soil and rhizosphere along various root segments; factor zone) of beech (*Fagus sylvatica* L.) saplings in a rhizobox experiment; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively (factor plant origin), and were grown in soil from the Bv horizon at BBR or from the Bh horizon at LUE (factor current soil); the analysis was performed either on the whole data set or on soil subsets; shown are *F* and *P* values for the factors and their interactions. Significant effects are indicated with *P* values in bold.

	Bacteria		**Fungi**	
Whole data set				
	Pseudo-F	P(perm)	Pseudo-F	P(perm)
Current soil	385.93	**0.001**	305.02	**0.001**
Plant origin	2.04	**0.001**	3.81	**0.001**
Zone	1.30	**0.009**	2.96	**0.001**
Soil:plant	1.93	**0.001**	3.26	**0.001**
Soil:zone	1.26	**0.016**	0.75	0.998
Plant:zone	1.00	0.476	1.24	**0.027**
Soil:plant:zone	1.03	0.318	1.25	**0.016**
BBR soil subset				
Plant origin	1.43	**0.003**	1.89	**0.001**
Zone	1.54	**0.001**	2.34	**0.001**
Plant:zone	1.08	0.077	1.01	0.44
LUE soil subset				
Plant origin	2.52	**0.002**	5.07	**0.001**
Zone	1.49	**0.005**	2.23	**0.001**
Plant:zone	1.19	0.099	1.75	**0.001**

Plant and zone effects on alpha and beta diversity

Considering the dominant effect of soil type, the influence of the site of origin of the beech saplings (factor „plant origin") and the sampling location (factor „zone") were analyzed on the one hand for the whole data set and for the data from the two soils separately.

In the PCO analysis, the relative abundances in the samples from different plant origins and different zones overlapped within the groups defined by the two soils (Figure 4.1). While the effect of plant origin on Shannon diversity was significant only for fungi in the LUE soil with low P availability, the impact of the factor zone was significant only for bacteria in the BBR

soil with high P availability (Table 4.3). By contrast, considering beta diversity, both plant and zone effects were significant in all cases, i.e., considering the whole data set and both soil subsets (Table 4.4).

Canonical analysis of principal coordinates (CAP) of bacterial and fungal community structures with fixed factor „zone" on soil subsets revealed a distinct separation of bulk soil and rhizosphere zones (Figure 4.2).

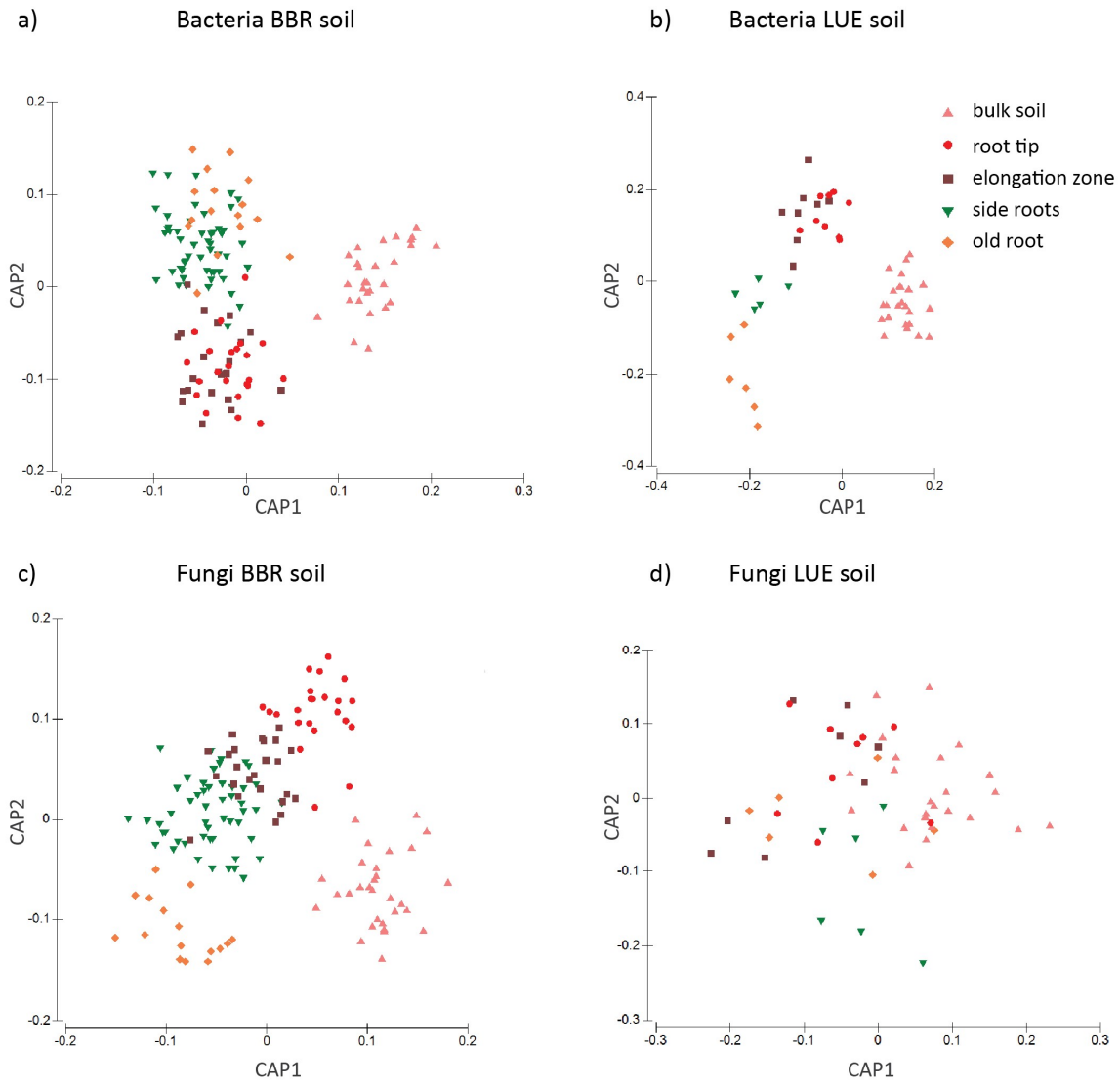


FIGURE 4.2| Canonical Analysis of Principal Coordinates (CAP) of soil bacterial (BBR soil (a);LUE soil (b)) and fungal (BBR soil (c); LUE soil (d)) community structures in a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively, and were grown in soil from the Bv horizon at BBR or from the Bh horizon at LUE; the CAP analysis was performed with the factor "zone" constrained (levels: bulk soil, root tip, elongation zone, side roots, older root).

Except for fungi in the LUE soil, the grouping indicates a „succession" of communities in the order bulk soil, root tip, elongation zone, side roots, older roots (Figure 4.2). Pair-wise comparison of the beta-diversity within the different zones in individual soil subsets revealed significant differences between rhizosphere zones and bulk soil for bacteria and fungi in both soils (Table 4.5). For bacteria and fungi in the BBR soil, this analysis, also, indicated a separation into two groups of rhizosphere zones, one comprising root tip and elongation zone, the other side roots, and older roots.

TABLE 4.5| *P*-values of pair-wise comparison among all zones after PERMANOVA analysis in soil subsets for bacterial (a) and fungal (b) community structures in a rhizobox experiment with beech (Fagus *sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively and were grown in soil from the Bv horizon at BBR or from the Bh horizon at LUE. Significant differences are indicated in bold.

a) Bacterial community

BBR soil

	Bulk soil	Older root	Side root	Elongation zone
Older root	**0.001**			
Side root	**0.001**	0.146		
Elongation zone	**0.001**	0.089	**0.001**	
Root tip	**0.001**	**0.024**	**0.011**	0.479

LUE soil

	Bulk soil	Older root	Side root	Elongation zone
Older root	**0.005**			
Side root	**0.009**	0.076		
Elongation zone	**0.004**	0.152	0.322	
Root tip	**0.012**	0.108	0.084	0.739

b) Fungal community

BBR soil

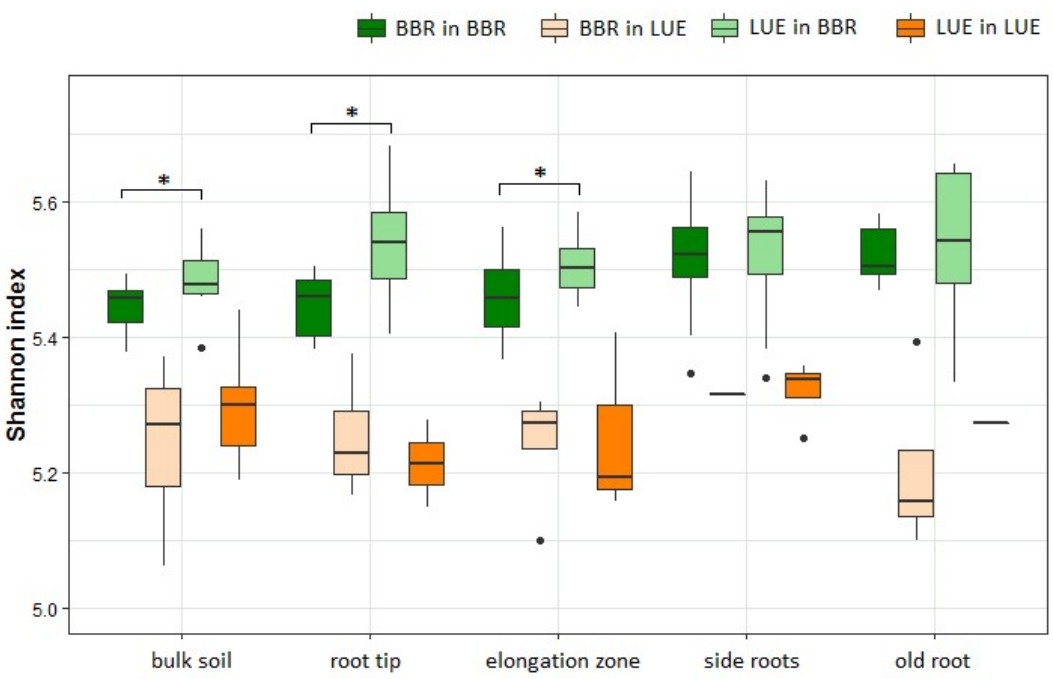
	Bulk soil	Older root	Side root	Elongation zone
Older root	**0.001**			
Side root	**0.001**	**0.026**		
Elongation zone	**0.001**	**0.007**	**0.008**	
Root tip	**0.001**	**0.007**	**0.007**	0.513

LUE soil

	Bulk soil	Older root	Side root	Elongation zone
Older root	**0.015**			
Side root	**0.001**	0.16		
Elongation zone	**0.004**	0.435	0.216	
Root tip	**0.003**	0.222	0.208	0.93

Considering the effects of the factor plant origin in individual zones, Shannon diversity of bacteria in bulk soil, root tip, and elongation zones of BBR soil were significantly higher in the case of beech saplings from LUE than from BBR (Figure 4.3).

a)



b)

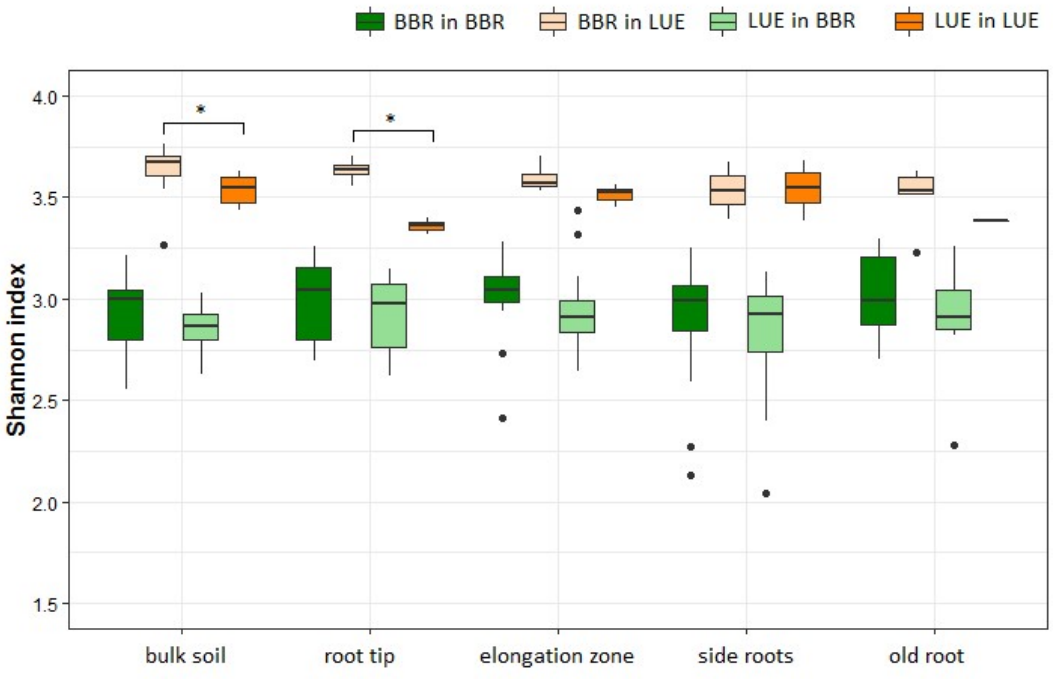


FIGURE 4.3| Shannon index of bacterial (a) and fungal (b) communities in a rhizobox experiment with beech (*Fagus sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively, and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE; shown are the indices in different zones for four experimental treatments. A significant effect of the factor "plant origin" is indicated with an asterisk (P<0.05), according to the Tukey post-hoc test.

The opposite behavior was observed for the Shannon diversity of fungi in bulk soil and around the root tip in the LUE soil, which were higher in the case of saplings from BBR than from LUE. The effect of the factor plant origin on beta-diversity was strongly significant in the elongation zone for both bacteria and fungi, and fungi also around the root tip (Table 4.6). Furthermore, there was a weakly significant plant effect on bacteria and fungi in the bulk soil.

TABLE 4.6| PERMANOVA analysis of current soil and plant origin effect on sampling zone subsets of bacterial and fungal community structures in a rhizobox experiment with beech (Fagus *sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively (factor plant origin), and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE (factor current soil); shown are Pseudo-*F* and *P* values for the factors and their interactions. Significant effects are indicated with *P* values in bold.

	Bacteria		**Fungi**	
	Pseudo-F	P(perm)	Pseudo-F	P(perm)
Bulk soil				
Current soil	251.78	**0.001**	226.78	**0.001**
Plant origin	1.48	**0.01**	1.40	**0.014**
Soil x plant	1.50	**0.004**	1.45	**0.011**
Root tip				
Current soil	85.04	**0.001**	57.40	**0.001**
Plant origin	1.49	0.054	2.17	**0.002**
Soil x plant	1.48	**0.035**	1.61	**0.013**
Elongation zone				
Current soil	101.58	**0.001**	69.87	**0.001**
Plant origin	1.68	**0.004**	2.29	**0.002**
Soil x plant	1.73	**0.002**	2.95	**0.001**
Side roots				
Current soil	52.80	**0.001**	52.28	**0.001**
Plant origin	1.04	0.339	1.33	0.108
Soil x plant	0.94	0.713	1.13	0.239
Old root				
soil	46.39	**0.001**	31.84	**0.001**
Plant origin	0.82	0.837	1.24	0.11
Soil x plant	0.88	0.73	1.11	0.223

Community composition of the two soils

There was a large number of microbial OTUs that were found in all four plant-soil combinations („treatments") (bacteria: 904 (including 49% of all LUE soil, and 23% of all BBR soil OTUs), fungi: 278 (including 36% of all LUE soil, and 20% of all BBR soil OTUs), Figure 4.4).

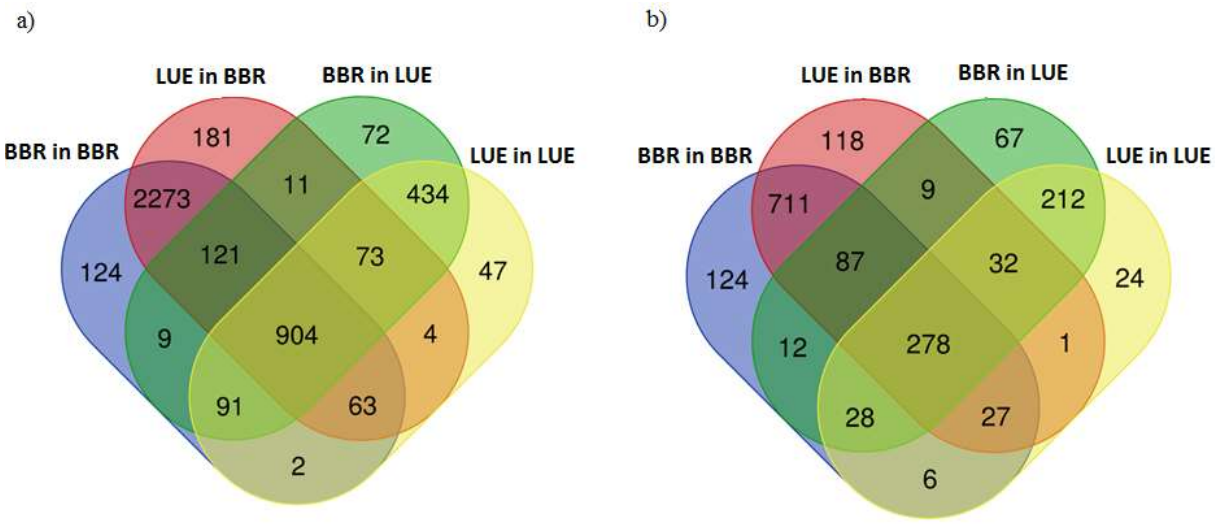


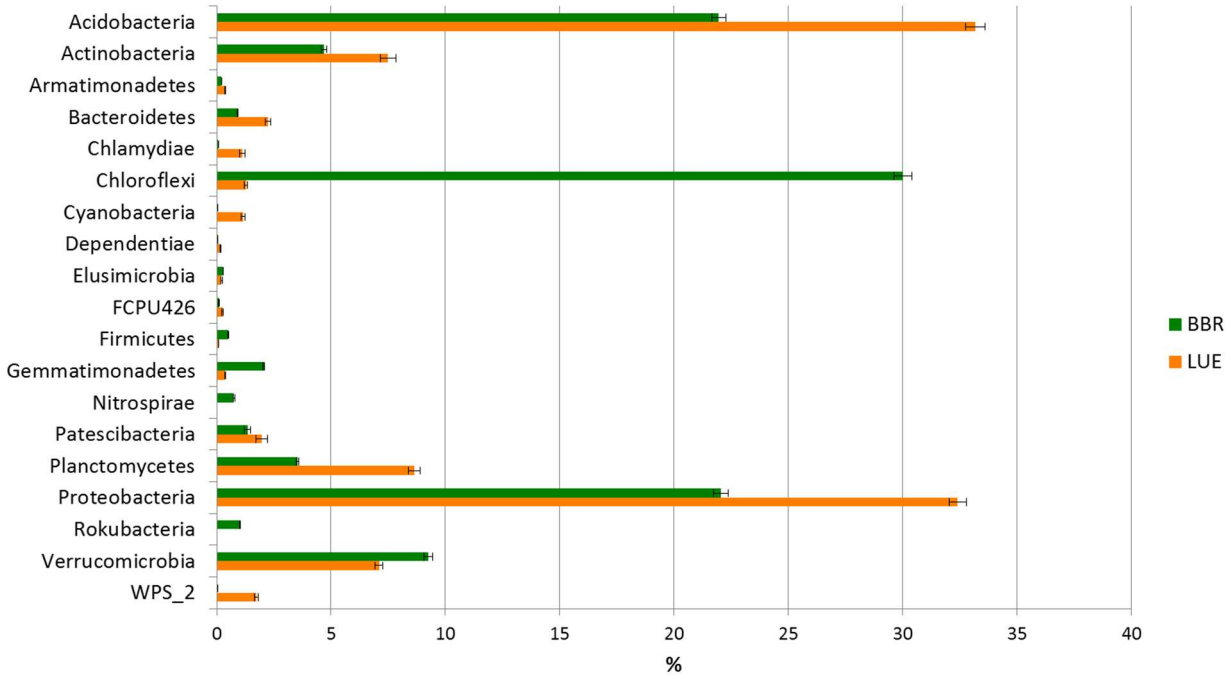
FIGURE 4.4| Venn diagrams showing the overall overlap of taxonomic units (OTUs) among four treatments for soil bacterial (a) and fungal (b) microbial communities sampled in a rhizobox experiment with beech (Fagus *sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively, and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE.

Based on taxonomical analysis (Figure 4.5), the two soils differed strongly in the relative abundance of main phyla and genera of bacteria and fungi. Considering the most dominant bacterial phyla, the BBR soil was characterized by a higher fraction of Chloroflexi and Verrucomicrobia, whereas the LUE soil had more Acidobacteria, Proteobacteria, and Planctomycetes. At the order level, we found several taxa identified by Bergkemper et al. (2016a) as being involved in P cycling like Solibacterales, Acidobacteriales, and Rhizobiales, however, with not much difference between the two soils (Fig 4.5c).

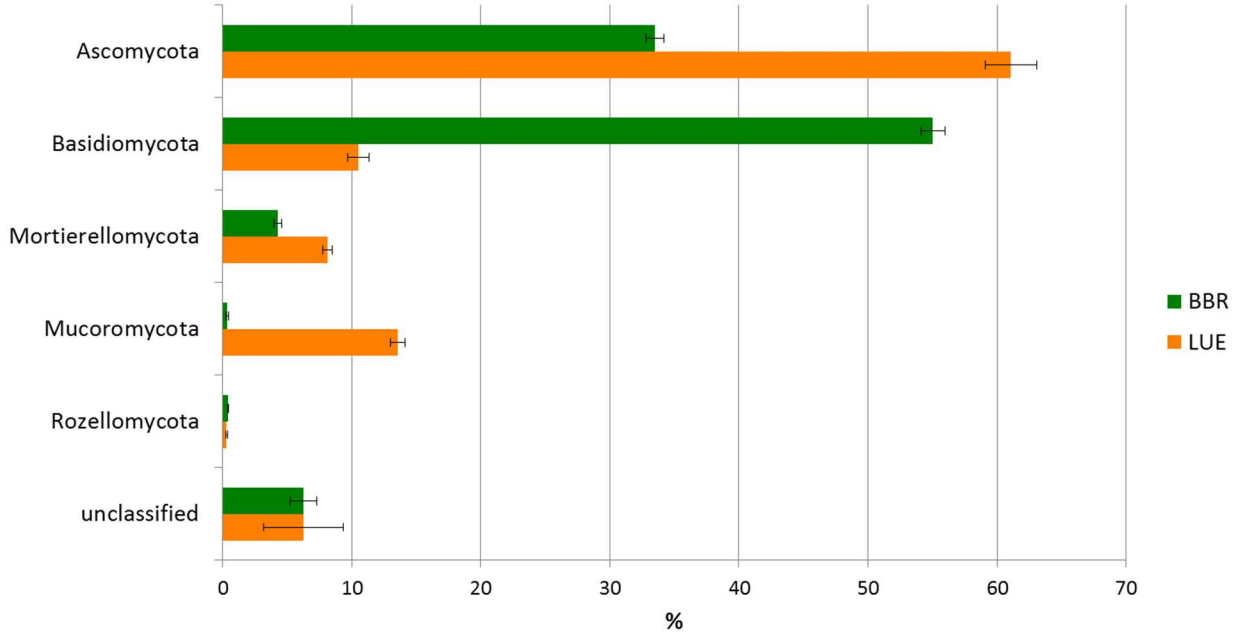
Dominant fungal phyla were Basidiomycota and Ascomycota in the BBR soil and Ascomycota and Mucoromycota in the LUE soil. FUNGuild analysis of the fungal genera revealed a higher fraction of symbiotrophs for the BBR soil with high P availability, but a higher proportion of saprotrophs and pathotrophs for the LUE soil with low P availability (Figure 4.6). For samples

from the BBR soil, 46% of the fungal OTUs were assigned to a single ectomycorrhizal genus – *Inocybe*. In samples from the LUE soil, the fungal genera *Umbelopsis* (saprotroph), *Oidiodendron* (pathotroph / symbiotroph), *Mortierella* (saprotroph/ symbiotroph) were most dominant (14%, 10%, 9%, respectively).

a)



b)



c)

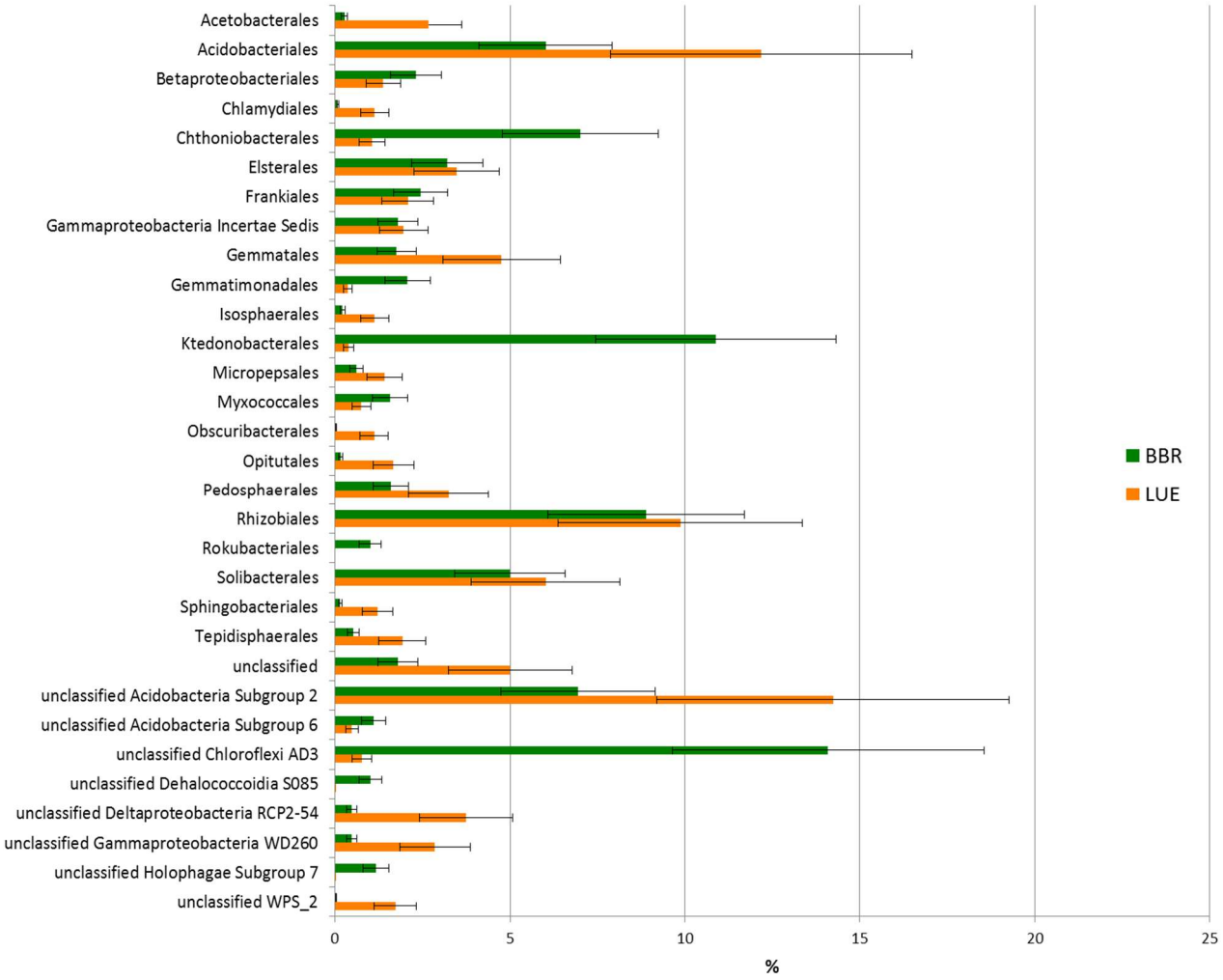
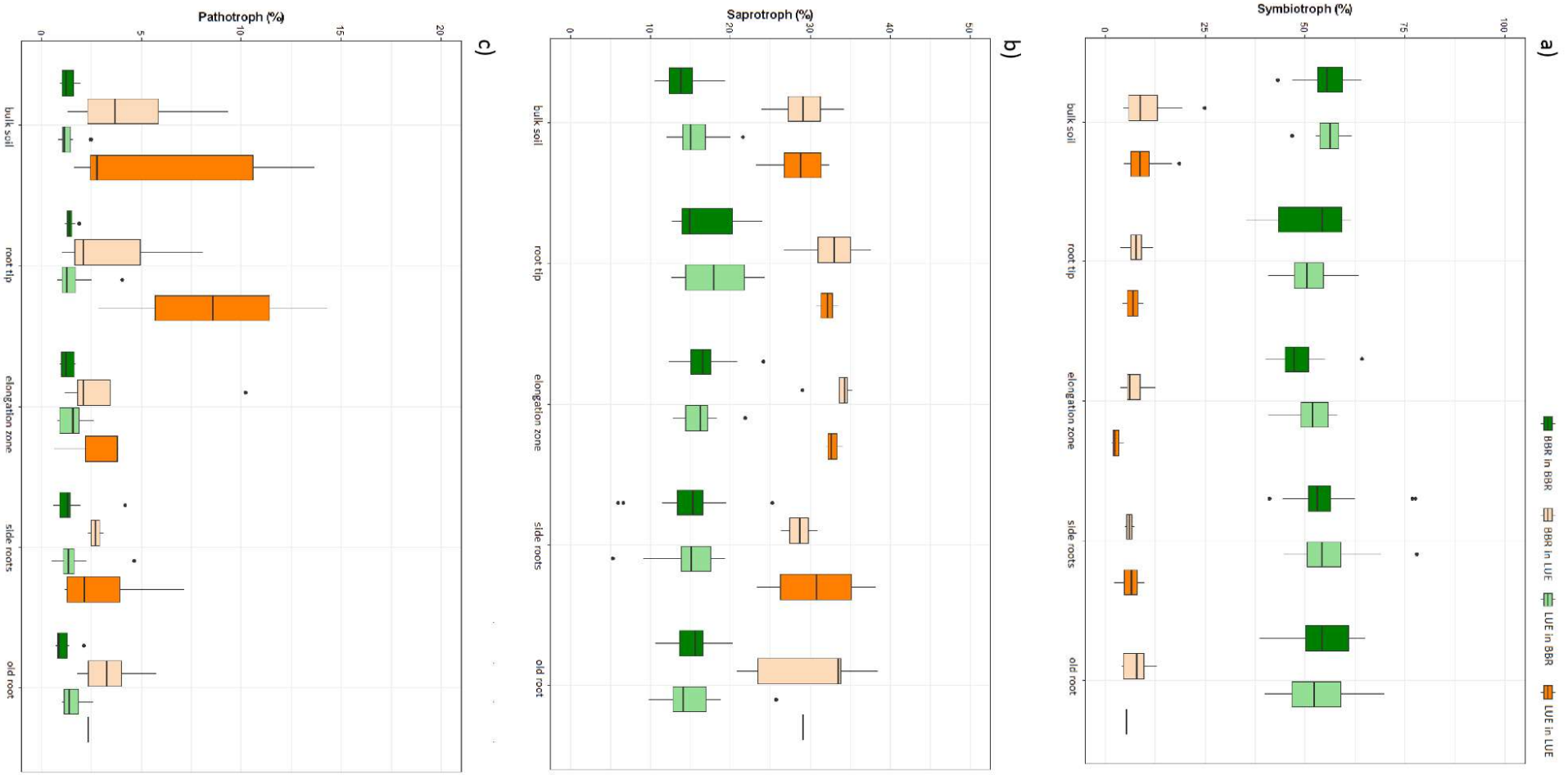


FIGURE 4.5| Relative abundance of bacterial (a) and fungal (b) phyla and bacterial orders (c) in two soils (threshold >1% abundance), sampled in a rhizobox experiment with beech (Fagus *sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE. If group was not classified at the order level, first higher taxonomic name is given. Error bars signify the standard error of the mean (n_{LUE}=8, n_{BBR}=10).

FIGURE 4.6| Relative abundance of all sequences belonging to fungal genera assigned to trophic mode according to FUNGuild analysis ((a) symbiotroph: gaining nutrients by exchanging some benefit with host cells; (b) saprotroph: gaining nutrients by breaking down dead host cells; (c) pathotroph: gaining nutrients by harming host cells), in soil samples from a rhizobox experiment with beech (Fagus *sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively and were grown in soils from the Bv horizon at BBR or from the Bh horizon at LUE; shown are values for four treatments in different zones.



Plant and zone effects on the relative abundance of main bacterial and fungal phyla and genera

While plant origin exhibited a strongly significant effect only on several less abundant bacterial phyla, the factor zone was strongly significant for the abundant phyla Proteobacteria and Chloroflexi (Table 4.7a). These effects were expressed as a slight increase in abundance of Proteobacteria from bulk soil to the elongation zone along the root axis (Figure 4.7a) and a slightly higher abundance of Chloroflexi in the bulk soil than in rhizosphere zones (Figure 4.7b). Of the dominant bacterial orders potentially involved in P mobilization (see above), the abundance of Solibacterales in the LUE soil was higher in the bulk soil than the rhizosphere. Furthermore, the abundance of this order was affected by plant origin in the bulk soil (LUE soil only) and in the elongation zone (both soils) with higher values in the case of saplings from LUE (Fig. 4.7c).

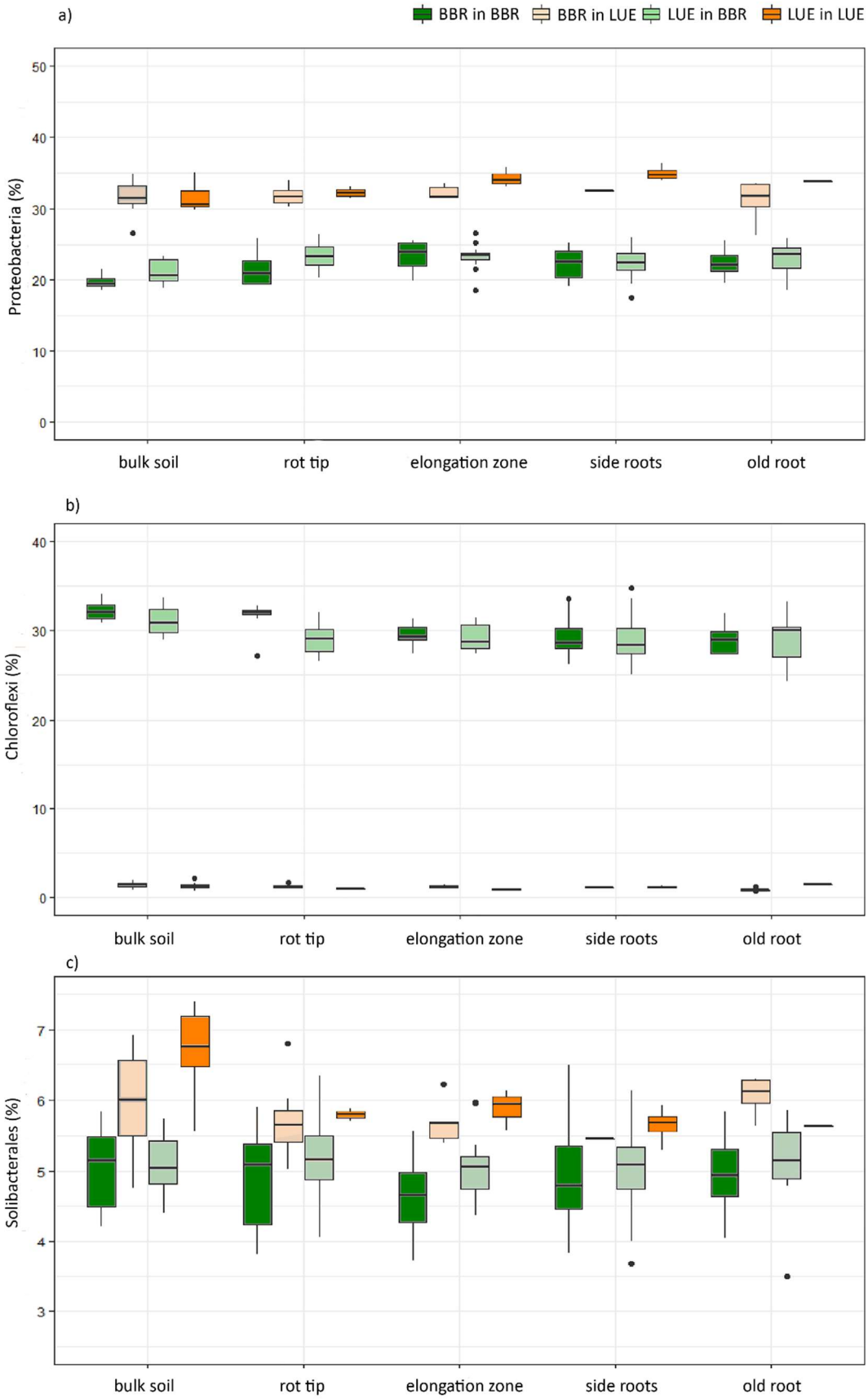
TABLE 4.7 |Analysis of variance of bacterial (a) and fungal (b) phyla sampled in different zones (bulk soil and rhizosphere along various root segments; factor zone) of beech (*Fagus sylvatica* L.) saplings in a rhizobox experiment; the saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively (factor plant origin) and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE (factor current soil); statistical significance is indicated as $***P < 0.001$, $**P < 0.01$, $*P < 0.05$. Significant effects are indicated in bold.

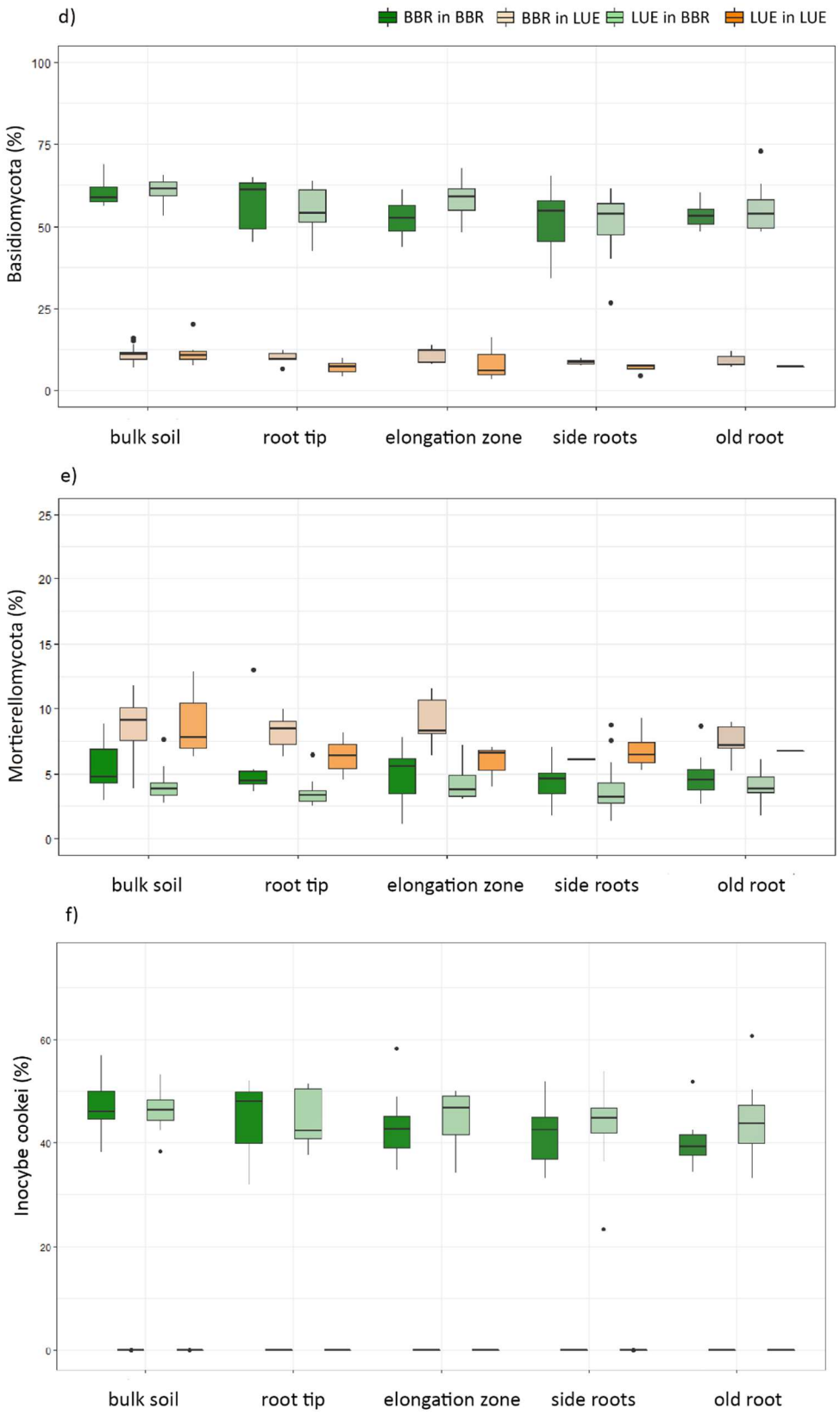
a)

	Current soil	Plant origin	Zone	Soil x plant	Plant x zone	Soil x zone	Soil x plant x zone
Acidobacteria	***					*	*
Proteobacteria	***	*	***				
Planctomycetes	***					**	
Actinobacteria	***			***	**	***	
Verrucomicrobia	***			***	*		**
Bacteroidetes	***	*				***	
Patescibacteria	***	***	*	**			
WPS_2 (sqrt)	***			***			**
Cyanobacteria (sqrt)	***	***	**				
Chloroflexi	***	*	***			*	
Chlamydiae (sqrt)	***	***		***		**	
Gemmatimonadetes	***	**	**				
Armatimonadetes	***			**			
FCPU426	***	***	*	***			
Elusimicrobia	***	**		**			
Dependentiae	***					***	**
Firmicutes	***		**			**	
Nitrospirae	***	**					
Rokubacteria	***						

b)

	Current soil	Plant origin	Zone	Soil x plant	Soil x zone	Plant x zone	Soil x plant x zone
Basidiomycota	***		***				
Ascomycota	***	*	***	***	***		***
Mortierellomycota	***	***	*				
Rozellomycota	**			**			
Mucoromycota	***	**		***		*	**


a)
BBR in BBR
BBR in LUE
LUE in BBR
LUE in LUE
Proteobacteria (%)
0
10
20
30
40
50
bulk soil
rot tip
elongation zone
side roots
old root
b)
Chloroflexi (%)
0
10
20
30
40
bulk soil
rot tip
elongation zone
side roots
old root
c)
Solibacterales (%)
3
4
5
6
7
bulk soil
rot tip
elongation zone
side roots
old root


d)
BBR in BBR
BBR in LUE
LUE in BBR
LUE in LUE
Basidiomycota (%)
100
75
50
25
0
bulk soil
root tip
elongation zone
side roots
old root
e)
Mortierellomycota (%)
25
20
15
10
5
0
bulk soil
root tip
elongation zone
side roots
old root
f)
Inocybe cookei (%)
60
40
20
0
bulk soil
root tip
elongation zone
side roots
old root

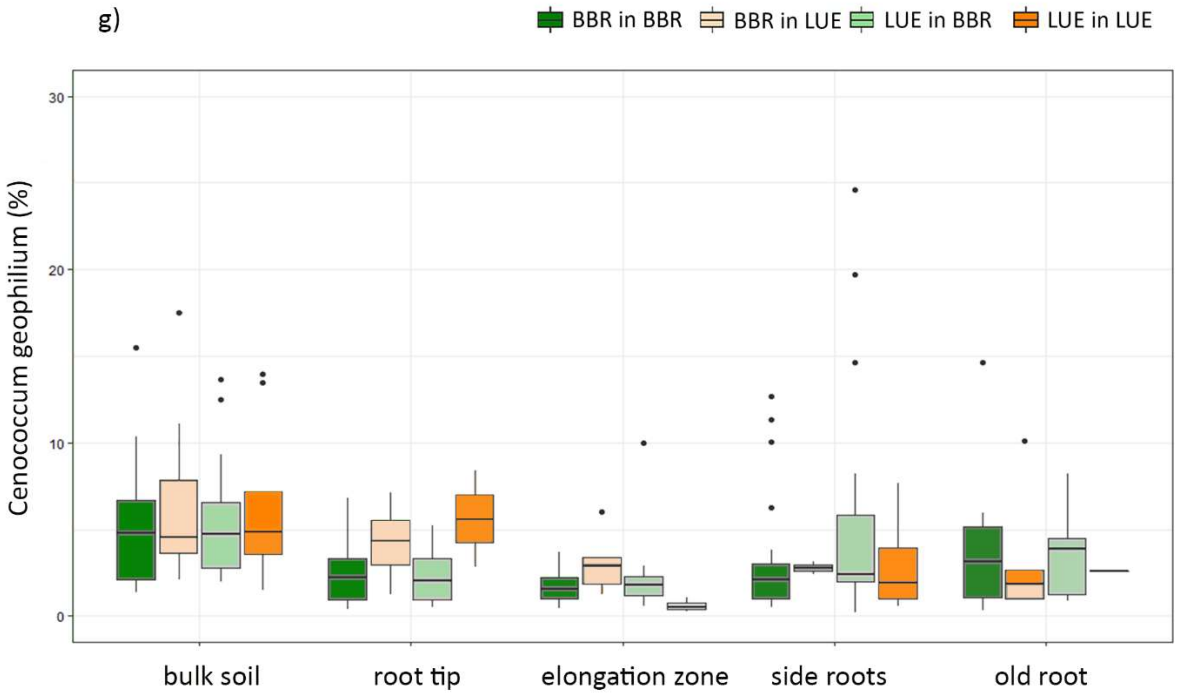


FIGURE 4.7| Relative abundance of the bacterial phyla Proteobacteria (a) and Chloroflexi (b), the bacterial order Solibacterales (c), the fungal phyla Basidiomycota (d) and Mortierellomycota (e), as well as the ectomycorrhizal fungal species *Inocybe cookei* (f) and *Cenococcum geophilum* (g) in soil samples from a rhizobox experiment with beech (Fagus *sylvatica* L.) saplings; saplings originated from the sites Bad Brückenau (BBR) and Unterlüss (LUE) with high and low soil P availability, respectively and were grown in material from the Bv horizon at BBR or from the Bh horizon at LUE; shown are values for four treatments in different zones; for the reasoning of the selection see text.

For the fungal community, plant origin exhibited a substantial effect on Mortierellomycota (Table 4.7b), expressed as a lower abundance in bulk soil and some rhizosphere zones in treatments with saplings from LUE (Fig. 4.7e). On the other hand, Basidiomycota was strongly affected by the factor "zone", expressed as a slight decrease from bulk soil to the old root zone along the root axis (Fig. 4.7d). The factor zone, but not plant origin, affected the relative abundance of ectomycorrhizal fungi, with some being more abundant in the rhizosphere than in the bulk soil, e.g. the generally highly abundant *Inocybe cookei* (Fig. 4.7f), and some less abundant in the rhizosphere than in the bulk soil, e.g. *Cenococcum geophilum* (Fig. 4.7g).

Discussion

In this study we compared the diversity and community structure of the microbiome in the rhizosphere of beech saplings originating from two forest sites differing strongly in soil P availability, and thus exhibiting a different nutritional status as shown by Meller et al. (2019) Chapter 2 and Chapter 3. By growing the saplings in mineral soil either from their site of origin or from the other site, we created four experimental model systems representing different situations. We considered the treatments with saplings growing in soil from their site of origin to represent plants and soils with their microbiome adapted to each other, and treatments with saplings growing in soils from the other site to stand for plants acclimating to altered soil conditions.

In our discussion below we assume that the newly grown and mostly non-mycorrhizal roots the rhizosphere of which we investigated, represented the major part of the root system, and thus the collected samples are not or only little influenced by non-visible roots and ectomycorrhizal hyphae. Further underlying assumptions and limitations of the approach in terms of soil properties are discussed by Meller et al. (2019) Chapter 2 and in Chapter 3 of this thesis.

Microbial community structure of the BBR and LUE soils

As shown in Table 4.1, the soil materials from BBR and LUE differed strongly in properties such as pH, organic carbon and major nutrient concentrations, all of which have been shown to exert a strong influence on soil microbial communities (Fierer and Jackson, 2006; Fierer et al., 2007; Ramirez et al., 2010; Tan et al., 2013; Nicolitch et al. 2016). Therefore, the dominant effect of the factor “current soil” and, as a consequence, the distinct separation of the bacterial and fungal communities inhabiting the two experimental soils, is of no surprise and in good agreement with findings from earlier studies with soils from the same sites (Bergkemper et al., 2016b; Zavišić et al., 2016; Spohn et al., 2018). Despite the large differences, the soils in all four plant-soil combinations shared a common core microbiome, comprising 49% of LUE and 23% of BBR soil bacterial OTUs, and 36% of LUE and 20% of BBR soil fungal OTUs. This result is in good agreement with identification of a common microbiome of 43% of all detected bacterial OTUs in Ah horizons along a geosequence of five sites differing in soil P availability (Bergkemper et al., 2016b). Considering the mature beech stands on all of these sites, this effect was attributed to the community shaping effect induced by the

dominant tree species (Urbanova et al., 2015, Bergkemper et al., 2016b). Nevertheless, it cannot be excluded that in our case some overlap was introduced due to our experimental setup, where rhizoboxes with different soils were handled in close vicinity to each other.

The higher bacterial and fungal richness in the BBR than the LUE soil can be explained by the more favorable growing conditions (higher pH, carbon and nutrient concentrations) (Fierer et al., 2006; Lang, 2017; Table 4.1) and is in good agreement with the higher soil microbial C as measured in the second growing season of this experiment (Chapter 3). Similarly, Bergkemper (2016b) reported a higher abundance of 16S rRNA genes in the Ah horizon from BBR than in the one from LUE, and along the entire geosequence of 5 sites mentioned above, they found the 16S rRNA abundance to be correlated with organic C, N and P contents. Furthermore, Spohn (2018) found a higher species richness of ectomycorrhizal fungi on beech fine roots from BBR than from LUE. The apparent discrepancy of a lower fungal Shannon index in the BBR than the LUE soil in our experiment may be attributed to the strong dominance of *Inocybe* sp. in the BBR soil, because this index is impacted by the distribution of species abundance.

Taxonomically, for bacteria on a phylum and order level, overall, our results for the two soils are in good agreement with the metagenomic analysis of Bergkemper et al. (2015). This concerns in particular the dominance of oligotrophic Acidobacteria and copiotrophic Proteobacteria in both soils and the significantly higher abundance of oligotrophic Chloroflexi in the BBR soil. Also, Bergkemper et al. (2016a) found mostly similar abundances of bacterial orders potentially involved in P mobilization (Acidobacteriales, Solibacterales) as in our experiment. Except, in their study the particularly important order Rhizobiales, harboring several genes coding for enzymes involved in P cycling (phosphatases, C-P lyases, quinoprotein glucose dehydrogenase solubilizing inorganically bound P, phosphate transporters), was significantly more abundant in the BBR soil, whereas in our study it was equally abundant in both soils. Members of the Rhizobiales order are also known as effective plant growth-promoting bacteria (Rodriguez and Fraga, 1999).

The higher relative proportion of saprotrophic fungal genera in the LUE than the BBR soil is probably best explained by the potentially higher proportion of labile organic carbon in the sandy LUE than the loamy BBR soil. Furthermore, the lack of an effect of the factor “plant origin” on the relative abundance of functional groups of fungal genera argues against flow

of plant-derived carbon from the roots affecting the ratio between symbiotrophs and saprotrophs, as was shown earlier (Gadgil and Gadgil, 1971, 1975; Lindahl and Tunlid, 2015; Averill and Hawkes 2016). While our results may be explained by the soil properties, they are in contradiction to the much better correlation of saprophytic than ectomycorrhizal ergosterol with soil P availability in mineral soils along the geosequence mentioned above (Zavišić et al., 2016). Nevertheless, our findings corroborate the conclusion by the latter authors that free soil microbes may play an important role in P mobilization in mineral soil layers. The study by Talbot et al. (2013) suggests that this can also be the case in organic soil layers. They found that enzymes correlated with the decomposition of recalcitrant N-containing compounds were of ectomycorrhizal fungal origin, while enzymes correlated with the decomposition of carbohydrate- and P-containing molecules were of saprophytic origin.

The development of a rhizosphere microbial community along the root axis

The significant differences we found between bulk soil and the rhizosphere zones indicate a strong rhizosphere effect in both soils, irrespective of the origin of the beech sapling growing in them. The rhizosphere is well-known as microbial hot-spot, fueled by root exudation of easily degradable organic compounds (e.g. Kuzyakov and Blagodatskaya, 2015). Specifically, rhizosphere effects resulting in changes in microbial abundance, composition or activity were repeatedly reported also for beech in a variety of soils (Colin et al., 2017; Nicolitch et al., 2016; Hoffmann et al., 2016). The fact that the microbial communities in all rhizosphere zones were strongly separated from the bulk soil community, indicates that roots can influence the soil microbial community not only next to segments with strong root exudation.

The spatial continuum of diverging microbial communities along the root axis has often been hypothesised, but rarely demonstrated (Marschner et al., 2011). Previous studies have shown spatial variability of bacteria next to individual root segments (De Leij, 1994; Gilbert, 1998; Lemanceau, 1995; Marschner et al., 2004; Yang & Crowley, 2000), but here for the first time, we reveal the gradual development of bacterial and fungal communities from bulk soil to the rhizosphere around the root tip and then along the root axis to old roots. This gradual change can be explained with differences in type and quantity of organic carbon available along the root axis (Yang and Crowley, 2000). Based on a model proposed by Marschner (2011), microbial colonization begins just behind the root tip by chemotaxis or as a legacy effect from the border cells sloughed off the root cap. In the elongation zone, root exudation increases

and releases low-molecular weight sugars, organic acid anions, phenols, and amino acids to the surrounding soil (Hoffland et al., 1989; Roemheld,1991; Marschner et al., 2011). This high abundance of easily available carbon stimulates microbial growth and attracts more soil microorganisms to the root surface. In the subsequent zones around side roots and older roots, root exudation is lower and cellulose and other recalcitrant cell wall materials from sloughed-off root cortex tissues become the primary substrates for microbial growth. The less pronounced differences among the rhizosphere zones in the LUE soil may be explained on the one hand by the smaller sample size, resulting mainly from plant mortality in the LUE in LUE treatment, but on the other hand also by specific properties of the LUE soil, e.g. its sandy texture.

Considering the maximum root exudation in the elongation zone, microbial growth rates and activity are expected to decrease with increasing distance from this zone (Nguyen and Guckert, 2001). This differentiation in C availability along the root axis could potentially be reflected in the relative abundance of bacterial phyla displaying different nutritional strategies, i.e. in the bulk soil and rhizosphere zones next to older root segments a higher relative abundance of oligotrophic taxa would be expected, and in the rhizosphere next to younger root segments a higher abundance of copiotrophic taxa. In agreement with this hypothesis is our finding of a higher abundance of the oligotrophic order Solibacterales in the bulk soil than in the rhizosphere of the LUE soil. This bacterial order within the phylum Acidobacteria harbors various metabolic, defensive and regulatory traits enabling its growth under unfavorable environmental conditions (Challacombe et al., 2011), and has been shown to be potentially important for the mobilization of inorganically bound P in both used soils (Bergkemper et al., 2016a).

Considering the importance of ectomycorrhizal symbiosis for the mineral nutrition of beech (Plassard and Dell, 2010, Chen et al., 2016), a higher relative abundance of respective fungi (EMF) in the rhizosphere than the bulk soil can be expected. The slight increase in abundance of the dominant EMF *Inocybe cookei* in both used soils from the bulk soil to the elongation zone supports this hypothesis. The opposite behavior of the generally much less abundant *Cenococcum geophilum* then indicates that root exudation by beech in both soils further selects for the dominant EMF species. This further corroborates the notion of a community

shaping effect induced by beech as the dominant tree species at both the BBR and LUE sites, as discussed above.

Effects of the plant nutritional status on bulk soil and rhizosphere microbiome

Considering the strong interaction between plants and the rhizosphere microbiome via secretion and detection of signaling compounds including flavonoids, strigolactones, cutin monomers, and others yet unidentified low molecular weight compounds (Venturi, 2016) we expected to find an impact of the plant P nutritional status on the rhizosphere microbial community. Such an effect of plant nutritional status was demonstrated before for iron nutrition of some crop plants (Yang et al., 2000; Pii et al., 2015). In agreement with our expectations, our data show a general influence of plant origin on beta diversity in both soils. Further considering the maximum rate of exudation in the elongation zone (see discussion above), the highest plant effects on beta diversity of bacteria and fungi in the rhizosphere next to this zone are no surprise. The weaker but still significant effect of plant origin in the bulk soil may be explained by the exploration of this zone by undetected ectomycorrhizal hyphae, suggesting that it rather should be called mycorrhizosphere than bulk soil.

In the bulk soil and some rhizosphere zones, we found a higher Shannon diversity of bacteria (BBR soil) and Fungi (LUE soil) in the treatments with beech saplings acclimating to altered soil conditions than for treatments with beech saplings adapted to a given soil (for respective assumptions see introductory discussion paragraph). Overall, this indicates that acclimating beech saplings introduce some new microbial species to the soil or stimulate additional microorganisms compared to the adapted saplings. The fact that in the BBR soil with high P availability only bacteria and in the LUE soil with low P availability only fungi were concerned, may be explained by the following reasoning based on results presented in Chapter 3 of this thesis. The beech saplings with a low P nutritional status from LUE acclimating to the higher P availability in the BBR soil were shown to transfer less carbon to the rhizosphere by root exudation than saplings from BBR adapted to this soil, whereas the opposite was observed for the case of saplings with a high P nutritional status acclimating to lower P availability in the LUE soil. This may suggest on one hand that it needs a higher flow of carbon into the rhizosphere to affect fungal diversity than bacterial diversity, and on the other hand, once fungal diversity is affected, there is too little carbon flow left to affect bacterial diversity. These hypotheses are supported by results demonstrating the importance of high carbon flow

into the rhizosphere for a high EMF diversity found in a common garden experiment that strong shading of beech saplings suppressed colonization by EMF but also resulted in a low EMF diversity. In a girdling experiment with beech Pena (2010) found no effect of the reduced carbon flow on root colonization but a significant related decrease of EMF Shannon diversity. From results of a combined girdling and shading experiment, Druebert (2009) concluded that for EMF diversity the flow of carbon and not its source (recent photosynthesis or stored carbohydrates) is important.

The well documented importance of root exudation of organic compounds for symbiotic fungi in the rhizosphere may also explain the lower abundance of Morteriellomycota in some zones in the treatments with beech saplings from LUE, characterized by a lower rate of root exudation than saplings from BBR, as discussed above. The opposite argument may then explain the higher abundance of the oligotrophic Solibacterales in some zones in the same treatments. Considering the potential role of this bacterial order in mobilization of inorganically bound P, and should the plant be able to profit from this process, this behavior may actually offer an advantage for saplings with a low P nutritional status.

Conclusions

Altogether, our results showed that soil is the primary factor determining bacterial and fungal diversity and community structure in the rhizosphere of beech saplings, thereby confirming our first hypothesis. For the first time, we could show a succession of bacterial and fungal communities developing in the rhizosphere along the axis of newly grown beech roots, confirming also our second hypothesis. We also detected small but significant effects of the plant P nutritional status on bacterial and fungal diversity in the rhizosphere around the root tip and along the elongation zone, thus partly confirming our third hypothesis. On the other hand, the only indication of a plant effect potentially offering an advantage with respect to P nutrition, was a higher relative abundance of a bacterial order potentially important for the mobilization of inorganically bound P in some zones in treatments with beech saplings exhibiting a low P nutritional status. Generally, it is difficult to disentangle how important a given group of bacteria or fungi is for P mobilization in the rhizosphere. Many groups can harbor multiple genes coding for enzymes mobilizing P from organic and inorganic sources (Bergkemper et al., 2016a; Uroz et al., 2007; Nicolitch et al., 2016). Thus, based on our data we are not able to confirm or reject our fourth hypothesis. On the other hand, we could show

that acclimation of beech saplings with a given plant nutritional status to altered soil P availability significantly affected microbial diversity in the bulk soil and active rhizosphere zones. Furthermore, we found several indications supporting the notion of a community shaping effect of beech as a stand dominating tree species which to a large degree is site independent.

CHAPTER 5
General discussion and outlook

In the general discussion and outlook, first, I present the objectives and principles of the experiment; second, I revisit the hypotheses and discuss main findings in the context of beech (*Fagus sylvatica* L.) acclimation strategies to soil P availability. Next, I discuss the implications of the results on organ and ecosystem levels. In the end, I present the limitations of the study and the outlook.

Objectives

In the context of exploring the acclimation of beech from high to low and from low to high soil P availability, we aimed to:

- identify and quantify the main responsive traits of *Fagus sylvatica* L. saplings, in terms of biomass and P allocation, contributing to acclimation,
- investigate root-soil interactions involved in the acclimation of beech in terms of root architecture and morphology, mycorrhization, and the occurrence of P-mobilizing compounds in the rhizosphere,
- determine the impact of beech on the composition of the rhizosphere microbiome and its potential role in plant P nutrition, using a fine resolution sampling next to different root segments.

Principles of the Experiment

We grew two groups of 12–15-year-old beech saplings originating from sites characterized generally with high (BBR) and low (LUE) soil nutrient (in particular P) availability for two years in mineral soil from their site and in soil from the other site. During the first growing season, we assessed mid-season and senescent leaves P concentration, as well as rhizosphere and bulk soil properties potentially related to P mobilization, and collected soil around root zones along the root axis for microbial DNA analysis. During the second growing season, we repeated the assessment of rhizosphere and bulk soil properties and destructively sampled the seedlings to measure plant traits related to root architecture and morphology, mycorrhization of root tips as well as biomass and P contents of plant compartments (leaves, stem, coarse roots, fine roots). Afterward, we tested if trait differences between the plant-soil combinations could be explained by the influence of factors “current soil” (the soil in which plants were grown during the experiment) or “plant origin” (the site/soil from which saplings were collected). The results of the experiment thus allow to discuss the influence of

plant nutritional status resulting from different origin on the plant allocation of P and biomass, root architecture, P mobilization in the rhizosphere, and relation to soil microbial community for beech saplings growing during the experiment in soil with different P availability. We consider the treatments with saplings growing in the soil from their site to represent the situation of plants adapted to a given soil situation. We furthermore assume that the results from the treatments with saplings growing in the soil from the other site provide clues on the plastic response of beech that allows it to acclimate to changes in soil nutrient conditions.

Main Results

1) Phosphorus allocation to leaves of beech saplings reacts to soil phosphorus availability.

We hypothesized that the acclimation of beech saplings to new soil conditions in terms of biomass and P allocation to different plant compartments is driven by the plant striving to achieve or maintain high foliar P concentrations.

Our results clearly rebut this hypothesis, highlighting instead the role of a sensitive signaling network that allows the plant to produce as much biomass as possible under given soil conditions by regulating mass and nutrient allocation accordingly. P concentrations in leaves and stem, as well as mass allocation to leaves and fine roots, were affected by both soil and plant origin. By contrast, relative P allocation to leaves and fine roots, as well as P concentrations in fine roots, were determined almost entirely by the experimental soil, making these plant traits most suitable to assess the plasticity of beech in response to soil P availability.

2) Plant nutritional status explains the modifying effect of provenance on the response of beech sapling root traits to differences in soil nutrient supply.

We hypothesized, (i) that the assessed root traits and rhizosphere parameters are determined mainly by the factor “current soil”, (ii) that the factor „plant origin“ modifies the effects of the soils, and that the modifying effect can be attributed mainly to differences in the plant nutritional status.

Our results showed that beech saplings exhibited a high plasticity in adapting their root system to soils, differing in their nutrient supply, in terms of root traits related to growth, architecture and morphology. We confirmed our first hypothesis that the plastic reactions

were determined mainly by the soil properties. Confirming our second hypothesis, plant provenance had a modifying effect, that was consistent with an influence of the plant status in those nutrients, which were not in sufficient supply in the soil. However, we cannot completely rule out an additional genotypic difference between the beech saplings from LUE and BBR in their sensitivity to differences in soil nutrient supply.

Compared to root traits, differences among treatments in mycorrhizal inoculation and rhizosphere parameters related to P mobilization were small and mainly determined by the soil properties. Plant origin had only a minor modifying effect, possibly due to differences in the ability to transfer carbon compounds from the shoot to the root and the fungal partner.

3) Phosphorus nutritional status of beech saplings (*Fagus sylvatica* L.) affects the rhizosphere microbiome around the elongation zone and the root tip.

We hypothesized that (i) the soil with its physico-chemical properties is the main factor defining the microbial pool from which microorganisms are recruited into the rhizosphere by beech roots, (ii) the rhizosphere microbiome differs among zones related to root segments differing in their functionality, (iii) the plant nutritional status exerts the largest modifying effect on the rhizosphere microbiome in the elongation zone which is expected to be a hot spot of root exudation, and (iv) in the rhizosphere of beech saplings with a low P nutritional status microbial groups are fostered that have the potential to improve P mobilization.

Our results showed that soil is the primary factor determining bacterial and fungal diversity and community structure in the rhizosphere of beech saplings, thereby confirming our first hypothesis. For the first time, we could show a succession of bacterial and fungal communities developing in the rhizosphere along the axis of newly grown beech roots, confirming also our second hypothesis. We also detected small but significant effects of the plant P nutritional status on bacterial and fungal diversity in the rhizosphere around the root tip and along the elongation zone, thus partly confirming our third hypothesis. On the other hand, the only indication of a plant effect potentially offering an advantage with respect to P nutrition, was a higher relative abundance of a bacterial order potentially important for the mobilization of inorganically bound P in some zones in treatments with beech saplings exhibiting a low P nutritional status. Generally, it is difficult to disentangle how important a given group of bacteria or fungi is for P mobilization in the rhizosphere. Many groups can harbor multiple

genes coding for enzymes mobilizing P from organic and inorganic sources (Bergkemper et al., 2016a; Uroz et al., 2007; Nicolitch et al., 2016). Thus, based on the data, we are not able to confirm or reject our fourth hypothesis. On the other hand, we could show that acclimation of beech saplings with a given plant nutritional status to altered soil P availability significantly affected microbial diversity in the bulk soil and active rhizosphere zones. Furthermore, we found several indications supporting the notion of a community shaping effect of beech as a stand dominating tree species which to a large degree is site independent.

Adaptation vs. acclimation of beech saplings to soil P availability

In our experiment, when we collected saplings originating from environments contrasting in nutrient (P) availability, we selected plants that survived in their environments between 12 to 15 years already. Depending on the source of the seeds, they might have been genetically adapted to the given condition thanks to the genetic make-up inherited from their parents or were plastic enough to alter their traits to aid with survival after sprouting. Either way, the saplings were expected to display a set of adaptive traits characteristic for the site of origin. However, important to note is that traits of living organisms are not independent of each other, but are always connected and influencing each other. Therefore, a set of interconnected traits across leaves, stems, and roots could be merged into an ecological strategy (Reich 2014).

Reflecting on resource availability in the two soils, the plants originating from BBR and LUE could be described as following “fast”- productive and “slow”- conservative life strategies, respectively (Grime 1977). In comparison to LUE plants, BBR plants were characterized by higher mass and P investments in foliage and fine root biomass, faster growth, photosynthetic efficiency, and stomatal conductance (Yang et al. 2016). However, at the same time, there were prone to potentially high P losses due to low P resorption from senescent leaves and a higher fraction of fine roots of smaller diameter (<0.1mm), which has been related to a shorter root life span (Eissenstat et al., 2000). The LUE plants exhibited a different set of traits, such as lower allocation of mass and P to leaves and fine roots, higher allocation to the stem, higher resorption of foliar P, and a small, less branched root system with potentially longer-living roots related to their larger diameter. The question was if beech saplings can alter their life strategy in response to soil nutrient availability in a short time, or the observed strategies

were the result of long-term adaptation (multigenerational genetical changes) and in consequence the observed traits were not prone to change.

In general, we observed rather high plasticity of P allocation and recovery from senescent leaves in comparison to mass allocation. Mass allocations in crossed treatments (LUE in BBR, BBR in LUE) tended to fall in-between values of adapted treatments (e.g., Leaves; Figure 5.1). Similarly, root architecture did not achieve LUE in LUE characteristics in BBR in LUE plants. The reason for that might be the length of the experiment. Some traits governed by fast processes like P transport from the vacuole to cytoplasm in leaf meristematic tissue are potentially more plastic than relatively slow growth of the new roots. Changes in biomass allocation, even for young saplings, need more time. Therefore, as we see a tendency for beech saplings to alter their biomass allocation, two years might be just too short time to observe it. As for the rhizosphere properties and microbial community interaction of the roots, plant origin effect was rather small indicating that acclimation to the new soil was rapid.

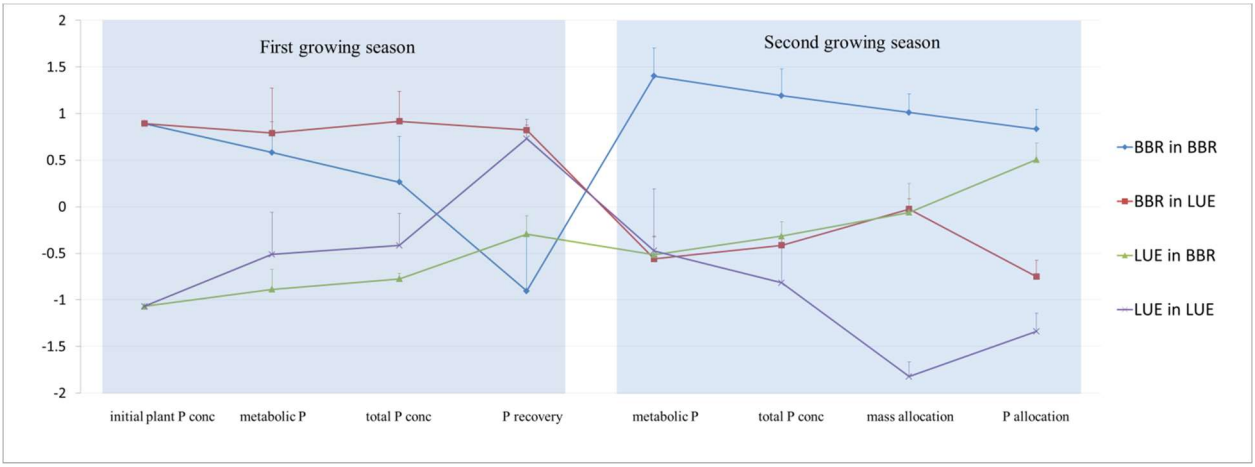


Figure 5.1| An overview of leaf traits measured at 1st and 2nd growing season in the four treatments; BBR in BBR and LUE in LUE represent the systems adapted to high and low soil P availability. The BBR in LUE and LUE in BBR treatments represent acclimation from high to low and low to high soil P availability, respectively. Data was collected in August in the first and second growing season, except P recovery data was collected after leaf abscission (November) after the first growing season. Data points are scaled, centered, and averaged per treatment. Lines are a visual aid; x-axis is not continuous; error bars represent standard error of the mean; initial plant P concentration is set arbitrary to match contrasting soil P availability.

In conclusion, it is unlikely that plants we sampled carried any unique adaptation to local soil conditions on a genetic level; they instead displayed acclimation allowed by high plasticity of their traits. Nevertheless, to fully demonstrate the plasticity of the saplings for more traits, longer observation times are needed. Similar acclimation strategies were reported for other

environmental factors like water and light availability (Jarcuska and Barna, 2011; Stojnić et al., 2015a), confirming the robustness of this species in terms of dealing with environmental change.

Beech internal P cycling – organ level

A complicated sensing and signaling networks on the organ level are behind these seemingly simple changes in allocation patterns on the whole plant level. In the last decade, significant progress has been made towards the identification of the molecular components involved in the control of plant responses to P starvation, with a focus mostly on herbaceous plants (Yuan and Liu, 2008). Phosphorus uptake and growth in perennial woody plants can be temporarily decoupled, i.e., the growth of young leaves may strongly depend on the transport of nutrients stored in the previous season in organs such as stem and coarse roots, but also from older leaves during a growing season (Schachtman et al., 1998; Güsewell, 2004; Zavišić et al., 2018). Therefore, trees like beech require an additional seasonally controlled sensing of P in their storage compartments and the soil (Zavišić et al., 2018), and mechanisms commonly found for herbaceous plants might not apply to trees. For example, a typical morphological and developmental response to P starvation observed in many plant species is a change in root system architecture, which includes reduced growth of primary roots and increased length and density of root hairs and lateral roots (Williamson et al., 2001; Ma et al., 2001; Schmidt and Schikora, 2001; Lopez-Bucio et al., 2002; Lopez-Bucio et al., 2003). What we found in our system is the opposite reaction: plants growing in soil with low P availability displayed a decrease in root length and branching.

Furthermore, the classical response to a decline in photosynthesis in P-deprived plants, involving systemic sugar signaling regulating the Pi starvation responsive genes, might not be of high relevance in trees due to the buffering capacity of P stored in the stem and coarse roots and to high P use efficiency in the leaves (Hauenstein et al., 2018). Further possibilities of reactions in response to P starvation could be inspired by the N nutrition of beech, as observed for the glutamate- and cytokinin-mediated control of nitrate uptake (Gessler et al. 2004, Castro-Rodriguez et al., 2017).

Beech internal P cycling – ecosystem level

Changes in the P and mass allocation patterns of trees can have an impact on forest nutrient cycling at the ecosystem level. Accumulating P in storage organs and limiting losses, via high

P resorption from senescing leaves, slows down the plant–soil-plant P cycle and helps avoid P losses by leaching. As P resorption from leaves is a very plastic trait reacting fast to changes in soil P availability, it can have a rapid impact on the quality of forest litter. This point was demonstrated in our experiment, where the N/P ratio of senescent leaves approximately doubled (from 11 to 20) during acclimation from high-P to low-P soil and decreased from 30 to 18 during acclimation from low-P to high-P soil already after the first growing season. The N/P ratio in the litter can strongly affect plant litter decomposition, the relative importance of fungi and bacteria in litter-associated microbial communities, and litter nutrient dynamics. For example, the relative importance of bacteria in the decomposition of litter is higher at a lower litter N content, whereas low P contents have been shown to favor fungi (Güsewell, 2008). Besides, Reich (2009) showed that P-deficiency in plants could limit an increase in productivity, related to enhanced N availability, by limiting a respective increase in photosynthetic capacity. In good agreement with this, Yang (2016) observed a lower photosynthetic activity of beech saplings from LUE than from BBR. In the latter study, the relation to foliar N concentrations was not tested, but the underlying mechanism was identified as a stomatal limitation due to anatomically smaller stomatal pore widths, not as the result of a biochemical limitation of photosynthesis. This suggests that a decrease in photosynthetic activity due to alteration of leaf anatomy could be an acclimation strategy and not a stress response. Since a reduction of photosynthesis reduces the amount of captured carbon, also the biomass production and root deposition could decrease, lowering carbon input to the environment.

Furthermore, a conservative nutrient use strategy of trees could also affect reproduction. I observed higher rejuvenation and seed quality (higher germination rate) at the forest site with higher P stocks. This could affect the capacity of the forest to regenerate after a disturbance.

Limitations of the design

Addressing questions from plant ecophysiology is challenging due to many factors influencing the research systems. Therefore, to obtain interpretable results, it is a necessity to decrease complexity and introduce a significant contrast in manipulated factors. Considering this, employing a rhizobox system with homogenized mineral soil and beech saplings seemed like a good compromise decreasing the number of variables but maintaining close-to natural soil

conditions (in contrast to hydroponics). Significant advantages are the possibility to manipulate the system and track changes in a reasonable time frame, as well as the potential to identify processes on a small scale. The limitations of this intermediate-complexity approach are, on the one hand, a loss of ecosystem-level interpretability and, on the other hand, limited insight into detailed mechanisms behind the observations. Furthermore, the statistical power was limited as well because of replications being restricted to what could be handled in the greenhouse in the given time.

Choice of soils

We selected Bh (LUE) and Bv (BBR) horizons from the two extreme sites in terms of P stock within the geosequence set-up by the Priority Program SPP 1685 "Ecosystem Nutrition" in order to detect a maximum effect of soil P availability on plant traits. As organic horizons had a higher P content, we excluded them from the experiment, in particular to impose a stronger P limitation in the soil from the low-P site LUE where 50% of the P stock is stored in the organic surface layer (Lang et al., 2017) and therefore plant roots were concentrated. In this way, with a rhizobox system and B (Bh/Bv) soil horizons, we created two well-defined model systems with homogeneous conditions of contrasting P availability.

One of the major concerns that has to be taken into account is that the soils used in our study differed not only in P content (/availability) but also in the proportions of different P forms (organic/inorganic), N content, texture, microbial biomass, pH and exchangeable aluminum (Al). All these factors complicate interpretation of results, in particular in the rhizosphere, due to differences in sorption (impact of texture), the decomposition rate of organic compounds (microbial biomass), and plant response (N content).

Choice of plants

The important assumption of a difference in P nutritional status of the beech saplings, linked to their site of origin, was supported by the measurements of total plant P at the end of the experiment. However, as the plants originated from a natural forest, their status regarding other nutrients was also different. Because the two sites, where we sampled the tree saplings, were far apart, possible genetical differences (provenances) could also influence the results. Furthermore, the sampling and re-planting processes could have introduced additional stress due to the loss of the original fine root system. However, this had the advantage that all the analyzed roots were created in the new soil.

The difficulty in translating results from young plants to adult individuals is that perennials, in particular, display different growth strategies with increasing age. Young trees rely more on current soil nutrient availability and prioritize growth, whereas older plants build nutrient storage in wood, allowing remobilization in spring and mast years (Johnson and Abrams, 2009). In the context of P nutrition, a particularly significant difference regarding tree age is storage size. The P nutrition of beech saplings has shown distinct differences compared to adult trees, indicating a reduced P-storage and P-mobilization capacity during annual growth (Netzer et al., 2017).

OUTLOOK

Rapid change of soil properties may seem like an unlikely scenario in the context of slow ecosystem development. However, significant changes can happen. One of the consequences of climate change is the increased frequency of extreme weather events like intense storms, extreme heat, floodings, and wildfires. They all have the potential to cause permanent damage to existing forests, and their recovery might depend on the acclimation capacity of the young trees. Furthermore, human management practices can lead to drastic changes in nutrient availabilities by removing significant P pool from the cycling by harvesting, or damage/removal of the soil organic layer. It is also vital in new plantings to understand what is the capability of beech to acclimatize to the new soil conditions.

To further explore beech plasticity and frame it in the context of a real ecosystem, it would be interesting to investigate allocation patterns of adult beech trees along a P gradient. Such an opportunity may present itself in the framework of the planned project “Test pflanzungen” at WSL. On about 60 forest sites across Switzerland, the current plant cover will be removed to make room for targeted plantations with saplings of selected tree species to test their resilience to climatic changes. Depending on the possibilities for combining tree harvest with weighing, the removal of the current plant cover might offer the unique opportunity to obtain both nutrient contents and biomass of different compartments of adult trees.

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LITERATURE

Aerts, R. (1996). Nutrient resorption from senescing leaves of perennials: are there general patterns? J. Ecol. 84, 597–608.

Agriculture, forestry and fishery statistics. (2018). Eurostat; ISBN 978-92-79-94757-5, doi: 10.2785/340432 , Cat. No: KS-FK-18-001-EN-N.

Averill, C. and Hawkes, C. V. (2016). Ectomycorrhizal fungi slow soil carbon cycling. Ecol. Lett., 19: 937-947.

Bahr, A., Ellström, M., Akselsson, C., Ekblad, A., Mikusinska, A., and 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. doi.org/10.1016/j.soilbio.2013.01.004.

Batjes, N. H. (2011). Global distribution of soil phosphorus retention potential. Wageningen: ISRIC - World Soil Information, 42.

Baudoin, E., Benizri, E., and Guckert, A. (2002). Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Appl. Soil Ecol. 19, 135–145.

Baudoin, E., Benziri, E., Guckert, A. (2001). Metabolic fingerprint of microbial communities from distinct maize rhizosphere compartments. Eur. J. Soil Biol. 37, 85-93.

Berg G. and Smalla K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol. Ecol. 68(1): 1–13.

Bergkemper, F., Schöler, A., Engel, M., Lang, F., Krüger, J., Schloter, M., Schulz, S. (2015). Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. Environ. Microbiol. 18(6):1988-2000. doi:10.1111/1462-2920.13188

Bergkemper, F., Kublik, S., Lang, F., Krüger, J., Vestergaard, G., Schloter, M., Schulz, S. (2016a). Novel oligonucleotide primers reveal a high diversity of microbes which drive phosphorous turnover in soil. J. Microbiol. Methods 125: 91–97.

Bergkemper, F., Welzl, G., Lang, F., Krüger, J., Schloter, M., Schulz, S. (2016b). The importance of C, N and P as driver for bacterial community structure in German beech dominated forest soils. J. Soil Sci. Plant Nutr. 179(4): 472–80.

Blossfeld, S. and Gansert, D. (2007). A novel non-invasive optical method for quantitative visualization of pH dynamics in the rhizosphere of plants. Plant Cell Environ. 30, 176–186. doi.org/10.1111/j.1365-3040.2006.01616.x.

Borken, W., Kossmann, G., and Matzner, E. (2007). Biomass, morphology and nutrient contents of fine roots in four Norway spruce stands. Plant Soil 292, 7-93. doi.org/10.1007/s11104-007-9204-x.

Bowsher, A.W., Miller, B.J., and Donovan, L.A. (2016). Evolutionary divergences in root system morphology, allocation, and nitrogen uptake in species from high- versus low-fertility soils. Funct. Plant Biol. 43, 129-140. doi.org/10.1071/FP15162.

Brady, N.C. and Weil, R.R. (1999). The nature and properties of soils. 12th ed. New Jersey, USA: Prentice Hall Inc.

Brant, A. N. and Chen, H. Y. H. (2015). Patterns and mechanisms of nutrient resorption in plants. CRC Crit. Rev. Plant Sci. 34, 471–486. doi: 10.1080/07352689.2015.1078611.

Braun, S., Thomas, V. F. D., Quiring, R., and Flückiger, W. (2010). Does nitrogen deposition increase forest production? The role of phosphorus. Environ. Pollut. 158, 2043–2052. doi: 10.1016/j.envpol.2009.11.030.

Brunet, J., Fritz, Ö., and Richnau, G. (2010). Biodiversity in European beech forests - a review with recommendations for sustainable forest management. Ecol. Bull. 53, 77–94.

Brunner, I. and Brodbeck, S. (2001). Response of mycorrhizal Norway spruce seedlings to various nitrogen loads and sources. Environ. Poll. 114, 223-233.

Buiteveld, J., Vendramin, G.G., Leonardi, S., Kamer, K., and Geburek, T. (2007). Genetic diversity and differentiation in European beech (*Fagus sylvatica* L.) stands varying in management history. For. Ecol. Manag. 247, 98-106. doi:10.1016/j.foreco.2007.04.018.

Bünemann, E.K., Augstburger, S., and Frossard, E. (2016). Dominance of either physicochemical or biological phosphorus cycling processes in temperate forest soils of contrasting phosphate availability. Soil Biol. Biochem. 101, 85–95. doi.org/10.1016/j.soilbio.2016.07.005.

Bünemann, E.K., Bossio, D.A., Smithson, P.C., Frossard, E., and Oberson, A. (2004). Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. Soil Biol. Biochem. 36, 889–901. doi.org/10.1016/j.soilbio.2004.02.002.

Caldwell, M.M., Dudley, L.M., Lilieholm, B. (1992) Soil solution phosphate, root uptake kinetics and nutrient acquisition: implications for a patchy soil environment. Oecologia. 89(3):305-309. doi:10.1007/BF00317406.

Castro-Rodríguez, V., Cañas, R.A., de la Torre, F.N., Pascual, M.B., Avila, C., Cánovas, F.C. (2017). Molecular fundamentals of nitrogen uptake and transport in trees. J. Exp. Bot. 68(10): 2489–2500.

Challacombe, J.F., Eichorst, S.A., Hauser, L., Land, M., Xie, G., Kuske, C.R. (2011). Biological consequences of ancient gene acquisition and duplication in the large genome of *Candidatus Solibacter* usitatus Ellin6076. PLoS ONE. 6. e24882.

Chalot, M. and Plassard, C. (2011). Ectomycorrhiza and nitrogen provision to the host tree. In Ecological Aspects of Nitrogen Metabolism in Plants, First Edition. eds. J.C. Polacco, and C.D. Todd. John Wiley & Sons, Inc., 69-94.

Chalot, M., Javelle, A., Blaudez, D., Lambilliote, R., Cooke, R., Sentenac, H., Wipf, D., Botton, B. (2002). An update on nutrient transport processes in ectomycorrhizas. Plant Soil 244:165–175.

Chen, W., Koide, R.T., Adams, T.S., DeForest, J.L., Cheng, L., and Eissenstat, D.M. (2016). Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. Proc. National Acad. Sci. 113, 8741–8746. doi.org/10.1073/pnas.1601006113.

Clemensson-Lindell, A. and Asp, H. (1995). Fine-root morphology and uptake of ^{32}P and ^{35}S in a Norway spruce (*Picea abies* (L.) Karst.) stand subjected to various nutrient and water supplies. Plant Soil 173, 147-155.

Colin, Y., Nicolitch, O., Van Nostrand, J.D., Zhou, J.Z, Turpault, M.-P., and Uroz, S. (2017). Taxonomic and functional shifts in the beech rhizosphere microbiome across a natural soil toposequence. Sci. Rep. 7(1): 1–17.

Craine, J. (2009). Resource Strategies of Wild Plants. Princeton University Press, 352pp.

Cuervo-Alarcon, L., Arend, M., Müller, M., Sperisen, C., Finkeldey, R., and Krutovsky, K.V. (2018). Genetic variation and signatures of natural selection in populations of European beech (*Fagus sylvatica* L.) along precipitation gradients. Tree Genetics and Genomes 14, 84. doi.org/10.1007/s11295-018-1297-2.

Curt, T., Coll, L., Prèvosto, B., Balandier, P., and Kunstler, G. (2005). Plasticity in growth, biomass allocation and root morphology in beech seedlings as induced by irradiance and herbaceous competition. Ann. For. Sci. 62, 51–60. doi: 10.1051/forest:2004092.

Diaconu, D., Stangler, D. F., Kahle, H. P., and Spiecker, H. (2016). Vessel plasticity of European beech in response to thinning and aspect. Tree Physiol. 36,1260–1271. doi: 10.1093/treephys/tpw053.

Drew, M.C., Saker, L.R., and Ashley, T.W. (1975). Nutrient supply and the growth of the seminal root system in barley. J. Exp. Bot. 24(109): 1189–1202.

Druebert, Ch., Lang, Ch., Valtanen, K., and Polle, A. (2009). Beech carbon productivity as driver of ectomycorrhizal abundance and diversity. Plant Cell Environ. 32: 992–1003.

Duquesnay, A., Dupouey, J.L., Clement, A., Ulrich, E., and Le Tacon, F. (2000). Spatial and temporal variability of foliar mineral concentration in beech (*Fagus sylvatica*) stands in northeastern France. Tree Physiol. 20, 13–22. doi: 10.1093/treephys/20.1.13.

Durrant, T.H., de Rigo, D., and Caudullo, G. (2016). *Fagus sylvatica* and other beeches in Europe: distribution, habitat, usage and threats. European Atlas of Forest Tree Species, 130–131. doi.org/10.2788/038466.

Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10(10): 996–98.

Edgar, R.C. and Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics 31(21): 3476–82.

Eissenstat, D.M., Kucharski, J.M., Zadworny, M., Adams, T.S., Koide, R.T. (2015). Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. New Phytol. 208(1): 114–24.

Eissenstat, D.M., Wells, C.E., Yanai R.D., and Whitbeck, J.L. (2000). Building roots in a changing environment: implications for root longevity. New Phytol. 147, 3342.

Eldhuset, T.D., Swensen, B., Wickstrøm, T., and Wollebæk, G. (2007). Organic acids in root exudates from *Picea abies* seedlings influenced by mycorrhiza and aluminum. Journal Plant Nutr. Soil Sci. 170, 645–648. doi.org/10.1002/jpln.200700005.

Eschrich, W., Fromm, J., and Essiamah, S. (1988). Mineral partitioning in the phloem during autumn senescence of beech leaves. Trees 2, 73–83.

Fierer, N., and Jackson, R.B. (2006). The diversity and biogeography of soil bacterial communities. PNAS 103(3): 626–31.

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

Finér, L., Helmisaari, H.-S., Lõhmus, K., Majdi, H., Brunner, I., Børja, I., Eldhuset, T., et. al (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, 394-405.

Flückiger, W. and Braun, S. (2003). Critical limits for nutrient concentrations and ratios for forest trees - a comment. Empirical critical loads for nitrogen - Proceedings SAEFL, Bern.

Flückiger, W. and Braun, S. (1998). Nitrogen deposition in Swiss forests and its possible relevance for leaf nutrient status, parasite attacks and soil acidification. Environ. Pollut. 102: 69–76. doi: 10.1016/b978-0-08-043201-4.50 015-0.

Folman, L.B., Postma, J., Van Veen, J.A. (2001). Ecophysiological characterization of rhizosphere bacterial com- munities at different root locations and plant developmental stages of cucumber grown on rock- wool. Microb Ecol 42: 586–597.

Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F., Hartmann, M. (2016). Microbial diversity in European alpine permafrost and active layers. FEMS Microbiology Ecology. 92(3).

Gadgil, R.L., Gadgil, P.D. (1975). Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata.* NZJ Forest Sci. 5:35–41.

Gadgil, R.L., Gadgil, P.D. (1971). Mycorrhiza and litter decomposition. Nature 233, 133.

Garbaye, J. and Wilhelm, M.E. (1985). Facteurs limitants et aspects dynamiques de la mycorhization contrôlée de *Fagus silvatica* Lin. par *Hebeloma crustuliniforme* (Bull. ex Saint-Amans) Quél, sur tourbe fertilisée. Annal. Sci. Forestières, 42, 53–68. doi.org/10.1051/forest:19850104.

Gärtner, H., and Schweingruber, F. H. (2013). Microscopic preparation techniques for plant stem analysis. Remagen: Dr. Norbert Kessel, 78.

Gee, G.W., and Bauder, J.W. (1986). “Particle-size analysis”, in Methods of Soil Analysis: Part 1 - Physical and Mineralogical Methods, ed. A. Klute (Madison, WI: Soil Science Society of America), 383–411.

George, T.S., Fransson, A.-M., Hammond, J.P., and White, P.J. (2011). “Phosphorus nutrition: rhizosphere processes, plant response and adaptations,” in Phosphorus in Action, eds Bünemann, E.K., Oberson, A. and Frossard, E. Soil Biology, Vol. 26, Springer, Berlin, Heidelberg, 245–271.

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

Gessler, A., Kopriva, S., Rennenberg, H. (2004). Regulation of nitrate uptake of trees at the whole plant level: interaction between nitrogen compounds, cytokinins and carbon metabolism. Tree Physiol.24, 1313–1321.

Gessler, A., Schneider, S., Weber, P., Hanemann, U., Rennenberg, H. (1998). Soluble N compounds in trees exposed to high loads of N: A comparison between the roots of Norway spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees grown under field conditions. New Phytol. 138, 385–399. doi: 10.1046/j.1469-8137.1998.00134.x.

Ghalambor, C.K., McKay, J.K., Carroll, S.P., Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21(3):394-407.

Gianfreda, L., and Ruggiero, P. (2006). "Enzyme activities in soil" in Nucleic Acids and Proteins in Soil. eds. Nannipieri, P. and Smalla, K. Springer-Verlag Berlin Heidelberg, 257-311.

Gilbert, B., and Frenzel, P. (1998). Rice roots and CH_4 oxidation: the activity of bacteria, their distribution and the microenvironment. Soil Biol. Biochem. Vol. 30, No. 14, pp. 1903-1916.

Gong, L. and Zhao, J. (2019). The response of fine root morphological and physiological traits to added nitrogen in Schrenk's spruce (*Picea schrenkiana*) of the Tianshan mountains, China. PeerJ 7:e8194 DOI 10.7717/peerj.8194.

Göttlein, A. (2015). Ranges of threshold values for the nutritional assessment of the main tree species spruce, pine, oak and beech. Allg. Forst- und Jagd-Ztg. 186, 110-116.

Gratani, L., Meneghini, M., Pesoli, P., and Crescente, M.F. (2003). Structural and Functional Plasticity of *Quercus Ilex* seedlings of different provenances in Italy. Trees - Structure and Function 17(6): 515–21.

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111, 1169–94. doi: 10.1086/283244.

Gruber, B.D., Giehl, R.F.H., Friedel, S., and von Wirén, N. (2013). Plasticity of the *Arabidopsis* root system under nutrient deficiencies. Plant Phys. 163, 161-179. doi/10.1104/pp.113.218453.

Güsewell, S. (2004). N:P ratios in terrestrial plants: variation and functional significance. New Phytol. 164, 243–266. doi: 10.1002/ece3.2587.

Güsewell, S. and Gessner, M. (2008). N:P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Funct. Ecol.23. 211 - 219.

Hauenstein, S., Neidhardt, H., Lang, F., Krüger, J., Hofmann, D., Pütz, T., and 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.org/10.1007/s11104-018-3804-5.

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

Hayes, P., Turner, B. L., Lambers, H., and Laliberté, E. (2014). Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient acquisition strategies along a 2-million-year dune chronosequence. J. Ecol. 102, 396–410. doi: 10.1111/1365-2745.12196.

Hedley, M.J., White, R.E., and Nye, P.H. (1982). Plant-induced changes in the rhizosphere of Rape (*Brassica napus* ver. Emerald) seedlings. III. Changes in L value, soil phosphate fractions and phosphatase activity. New Phytol. 91, 45–56.

Heim, A., Brunner, I., Frey, B., Frossard, E., and Luster, J. (2001). Root exudation, organic acids, and element distribution in roots of Norway spruce seedlings treated with aluminum in hydroponics. J. Plant Nutr. Soil Sci. 164, 519–526.

Hertel, D., Strecker, T., Müller-Haubold, H., and 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? J. Ecol. 101, 1183–1200. doi.org/10.1111/1365-2745.12124.

Heuck, C., Weig, A., and Spohn, M. (2015). Soil microbial biomass C:N:P stoichiometry and microbial use of organic phosphorus. Soil Biol. Biochem. 85, 119–129. doi.org/10.1016/j.soilbio.2015.02.029.

Hinsinger, P. (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil, 237, 173–195.

Hinsinger, P., Brauman, A., Devau, N., Gérard, F., Jourdan, C., Laclau, J.-P., et al. (2011). Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail? Plant Soil 348, 29–61. doi.org/10.1007/s11104-011-0903-y.

Hodge, A. (2004). The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 162. 9–24.

Hodge, A. (2006). Plastic plants and patchy soils. J. Exp. Botany 57, 401-411. doi:10.1093/jxb/eri280.

Hodge, A. (2009). Root decisions. Plant Cell Environ. 32, 628–640. doi: 10.1111/j.1365-3040.2008.01891.x.

Hodge, A., Berta, G., Doussan, C., Merchan, F., and Crespi, M. (2009). Plant root growth, architecture and function. Plant Soil 321, 153–187. doi.org/10.1007/s11104-009-9929-9.

Hoffland, E., Findenegg, G.R., and Nelemans, J.A. (1989). Solubilization of rock phosphate by rape - II. local root exudation of organic acids as a response to P-starvation. Plant Soil 113(2): 161–65.

Hofmann, K., Heuck, C., and Spohn, M. (2016). Phosphorus resorption by young beech trees and soil phosphatase activity as dependent on phosphorus availability. Oecologia, 181, 369–379. doi.org/10.1007/s00442-016-3581-x.

Holdaway, R.J., Richardson S.J., Dickie, I.A., Peltzer D.A., Coomes, D.A. (2011). Species- and community-level patterns in fine root traits along a 120000-year soil chronosequence in temperate rain forest. Ecology 99(4): 954–63.

Holzmann, S., Missong, A., Puhlmann, H., Siemens, J., Bol, R., Klumpp, E., and von Wilpert, K. (2016). Impact of anthropogenic induced nitrogen input and liming on phosphorus leaching in forest soils. J. Plant Nutr. Soil Sci. 179, 443–453. doi.org/10.1002/jpln.201500552.

Ishida, T.A., Nara, K., and Hogetsu, T. (2007). Host effects on ectomycorrhizal fungal communities insight from eight host species in mixed conifer–broadleaf forests. New Phytol. 174, 430–440.

Jackson, R.B., Manwaring, J.H., and Caldwell, M.M. (1990). Rapid physiological adjustment roots to localized soil enrichment. Nature 344: 58–60.

Jacobsen, C., Rademacher, P., Meesenburg, H., Meiwes, K.J., 2003. Gehalte chemischer Elemente in Baumkompartimenten Ber. Forschungs zentr. Waldoekosyst. B 69, 81.

Jaeger, C.H., Lindow, S.E., Miller, S., Clark, E., Firestone, M.K. (1999). Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. Appl Environ Microbiol 65: 2685–2690.

Jarcuska, B. and Barna, M. (2011). Plasticity in above-ground biomass allocation in *Fagus sylvatica* L. saplings in response to light availability. Annals of Forest Science, 54(2), 151–160.

Johnson, S. and Marc, A. (2009). Age class, longevity and growth rate relationships: protracted growth increases in old trees in the eastern United States. Tree Physiol. 29. 1317-28.

Jonard, M., André, F., Dambrine, E., Ponette, Q., and Ulrich, E. (2009). Temporal trends in the foliar nutritional status of the French, Walloon and Luxembourg broad-leaved plots of forest monitoring. Ann. For. Sci. 66, 412–421.

Jonard, M., Fürst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., et al. (2015). Tree mineral nutrition is deteriorating in Europe. Global Change Biol. 21, 418–430. doi.org/10.1111/gcb.12657.

Jones, D., Dennis, P.G., Owen, A.G., van Hees P.A.W. (2003). Organic acid behavior in soils – misconceptions and knowledge gaps. Plant Soil 248: 31–41.

Kazantseva, O., Bingham, M., Simard, S.W., and Berch, S.M. (2009). Effects of growth medium, nutrients, water, and aeration on mycorrhization and biomass allocation of greenhouse-grown interior Douglas-fir seedlings. Mycorrhiza 20, 51–66. doi.org/10.1007/s00572-009-0263-0.

Kiba, T. and Krapp, A. (2016). Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. Plant and Cell Phys. 54, 707-714. doi:10.1093/pcp/pcw052.

Kjøller, R., Nilsson, L.-O., Hansen, K., Kappel Schmidt, I., Vesterdal, L., and Gundersen, P. (2012). Dramatic changes in ectomycorrhizal community composition, root tip abundance and mycelial production along a stand-scale nitrogen deposition gradient. New Phytol. 194, 278–286. doi: 10.1111/j.1469-8137.2011.04041.x.

Koranda, M., Schnecker, J., Kaiser, Ch., Fuchslueger L., Kitzler, B., Stange, C.F et al. (2011). Microbial processes and community composition in the rhizosphere of European beech - the influence of plant C exudates. Soil Biol. Biochem. 43(3): 551–58.

Kouno, K., Tuchiya, Y., and Ando, T. (1995). Measurement of soil microbial phosphorus by anion exchange membrane method. Soil Biol.Biochem. 27, 1353–1357.

Kramer, K. (1995). Phenotypic plasticity of the phenology of seven European tree species in relation to climatic warming. Plant Cell Environ. 18, 93–104. doi: 10.1111/j.1365-3040.1995.tb00356.x.

Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R., et al. (2012). Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecol. Letters 15, 378-392. Doi/10.1111/j.1461-0248.2012.01746.x.

Kuzyakov, Y., and Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: concept & review. Soil Biol. Biochem. 83: 184–99.

Lambers, H., Raven, J.A., Shaver, G.R., and Smith, S.E. (2008). Plant nutrient-acquisition strategies change with soil age. Trends Ecol. Evol. 23(2): 95–103.

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.org/10.1007/s10533-017-0375-0.

Leberecht, M., Dannenmann, M., Tejedor, J., Simon, J., Rennenberg, H., and Polle, A. (2016). Segregation of nitrogen use between ammonium and nitrate of ectomycorrhizas and beech trees. Plant, Cell and Environ. 39, 2691-2700. doi: 10.1111/pce.12820.

Lee, R. B., and Ratcliffe, R. G. (1992). Subcellular distribution of inorganic phosphate, and levels of nucleoside triphosphate, in mature maize roots at low external phosphate concentrations: measurements with ^{31}P-NMR. J. Exp. Bot.44, 587–598. doi: 10.1093/jxb/44.3.587.

Lee, R. B., Ratcliffe, R. G., and Southon, T. E. (1990). ^{31}P NMR measurements of the cytoplasmic and vacuolar Pi content of mature maize roots: relationships with phosphorus status and phosphate fluxes. J. Exp. Bot. 41, 1063–1078. doi:10.1093/jxb/41.9.1063.

Lemanceau, P., Corberand, T., Gardan, L., Latour, X., Laguerre, G., Boeufgras, J., Alabouvette, C. (1995). Effect of two plant species, flax (*Linum usitatissinum* L.) and tomato (*Lycopersicon esculentum* Mill.), on the diversity of soilborne populations of fluorescent Pseudomonads. Appl. Environ. Microbiol. 61. 1004-12.

Leuschner, C., Hertel, D., Schmid, I., Koch, O., Muhs, A., and Hölscher, D. (2004). Stand fine root biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. Plant Soil 258, 43-56.

Leuschner, C., Meier, I. C., and 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, 355–368. doi.org/10.1051/forest:2006016.

Lin, W.-Y., Huang, T.-K., Leong, S. J., and Chiou, T.-J. (2014). Long-distance call from phosphate: systemic regulation of phosphate starvation responses. J. Exp.Bot. 65, 1817–1827. doi: 10.1093/jxb/ert431.

Lin, W.-Y., Lin, S.-I., and Chiou, T.-J. (2009). Molecular regulators of phosphate homeostasis in plants. J. Exp. Bot. 60, 1427–1438. doi: 10.1093/jxb/ern303.

Lindahl, B.D. and Tunlid, A. (2015). Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. New Phytol. 205, 1443–1447.

Liu, J., Mei, L., Gu, J., Quan, X., and Wang, Z. (2009). Effects of nitrogen fertilization on fine root biomass and morphology of *Fraxinus mandshurica* and *Larix gmelinii:* A study with in-growth core approach. Shengtaixue Zazhi 28, 1-6.

López-Bucio, J., and Cruz-Ramírez, A., and Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. Curr. Opin. Plant Biol. 6. 280-7.

Lopez-Bucio, J., Hernandez-Abreu, E., Sanches-Calderon, L., Nieto-Jacobo, M.F., Simposon, J., and Herrera-Estrella, L. (2002). Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. Plant Physiol. 129: 244–256.

Lynch J, (1995). Root architecture and plant productivity. Plant Physiology 109, 7-13.

Lynch, J. and Brown, K. (2001). Topsoil foraging - An architectural adaptation of plants to low phosphorus availability. Plant Soil. 237(2):225-237.

Ma, Z., Walk, T.C., Marcus, A., and Lynch, J.P. (2001). Morphological synergism in root hair length, density, initiation and geometry for phosphorus acquisition in *Arabidopsis thaliana*: A modeling approach. Plant Soil 236: 221–235.

Marklein, A.R. and Houlton, B. Z. (2012). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytol. 193, 696–704. doi.org/10.1111/j.1469-8137.2011.03967.x.

Marschner, H. and Römheld, V. (1983). In vivo measurement of root-induced pH changes at the soil-root interface: effect of plant species and nitrogen source. Zeitschrift für Pflanzenphysiologie 111(3): 241–51.

Marschner, H., Kirkby, E.A, and Cakmak, I. (1996). Effect of mineral nutritional status on shoot-root partitioning of photo assimilates and cycling of mineral nutrients. J. Exp. Botany 47, 1255–1263. doi.org/10.1093/jxb/47.Special_Issue.1255.

Marschner, P., Yang, C.H., Lieberei, R., Crowley, D.E. (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem. 33. 1437-1445.

Marschner, P., Crowley, D., and Yang, C.H. (2004). Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. Plant Soil 261(1–2): 199–208.

Marschner, P., Crowley D., Rengel, Z. (2011). Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis—Model and research methods. Soil Biol Biochem. 43. 883-894.

Marschner H. (2012). Mineral Nutrition of Higher Plants 3d Edn. London: Academic Press.

Marschnert, H., Kirkby, E. A., and Engels, C. (1997). Importance of cycling and recycling of mineral nutrients within plants for growth and development. Botanica Acta, 110: 265-273.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17(1): 10–12.

Massalha, H., Korenblum, E., Tholl, D., and Aharoni, A. (2017). Small molecules below-ground: the role of specialized metabolites in the rhizosphere. Plant J. 90, 788–807.

Matesanz, S., Gianoli, E., and Valladares, F. (2010). Global change and the evolution of phenotypic plasticity in plants. Ann. N.Y. Acad. Sci. 1206, 35–55. doi: 10.1111/j.1749-6632.2010.05704.x.

Meier, I.C. and Leuschner, C. (2008). Genotypic variation and phenotypic plasticity in the drought response of fine roots of European beech. Tree Phys. 28, 297-309.

Meller, S., Frossard, E., and Luster, J. (2019). Phosphorus allocation to leaves of beech saplings reacts to soil phosphorus availability, Frontiers Plant Sci. 10, 744. doi.org/10.3389/fpls.2019.00744.

Mellert, K.H., and Göttlein, A. (2012). Comparison of new foliar nutrient thresholds derived from van den Burg's literature compilation with established central European references. Eur. J. Forest Res. 131, 1461-1472. DOI 10.1007/s10342-012-0615-8.

Mimura, T. (1995). Homeostasis and transport of inorganic phosphate in plants. Plant Cell Physiol. 36, 1–7.

Mimura, T., Sakano, K., and Shimmen, T. (1996). Studies on distribution, retranslocation and homeostasis of inorganic phosphate in barley leaves. Plant Cell Environ. 19, 311–320. doi: 10.1111/j.1365-3040.1996.tb00253.x.

Minotta, G., and Pinzauti, S. (1996). Effects of light and soil fertility on growth, leaf chlorophyll content and nutrient use efficiency of beech (*Fagus sylvatica* L.) seedlings. Forest Ecol. Manag. 86, 61-71.

Nadelhofer, K.J. (2000). The potential effects of nitrogen deposition on fine-root production in forest ecosystems. New Phytol. 147: 131-139.

Nannipieri, P., Giagnoni, L., Landi, L., and Renella, G. (2011). "Role of Phosphatase Enzymes in Soil" in Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling. eds. Bünemann, E.K., Oberson, A., and Frossard, E. Berlin, Heidelberg: Springer Berlin Heidelberg, 215–43.

Nannipieri, P., Giagnoni, L., Landi, L., and Renella, G. (2011). "Role of phosphatase enzymes in soil" in Phosphorus in Action, eds. Bünemann, E.K., Oberson, A., and Frossard, E. Soil Biology, Vol. 26, Springer, Berlin, Heidelberg, 215–243.

Netzer, F., Schmid, C., Herschbach, C., and Rennenberg, H. (2017). Phosphorus nutrition of European beech (*Fagus sylvatica* L.) during annual growth depends on tree age and P-availability in the soil. Environ. Exp. Bot. 137, 194–207.doi: 10.1093/treephys/tpx126.

Nguyen, C. and Guckert, A. (2001). Short-term utilisation of ^{14}C(U)glucose by soil microorganisms in relation to carbon availability. Soil Biol. Biochem. 33, 53-60.

Nguyen, N.H., Song, Z., Bates, S.T., Branco S., Tedersoo, L. (2015). FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol.: 1–8.

Nicolitch, O., Colin, Y., Turpault, M. P., and Uroz, S. (2016). Soil type determines the distribution of nutrient mobilizing bacterial communities in the rhizosphere of beech trees. Soil Biol Biochem. 103: 429–45.

Nicotra, A.B., Atkin, O.K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., et al. (2010). Plant phenotypic plasticity in a changing climate. Trends Plant Sci. 15, 684–692. doi: 10.1016/j.tplants.2010.09.008.

Nikolenko, S.I., Korobeynikov, A.I., and Alekseyev, M.A. (2013). BayesHammer: Bayesian clustering for error correction in single-cell sequencing. BMC Genomics 14, S7 (2013). https://doi.org/10.1186/1471-2164-14-S1-S7.

Niu, Y.F., Chai, R.S., Jin, G.L., Wang, H., Tang, C.X., and Zhang, Y.S. (2013). Responses of root architecture development to low phosphorus availability: A review. Annals Botany 112, 391–408. doi.org/10.1093/aob/mcs285.

Oburger, E., Jones, D.L., and Wenzel, W.W. (2011). Phosphorus saturation and pH differentially regulate the efficiency of organic acid anion-mediated P solubilization mechanisms in soil. Plant Soil 341(1–2): 363–82.

Oburger, E., Jones, D.L., and Wenzel, W.W. (2011). Phosphorus saturation and pH differentially regulate the efficiency of organic acid anion-mediated P solubilization mechanisms in soil. Plant Soil 341, 363–382. doi.org/10.1007/s11104-010-0650-5.

Pena, R., Offermann, C., Simon, J., Naumann, P. S., Gessler, A., et al. (2010). Girdling affects ectomycorrhizal fungal (EMF) diversity and reveals functional differences in EMF community composition in a beech forest. Appl Environ Microbiol. 76(6), 1831–1841. https://doi.org/10.1128/AEM.01703-09.

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., et al. (2013). Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nat. Commun. 4:2934. doi: 10.1038/ncomms3934.

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., Crecchio, C. (2015). Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol Fertil Soils 51, 403–415 https://doi.org/10.1007/s00374-015-0996-1.

Plassard, C., and Dell, B. (2010). Phosphorus nutrition of mycorrhizal trees. Tree Phys. 30, 1129–1139. doi.org/10.1093/treephys/tpq063.

Plassard, C., Louche, J., Ali, M.A., Duchemin, M., Legname, E., and Cloutier-Hurteau, B. (2011). Diversity in phosphorus mobilisation and uptake in ectomycorrhizal fungi. Annals For. Sci. 68, 33–43. doi.org/10.1007/s13595-010-0005-7.

Poorter, H. and Sack, L. (2012). Pitfalls and possibilities in the analysis of biomass allocation patterns in plants. Front. Plant Sci. 3:259. doi: 10.3389/fpls.2012.00259.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic acids research, 41(Database issue), D590–D596. https://doi.org/10.1093/nar/gks1219.

R Core Team (2014). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Raghothama, K. G. (1999). Phosphate acquisition. Annu. Rev. Plant Biol. 50, 665–693.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., and Fierer, N. (2010). Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91, 3463–3470.

Razaq, M., Zhang, P., Shen, H., and Salahuddin (2017). Influence of nitrogen and phosphorous on the growth and root morphology of *Acer mono*. PLoS ONE 12, e0171321. doi:10.1371/journal.pone.0171321.

Reich, P., Oleksyn, J., and Wright, I. (2009). Leaf phosphorus influences the photosynthesis-nitrogen relation: A cross-biome analysis of 314 species. Oecologia. 160. 207-12.

Reich, P.B. (2014). The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. Ecology 102(2): 275–301.

Rennenberg, H. and Herschbach, C. (2013). Phosphorus nutrition of woody plants: many questions - few answers. Plant Biol. 15(5): 785–88.

Rennenberg, H., Schneider, S., and Weber, P. (1996). Analysis of uptake and allocation of nitrogen and sulphur compounds by trees in the field. J. Exp. Bot. 47, 1491–1498. doi: 10.1093/jxb/47.10.1491.

Richardson, A.E. and Simpson, R.J. (2011). Soil microorganisms mediating phosphorus availability update on microbial phosphorus. Plant Physiology, 156(3), 989–996. https://doi.org/10.1104/pp.111.175448.

Richardson, A.E., Hocking, P.J., Simpson, R.J., and George, T.S. (2009). Plant mechanisms to optimise access to soil phosphorus. Crop Pasture Sci. 60, 124–143. doi.org/10.1071/CP07125.

Richardson, S. J., Peltzer, D. A., Allen, R. B., McGlone, M. S., and Parfitt, R. L. (2004). Rapid development of phosphorus limitation in temperate rain forest along the Franz Josef soil chronosequence. Oecologia 139, 267–276. doi: 10.1007/s00442-004-1501-y.

Riley, D., and Barber, S.A. (1971). Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root-induced pH changes at the root-soil interface. Soil Sci. Soc. Am. J. 35, 300-306. doi.org/10.2136/sssaj1971.03615995003500020035x.

Rodriguez, H. and Fraga, R. (1999) Phosphate solubilising bacteria and their role in plant growth promotion. Biotechnol Adv 17:319– 339.

Roemheld, V. (1991). The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: an ecological approach. Plant Soil 130, 127-134.

Rolo, V., Andivia, E., and Pokorny, R. (2015). Response of *Fagus sylvatica* and *Picea abies* to the interactive effect of neighbor identity and enhanced CO_2 levels. Trees Struct. Funct. 29, 1459–1469. doi:10.1007/s00468-015-1225-0.

Sardans, J. and Peñuelas, J. (2015), Phosphorus; the treasure nutrient. Glob. Ecol. Biogeogr., 24: 147-156.

Sardans, J. and Peñuelas, J. (2013). Tree growth changes with climate and forest type are associated with relative allocation of nutrients, especially phosphorus, to leaves and wood. Glob. Ecol. Biogeogr. 22, 494–507. doi: 10.1111/geb.12015.

Schachtman, D. P., Reid, R. J., and Ayling, S. M. (1998). Phosphorus uptake by plants. From soil to cell. Plant Physiol. 116, 447–453. doi: 10.1104/pp.116.2.447.

Schilling, G., Gransee, A., Deubel, A., Lezovic, G., Ruppel, S. (1998) Phosphorus availability, root exudates, and microbial activity in the rhizosphere. J. Soil Sci. Plant Nutr.161:465–478.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R, Hartmann, M. et al. (2009). Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75(23): 7537–41.

Schmidt, W., and Schikora, A. (2001). Different pathways are involved in phosphate and iron stress-induced alterations of root epidermal cell development. Plant Physiol. 125: 2078–2084.

Schneider, C.A.; Rasband, W.S., and Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis, Nature methods 9: 671-675, PMID 22930834.

Shahbaz, A.M., Oki, Y., Adachi, T., Murata, Y., and Khan, M. H. R. (2006). Phosphorus starvation induced root-mediated pH changes in solubilization and acquisition of sparingly soluble P sources and organic acids exudation by *Brassica* cultivars. Soil Sci. Plant Nutr. 52, 623–633. doi.org/10.1111/j.1747-0765.2006.00082.x.

Shen, J., Tang, C., Rengel, Z., and Zhang, F. (2004). Root-induced acidification and excess cation uptake by N_2-fixing *Lupinus albus* grown in phosphorus-deficient soil. Plant Soil 260(1–2): 69–77.

Shen, Q., Wen, Z., Dong, Y., Li, H., Miao, Y., and Shen, J. (2018). The responses of root morphology and phosphorus-mobilizing exudations in wheat to increasing shoot phosphorus concentration. AoB PLANTS, 10, ply054; doi.org/10.1093/aobpla/ply054.

Shi, S., Condron, L., Larsen, S., Richardson, A.E., Jones, E., Jiao, J., et al. (2011). *In situ* sampling of low molecular weight organic anions from rhizosphere of radiata pine (*Pinus radiata*) grown in a rhizotron system. Env. Exp. Bot. 70, 131–142. doi.org/10.1016/j.envexpbot.2010.08.010.

Simon, J., Dannenmann, M., Pena, R., Gessler, A., and Rennenberg, H. (2017). Nitrogen nutrition of beech forests in a changing climate: importance of plant-soil-microbe water, carbon, and nitrogen interactions. Plant Soil 418, 89-114.

Smith, S.E., Read, D.J. (2008) Mycorrhizal symbiosis, 3rd edition. Elsevier/Academic Press, Amsterdam, The Netherlands.

Spohn, M., and Kuzyakov, Y. (2013). Distribution of microbial-and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation–coupling soil zymography with ^{14}C imaging. Soil Biol. Biochem. 67, 106-113.

Spohn, M., Ermak, A., and Kuzyakov, Y. (2013). Microbial gross organic phosphorus mineralization can be stimulated by root exudates– a ^{33}P isotopic dilution study. Soil Biol. Biochem. *65*, 254-263.

Spohn, M., Zavišić, A., Nassal, P., Bergkemper, F., Schulz, S., Marhan, S. (2018). Temporal variations of phosphorus uptake by soil microbial biomass and young beech trees in two forest soils with contrasting phosphorus stocks. Soil Biol. Biochem. 117, 191–202. doi.org/10.1016/j.soilbio.2017.10.019

Stojnić, S., Sass-Klaassen, U., Orlovic, S., Matovic, B., and Eilmann, B. (2013). Plastic growth response of European beech provenances to dry site conditions. IAWA J. 34, 475–484. doi: 10.1163/22941932-00000038.

Stojnić, S., Orlović, S., Miljković, S., Galić, Z., Kebert, M., von Wuehlisch, G. (2015a) Provenance plasticity of European beech leaf traits under differing environmental conditions at two Serbian common garden sites. Eur J For Res. 134(6): 1109–25.

Stojnić, S., Orlović, S., Trudić, B., Živkovic, U., von Wuehlisch, G., Milijković, D. (2015b) Phenotypic plasticity of European beech (*Fagus sylvatica* L.) stomatal features under water deficit assessed in provenance trial. Dendrobiology 73: 163–73.

Sultan, S. E. (2004). Promising directions in plant phenotypic plasticity. Perspect. Plant Ecol. Syst. 6, 227–233. doi: 10.1078/1433-8319-00082.

Sun, Y., Gu, J.-C., Zhuang, H.-F., and Wang, Z.-Q. (2010). Effects of ectomycorrhizal colonization and nitrogen fertilization on morphology of root tips in a *Larix gmelinii* plantation in northeastern China. Ecol. Res. 25, 295-302. DOI 10.1007/s11284-009-0654-x.

Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Peay, K.G., (2013). Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil Biol. Biochem. 57, 282-291.

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

Tamburini, F., Pfahler, V., Bünemann, E. K., Guelland, K., Bernasconi, S. M., and Frossard, E. (2012). Oxygen isotopes unravel the role of microorganisms in phosphate cycling in soils. Environ. Sci. Technol. 46, 5956–5962. doi: 10.1021/es300311h.

Tan, S. Y., Yang, C. L., Mei, X. L., Shen, S. Y., Raza, W., Shen, Q. R., et al. (2013). The effect of organic acids from tomato root exudates on rhizosphere colonization of *Bacillus amyloliquefaciens* T-5. Appl. Soil Ecol. 64, 15–22.

Tedersoo, L., Bahram, M., Polme, Kõljalg, U., Yorou, N.S., et al., (2014). Global diversity and geography of soil fungi. Science 346 (6213): 1256688. doi: 10.1126/science.1256688.

Theodorou, C., and Bowen, G.D. (1993). Root morphology, growth and uptake of phosphorus and nitrogen of *Pinus radiata* families in different soils. For. Ecol. Manag. 56, 43-56.

Tiessen, H., and Moir, J.O. (2006). "Characterization of available P by sequential extraction." in: Soil Sampling and Methods of Analysis (2nd ed.). eds. Carter, M. and Gregorich, E. Canadian Society of Soil Science, Taylor and Francis, chapter 25.

Trolove, A.N., Hedley, M.J., Kirk, G.J.D., Bolan, N.S., Loganathan, P. (2003). Progress in selected areas of rhizosphere research on P acquisition. Aust. J. Soil Res. 41, 471-499.

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.

Uroz, S., Calvaruso, C., Turpault, M.P., Pierrat, J.C, Mustin, C., Frey-Klett, P. (2007). Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. Appl. Environ. Microbiol. 73 (9) 3019-3027; doi: 10.1128/AEM.00121-07.

Valladares, F., Chico, J. M., Aranda, I., Balaguer, L., Dizengremel, P., Manrique, E., et al. (2002). The greater seedling high-light tolerance of *Quercus robur* over *Fagus sylvatica* is linked to a greater physiological plasticity. Trees 16, 395–403.

Valladares, F., Sanchez-Gomez, D., and Zavala, M. A. (2006). Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J. Ecol. 94, 1103–1116. doi: 10.1111/j.1365-2745.2006.01176.x.

Van Egeraat A.W.S.M. (1975) The growth of *Rhizobium leguminosarum* on the root surface and in the rhizosphere of pea seedlings in relation to root exudates. Plant Soil 42, 367–379.

Van Heerwaarden, L. M., Toet, S., and Aerts, R. (2003). Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. Oikos 101, 664–669. doi: 10.1034/j.1600-0706.2003.12351.x.

Van Veldhoven, P.P., and Mannaerts, G.P. (1987). Inorganic and organic phosphate measurements in the nanomolar range. Anal. Biochem. 161, 45–48. doi: 10.1016/0003-2697(87)90649-x.

Vance, C.P., Uhde-Stone, C., and Allan, D.L. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol. 157: 423-447. doi:10.1046/j.1469-8137.2003.00695.x.

Venturi, V. and Keel, Ch. (2016). Signaling in the rhizosphere. Trends Plant Sci. 21(3):187-198. doi: 10.1016/j.tplants.2016.01.005.

Vergutz, L., Manzoni, S., Porporato, A., Novais, R. F., and Jackson, R. B. (2012). Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecol. Monogr. 82, 205–220. doi: 10.1890/11-0416.1.

Vitasse, Y., Bresson, C., Kremer, A., Michalet, R., and Delzon, S. (2010). Quantifying phenological plasticity to temperature in two temperate tree species. Funct. Ecol. 24, 1211–1218. doi: 10.1111/j.1365-2435.2010.01748.x.

Vitasse, Y., Delzon, S., Dufrêne, E., Pontailler, J.-Y., Louvet, J.-M., Kremer, A., et al. (2009). Leaf phenology sensitivity to temperature in European trees: do within-species populations exhibit similar responses? Agric. For. Meteorol. 149, 735–744. doi: 10.1016/j.agrformet.2008.10.019.

Voroney, R.P., Brookes, P.C., and Beyaert, R. P. (2006). “Soil microbial biomass C, N, P, and S.” in: Soil Sampling and Methods of Analysis (2nd ed.). eds. Carter, M. and Gregorich, E. Canadian Society of Soil Science, Taylor and Francis, chapter 49.

Wang, Q., Garrity, G.M., Tiedje, J.M, and Cole, J.R. (2007). Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73(16): 5261–67.

Weemstra, M., Mommer, L., Visser, E.J.W., van Ruijven, J., Kuyper, T.W., Mohren, G.M.J. and Sterck, F.J. (2016). Towards a multidimensional root trait framework: a tree root review. New Phytol. 211: 1159-1169. doi:10.1111/nph.14003.

Weemstra, M., Sterck, F. J., Visser, E. J. W., Kuyper, T. W., Goudzwaard, L., and Mommer, L. (2016). Fine-root trait plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on two contrasting soils. Plant Soil 415, 175–188.doi: 10.1007/s11104-016-3148-y.

Weemstra, M., Sterck, F.J., Visser, E.J.W., Kuyper, T.W., Goudzwaard, L., and Mommer, L. (2017). Fine-root trait plasticity of beech (Fagus sylvatica) and spruce (*Picea abies*) forests on two contrasting soils. Plant Soil 415, 175–188. doi.org/10.1007/s11104-016-3148-y.

Weintraub, M.N. (2011). “Biological phosphorus cycling in Arctic and Alpine soils”. in: Bünemann, E.K., Oberson, A., Frossard, E. (eds.), Phosphorus in Action. Biological processes in soil phosphorus cycling. Soil Biology 26, 295-316.

Wilcox, J. R., Premachandra, G. S., Young, K. A., and Raboy, V. (2000). Isolation of high seed inorganic P, low-phytate soybean mutants. Crop Sci. 40, 1601–1605.

Williamson, L.C., Ribrioux, S.P.C.P., Fitter, A.H, and Leyser, H.M.O. (2001). Phosphate availability regulates root system architecture in *Arabidopsis*. Plant Physiol. 126(2): 875–82.

Xuan, W., Beeckman, T., and Xu, G. (2017). Plant nitrogen nutrition: sensing and signaling. Curr. Opinion Plant Biol. 39, 57-65. doi.org/10.1016/j.pbi.2017.05.010.

Yan, G., Chen, F., Zhang, X., Wang, J., Han, S., Xing, Y., and Wang, Q. (2017). Spatial and temporal effects of nitrogen addition on root morphology and growth in a boreal forest. Geoderma 303, 178-187.

Yan, X.-L., Wang, C., Ma, X., and Wu, P. (2019). Root morphology and seedling growth of three tree species in southern China in response to homogeneous and heterogeneous phosphorus supplies. Trees 33, 1283-1297. doi.org/10.1007/s00468-019-01858-x.

Yang, Ch. and Crowley, D.E. (2000). Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Appl. Environ. Microbiol. 66(1): 345–51.

Yang, N., Zavišić, A., Pena, R., and Polle, A. (2016). Phenology, photosynthesis, and phosphorus in European beech (*Fagus sylvatica* L.) in two forest soils with contrasting P contents. J.Plant Nutr. Soil Sci. 179, 151–158. doi.org/10.1002/jpln.201500539.

Yang, X., Post, W. M., Thornton, P. E., and Jain, A. (2013). The distribution of soil phosphorus for global biogeochemical modeling. Biogeosciences. 10, 2525–2537.doi: 10.1016/j.chemosphere.2011.02.039.

Yuan, H. and Liu, D. (2008). Signaling components involved in plant responses. J. Integr. Plant Biol. 50(7): 849–59.

Zavišić, A., Nassal, P., Yang, N., Heuck, C., Spohn, M., et al. (2016). Phosphorus availabilities in beech (*Fagus sylvatica* L.) forests impose habitat filtering on ectomycorrhizal communities and impact tree nutrition. Soil Biol Biochem. 98: 127–37.

Zavišić, A., and 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 Physiol. 38, 37–51. doi: 10.1093/treephys/tpx146.

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

Zelenev, V. V., Van Bruggen, A. H. C., and Semenov, A. M. (2005). Modeling wave-like dynamics of oligotrophic and copiotrophic bacteria along wheat roots in response to nutrient input from a growing root tip. Ecol Modell. 188(2–4): 404–17.

Zemunik, G., Turner, B.L., Lambers,H., and Laliberté, E. (2015). Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. Nature Plants 1(April): 1–4.

Zhang, Y., Zhou, Z., and Yang, Q. (2013). Genetic variations in root morphology and phosphorus efficiency of *Pinus massoniana* under heterogeneous and homogeneous low phosphorus conditions. Plant Soil 364, 93-104. doi.org/10.1007/s11104-012-1352-y.

Zhou, X.-B., Jia, Z.-M., and Wang, D.-B. (2018). Effects of limited phosphorus supply on growth, root morphology and phosphorus uptake in citrus rootstocks seedlings. Int. J. Agric. Biol. 20, 431-436. doi.org/10.17957/IJAB/15.0553.

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