Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com

Temporal variations of phosphorus uptake by soil microbial biomass and young beech trees in two forest soils with contrasting phosphorus stocks

Marie Spohn[a, *, 1], Aljoša Zavišić[b, c, 1], Pascal Nassal[d], Fabian Bergkemper[e], Stefanie Schulz[e], Sven Marhan[d], Michael Schloter[e], Ellen Kandeler[d], Andrea Polle[b, c]

[a] Soil Biogeochemistry, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Germany
[b] Forest Botany and Tree Physiology, Georg-August University of Göttingen, Germany
[c] Laboratory for Radio-Isotopes, Georg-August University of Göttingen, Germany
[d] Institute of Soil Science and Land Evaluation, Soil Biology Department, University of Hohenheim, Stuttgart, Germany
[e] Research Unit Comparative Microbiome Analysis, Helmholtz Zentrum München, Germany

ARTICLE INFO

Keywords:
Forest phosphorus nutrition
Phosphorus uptake kinetics
Plant-microbe interactions
Ectomycorrhizal community
Seasonal dynamics
Phosphatase activity

ABSTRACT

The objective of this study was to determine temporal variations of phosphorus (P) uptake by young beech trees (*Fagus sylvatica* L.) and soil microorganisms in two forests with contrasting P stocks with the aim to better understand P dynamics in forest ecosystems. For this purpose, we conducted a mesocosm experiment and determined P uptake by *F. sylvatica*, total soil microbial biomass (SMB) and ectomycorrhizal fungi (EMF) at the root tip based on ^{33}P labeling at five times during the year. Furthermore, we measured EMF community composition, potential acid phosphatase activity (APA), and abundance of bacterial acid phosphatase (*phoN*) genes. The results showed that plant P uptake was elevated in summer and autumn in the mesocosms from the P-poor site, while it was elevated only in autumn in the mesocosms from the P-rich site. P uptake by SMB was higher in the organic layer at the P-poor site than in the organic layer at the P-rich site throughout the year, underlining the importance of the microbial P pool in the organic layer of P-poor forests. The finding shows that the SMB was able to compensate for the lower P availability in the soil of the P-poor site. The EMF community composition was very variable over the year, and plant P uptake seemed to be independent of EMF community composition. Despite the high species turnover in the EMF community, the potential APA was high throughout the year, indicating functional redundancy of the microbial community with respect to P mineralization. Taken together, our results show important differences in temporal patterns of P uptake by *F. sylvatica* and the SMB as well as in the total partitioning of P between the SMB and *F. sylvatica* across the sites. Moreover, decreasing P availability in forests would not only change the size of P stocks and of P cycling rates, but would also affect temporal dynamics of P uptake and the overall partitioning of P between different biotic compartments.

1. Introduction

Despite the importance of phosphorus (P) for plant nutrition, little is known about the temporal patterns of plant P uptake in forest ecosystems and about the factors that control them (Vance et al., 2003; Plassard and Dell, 2010). During the last decades, foliar P concentrations of several tree species in temperate forests have decreased, and the reasons for this decrease are not yet known (Flückiger and Braun, 1998; Duquesnay et al., 2000; Ilg et al., 2009; Braun et al., 2010; Crowley et al., 2012; Jonard et al., 2015; Talkner et al., 2015). This calls for a better understanding of P dynamics in the interplay of soil, ectomycorrhizal fungi (EMF), soil microbial biomass (SMB), and plant and microbial activities in temperate forests.

P availability in soil is largely affected by sorption (Hinsinger, 2001; Giesler et al., 2002). Since phosphate is rapidly sorbed to the soil matrix, only a small proportion of the total soil P is plant available. Thus, plants, bacteria and especially EMF have developed several mechanisms to solubilize bound inorganic P by releasing organic acid anions, protons and siderophores (Jones and Oburger, 2011; Jansa et al., 2011; Smits et al., 2012). Furthermore, they can mineralize organic

* Corresponding author. Dr. Hans-Frisch-Str. 1-3, 95448 Bayreuth, Germany.
Email address: marie.spohn@uni-bayreuth.de (M. Spohn)
[1] Shared first authorship.

https://doi.org/10.1016/j.soilbio.2017.10.019
Received 27 June 2017; Received in revised form 19 October 2017; Accepted 20 October 2017
Available online xxx
0038-0717/

P by releasing extracellular phosphatases, which renders P plant available (Plassard and Dell, 2010; Nannipieri et al., 2011). Microbial organic phosphorus mineralization in the vicinity of the root can increase the plant available inorganic P concentration (Richardson et al., 2009; Spohn et al., 2013). Besides mobilizing P, EMF can be very efficient in P uptake from soil because their hyphae reach micropores that are not accessible to roots and have a very high surface area-to-volume ratio (Jansa et al., 2011).

Only few studies explored P uptake kinetics of ectomycorrhizal-forming tree species (Van Tichelen and Colpaert, 2000; Brandtberg et al., 2004; Jonard et al., 2009; Desai et al., 2014; Kavka and Polle, 2016). Tracer studies with ^{33}P showed that P uptake systems of non-mycorrhizal roots are limited because their Michaelis-Menten constants (K_m) are higher than typical concentrations of free P_i in the soil solution (Van Tichelen and Colpaert, 2000; Desai et al., 2014; Kavka and Polle, 2016). In mycorrhizal trees, the K_m is strongly decreased and the uptake rate drastically enhanced (Van Tichelen and Colpaert, 2000; Desai et al., 2014), which underpins the relevance of EMF for plant P uptake. It is not known yet, whether high plant P uptake is associated with a specific EMF community or whether there is a functional redundancy in EMF communities with respect to P mobilization. Moreover, since previous experiments (Van Tichelen and Colpaert, 2000) were conducted in hydroponic solutions in the absence of soil bacteria and saprotrophic fungi, which may strongly affect tree nutrient uptake, the environmental factors that influence P acquisition of EMF and their host trees are still unknown.

Microbial biomass P can represent a substantial fraction of the total soil P. In temperate coniferous and broadleaf forests it amounts on average to 4.3 and 8.6% of the total P in the mineral soil, respectively (Xu et al., 2013). In the organic layer of beech forests, about 22–47% of the total P is sequestered in the microbial biomass (Zederer et al., 2017). Especially in relatively nutrient poor temperate forests with the humus form moder, a large proportion of the total soil P is stored in the SMB (Zederer et al., 2017).

While there are only few studies that explored P uptake kinetics of trees and microorganisms using ^{33}P, uptake kinetics have been studied more intensively for nitrogen (N) using ^{15}N. In many studies, in which N uptake by trees and SMB was compared, it was found that initially the SMB took up a significantly larger percentage of the added ^{15}N than the tree. This was documented for *Acer saccharum* in northern hardwood forests (Zogg et al., 2000), for *Quercus douglasii* in California (Cheng and Bledsoe, 2004), for birch forests in subarctic Sweden (Grogan and Jonasson, 2003), for *Fraxinus excelsior* in France (Bloor et al., 2009), and for *Fagus sylvatica* in Germany (Pena et al., 2013; Leberecht et al., 2015; Dannenmann et al., 2016). ^{15}N immobilized in the SMB was only very slowly released during the following months (Zogg et al., 2000; Grogan and Jonasson, 2003).

In ecosystems with pronounced seasonality, such as temperate and alpine ecosystems, plant N uptake and microbial N uptake are often anticyclical in the way that plants take up N mostly during the growing season, while microbial N uptake is highest in autumn, stimulated by high inputs of leaf litter during this time of the year (Jaeger et al., 1999; Lipson et al., 1999; Kaiser et al., 2011). At the end of winter, microbial N decreases again due to thawing-and-freezing events that induce microbial cell lysis (Jaeger et al., 1999; Lipson et al., 1999; Kaiser et al., 2011). It is not known yet, whether such dynamics also occur in temperate forest soils with respect to P uptake.

The objective of this study was to determine temporal variations of P uptake by SMB and by young beech trees (*Fagus sylvatica*) along with the root-associated EMF assemblage, potential acid phosphatase activity (APA) and abundance of the bacterial acid phosphatase (*phoN*) genes in two forests differing in total P stocks. For this purpose, young beech trees were extracted with intact soil cores from two forest sites that differ in total soil P stocks and P availability (Zavišić et al., 2016), and were exposed to ambient conditions in a common garden study. We hypothesized, first, that uptake of P by beech trees is higher in summer, when the trees are photosynthetically more active than in autumn (Yang et al., 2016), while P uptake by the SMB is highest in autumn due to inputs of plant detritus during this time of the year. Second, we hypothesized that a larger proportion of P is taken up by the SMB in the P-poor forest compared to the P-rich forest throughout the year. Third, we hypothesized that P uptake by *F. sylvatica* is independent of specific EMF species due to a high diversity and functional redundancy of EMF species.

2. Materials and methods

2.1. Study site

Soils and juvenile trees were collected at two sites with contrasting soil P availability (Table 1). The site Bad Brueckenau (BBR) that has a high soil P availability is located in the Rhoen Mountains, close to the city of Fulda, Germany (N 50° 21.38′, E 9° 55.71′) at 825 m above sea-level. The mean annual rainfall is 1031 mm and the mean annual temperature is 5.8 °C. The soil is a Dystric Skeletic Cambisol derived from basalt, and the prevailing tree species is European beech (*Fagus sylvatica* L.). The site Luess (LUE) that has a low soil P availability is located in the Lueneburg Heath, close to the city of Hamburg, Germany (N 52° 50.32′, E 10° 16.06′) at 115 m above sea-level. The mean annual rainfall amounts to 730 mm and the mean annual temperature is 8 °C. The soil is a Hyperdystric Folic Cambisol developed from sandy Pleistocene sediments, and the dominant tree species is also *Fagus sylvatica* L. More details have been reported by Zavišić et al. (2016).

Table 1
Properties of the soils of the P-poor site LUE and the P-rich site BBR together with the soil chemical features of soil in the mesocosms from the two sites, and the average soil mass, fine root abundance, and water content during the experiment. Different letters indicate significant differences ($p < 0.05$) in soil chemical parameters between all four soil layers.

	Luess (LUE)		Bad Brueckenau (BBR)	
Soil order (WRB)	Cambisol		Dystric Skeletic Cambisol	
Parent material	Glacial sands		Basalt	
Humus form	Mor-like Moder		Mull-like Moder	
Depth of the organic layer [cm]	12		7	
	Organic layer	Mineral soil	Organic layer	Mineral soil
Dry weight per mesocosm [g]*	276 (±133)	1226 (±391)	180 (±76)	788 (±184)
Relative fine root abundance×	0.64 (±0.05)	0.36 (±0.05)	0.32 (±0.02)	0.68 (±0.03)
Average water content [%]*	18.8 (±4.6)	50.3 (±4.6)	33.9 (±6.7)	42.4 (±10.8)
Total C [g kg^{-1}]×	223.6 (±19.1)^{c}	48.4 (±4.9)^{a}	141.5 (±7.6)^{b}	62.7 (±2.7)^{a}
Total N [g kg^{-1}]×	8.96 (±0.76)^{c}	1.75 (±0.19)^{a}	7.88 (±0.36)^{c}	3.79 (±0.12)^{b}
Total P [g kg^{-1}]*	0.5 (±0.2)^{b}	0.1 (±0.0)^{a}	2.8 (±0.3)^{c}	2.8 (±0.4)^{c}
Labile P [mg kg^{-1}]*	52.7 (±37.8)^{b}	5.9 (±4.2)^{a}	120.0 (±81.9)^{c}	11.9 (±37.8)^{a}
Soil pH_{H2O}×	4.3^{b}	3.8^{a}	5.1^{c}	4.3^{b}

*n = 100, ×n = 5.

2.2. Sampling and experimental setup

Young beech trees of 0.3–0.4 m height were excavated on the sites BBR and LUE at the end of October 2013. For this purpose, polymer tubes with a diameter of 12 cm and a height of 20 cm were hammered into the soil around the young tree and subsequently extracted with the plant and the intact soil core. In total, 150 tubes with young beech trees and soil from each forest site (Table 1) were transported to the Forest Botanical Garden of the University of Goettingen, where they were kept outdoors, under natural climatic conditions throughout the experiment (for air temperature and soil water content during the experiments see Additional online material Fig. 1). In order to avoid confounding effects of position within the common garden, the mesocosms with the beech seedlings were randomly interspersed. The position of all mesocosms were changed randomly on a weekly basis throughout the experiment. Because root damage could not be entirely excluded, when harvesting field-grown young trees, the beech trees in the tubes were left undisturbed for six months before using them for experiments.

2.3. Labeling, harvest and biomass

At five dates during the first year, which corresponded to five phenological stages as described by Yang et al. (2016), 15 mesocosms from each of the two sites were labeled with ^{33}P. Additionally, another 15 mesocosms from each site were labeled in the following year in summer, leading to a total of six labeling dates; spring (April 2nd, 2014): bud swelling; early summer (May 12th, 2014): young leaves, late summer (July 21st, 2014): mature leaves; fall (September 22nd, 2014): leaf senescence; and winter (February 9th, 2015): dormancy (no leaves), and additionally, a second late summer (July 16, 2015). For this purpose, 1912 kBq carrier free $H_3{}^{33}PO_4$ (Hartmann Analytic GmbH, Braunschweig, Germany) was added in 40 ml of tap water, amounting to a total of 0.017 nmol P per plant. It should be noted that the amount of P added is negligible, and ^{33}P served only as a tracer without affecting the P availability. Subsequently, each plant was watered with 40 ml tap water in order to distribute the tracer throughout the soil core. To avoid loss of label via leaching, a plastic saucer was placed underneath each open soil core and if leachate appeared it was immediately re-applied on the soil. The homogeneous distribution of the tracer was previously tested with mesocosms from both sites using the dye coomassie blue. During each experiment, 20 mesocosms from each site were destructively harvested. Five non-labeled mesocosms of each site were destructively sampled at the day of tracer application. Labeled mesocosms of each forest site were destructively sampled 1, 7 and thirty days after labeling (n = 5 mesocosms per time point and site). Since there were six labeling dates and two sites, a total of 240 mesocosms were destructively harvested in this study. For this purpose, the plant with the soil core was completely removed from the tube. The organic layer and the mineral soil of each mesocosm were separated and weighed. Subsequently, the stem was cut at the shoot-root junction and the roots were carefully removed from the soil to avoid damaging EMF. The mineral soil and the organic layer were kept separately and each fraction was thoroughly mixed. Leaves, buds, coarse roots and fine roots (< 2 mm diameter) were separated using scissors, and an aliquot of the plant material was flash frozen and stored at −80 °C. The remaining plant material was oven-dried at 40 °C for one week. Fine roots were dried after morphotyping. Fine root distribution was determined for selected samples that were not included in other analyses. For this purpose soil cores were cut at the border of the mineral soil and organic layer, all roots per soil layer were carefully removed, separated according to coarse and fine roots and dried at 40 °C for one week. Total dry weight of all plant components (leaves, buds, stem, coarse roots, and fine roots) and mineral and organic soil was measured. An aliquot of each fresh soil fraction was stored at −80 °C for phosphatase activity and *phoN* gene abundance measurements. Further fresh soil aliquots were used for determination of microbial biomass C and P, labile soil P, soil moisture and dry mass. The mass of the ectomycorrhiza was determined as a mean based on 150 vital mycorrhizal root tips from six soil cores from each of the two sites. For this purpose, the tips were cut directly at the fungus mantle (as described in Valtanen et al., 2014). The 150 mycorrhizal tips of each soil core were dried and weighed together. The weight of one ectomycorrhiza was calculated as a mean based on the six independent replicates each calculated from 150 samples.

2.4. Ectomycorrhizal species composition

Vital and dead roots tips of each plant were identified by their turgescent or dry and shrunken appearance under a dissecting microscope (Leica M205 FA; Leica, Wetzlar, Germany). Labeling studies with ^{15}N showed that dry and shrunken root tips were physiologically inactive (Pena and Polle, 2014). Therefore these root tips were considered as dead root tips. Vital and dead root tips were counted. Ectomycorrhizal morphotypes were identified based on color, shape, branching pattern, mantle surface texture, hyphal morphology and rhizomorph connection/shape according to Agerer (2006). Each morphotype was collected (n = 5–20 root tips) and stored at −20 °C. Total genomic DNA was isolated from the morphotypes using the innuPREP Plant DNA Kit (Analytik Jena AG, Jena, Germany) as described by the manufacturer. The rRNA ITS-region was amplified by the polymerase chain reaction (PCR) with the primer pair ITS1F and ITS4 (Eurofins MWG Operon, Ebersberg, Germany) (White et al., 1990) using either the original genomic DNA sample or a dilution (1:10 or 1:100) to remove inhibitors of the PCR as described previously (Lang and Polle, 2011). Purification of all PCR products was done by addition of 35 µl 2-propanol (Roth, Karlsruhe, Germany). DNA was subsequently precipitated for 1 h at room temperature, and centrifuged for 30 min at 20,000×*g* (Centrifuge 5417R, Eppendorf, Hamburg, Germany). The obtained pellet was fully dehydrated for 10 min at 50 °C (Concentrator 5301, Eppendorf, Hamburg, Germany) and dissolved in 30 µl ultrapure H_2O (Applichem GmbH, Darmstadt, Germany). The purified DNA was used for Sanger sequencing (Applied Biosystems 3730XL DNA Analyzer, Seqlab GmbH, Göttingen, Germany). Sequence alignment was done using the Staden Package software (4.10, http://staden.sourceforge.net) and blasted in the NCBI GenBank. With a sequence homology of > 97% and a score > 900 bits, the name suggested by the database was accepted. Sequences were deposited in the NCBI Genbank with the accession numbers KX168637-KX168665 (see also Additional online material Table 1).

2.5. Soil microbial biomass, labile P and total C and N

Soil microbial biomass C and P (SMB_C and SMB_P), comprising C and P of archaea, protozoa, bacteria as well as saprotrophic and mycorrhizal fungi, were determined in fresh soil using the chloroform fumigation extraction method (Brookes et al., 1982; Vance et al., 1987). 10 g of fresh mineral topsoil and 5 g of organic layer were fumigated with chloroform for 24 h. P was extracted from the fumigated and the non-fumigated soil in Bray-1 solution (0.03 M NH_4F - 0.025 M HCl) and quantified by the molybdate blue assay using an injection flow photometer (FIA-LAB, MLE Dresden, Germany). A conversion factor of 2.5 was used to calculate SMB_P (Brookes et al., 1982). The inorganic P concentration in the Bray-1 extracts of the non-fumigated soils (see above) were considered the labile P fraction. C was extracted from the

fumigated and the non-fumigated soil in 40 ml 0.5 M K_2SO_4 and were measured using a TOC/TN analyzer (Multi N/C 2100S, Analytik Jena AG, Jena, Germany). A conversion factor of 2.2 was used to calculate SMB_C (Joergensen, 1996). Total C and N were determined using a CNS elemental analyzer (EA1108, Carbo Erba Strumentazione, Rodano, Italy).

2.6. ^{33}P activity

Plant components (leaves, buds, stem, coarse roots, and fine roots) and EMF samples were milled (Retsch MN 400, Haan, Germany) and extracted in 65% HNO_3 at 160 °C for 12 h according to Heinrichs et al. (1986). Total P in the extracts was measured by ICP-OES (iCAP 6000 Series ICP–OES, Thermo Fisher Scientific, Dreieich, Germany). ^{33}P in all HNO_3 and in Bray-1 extracts was determined by a Perkin-Elmer scintillation counter (Tri-Carb TR/SL 3180, Waltham, MA, USA), using a scintillation cocktail (Rotiszint eco plus, Roth, Karlsruhe, Germany). The signal was corrected for radioactive decay, and a background correction value was subtracted that was measured using only scintillation cocktail and extraction solution.

2.7. Potential acid phosphatase activity (APA)

Potential APA that is produced by plants and soil microorganisms (including EMF) was measured using a modified disodium phenylphosphate method. For this purpose, 5 g of soil was incubated in 10 ml of acetate buffer (pH 5) and 5 ml of 20 mM buffered disodium phenylphosphate solution at 37 °C for 3 h; the release of phenol was determined colorimetrically at 614 nm, using 2,6-dibromchinone-chlorimide as coloring reagent (Hoffmann, 1968; modified by Öhlinger, 1996).

2.8. Abundance of phoN genes

Total genomic DNA was extracted from the soil of day 0 of each labeling experiment using the "NucleoSpin Soil Kit" (Macherey-Nagel, Germany) according to the manufacturer's protocol. Quantification of the bacterial *phoN* gene was performed using quantitative real-time PCR and target specific primers *phoN*-FW and *phoN*-RW (Bergkemper et al., 2016) on a 7300 Real-Time PCR System (Applied Biosystems, Germany) together with SybrGreen (Life Technologies, USA) as fluorescent dye. After each qPCR run a melting curve analysis was performed to verify the amplicon specificity. The quantification of the target gene was conducted by using serial dilutions of plasmid encoded *phoN* genes (10^7 to 10^1 gene copies μl^{-1}) derived from *Salmonella enterica* DSM 10062. Based on a previous dilution test all samples were diluted in a 1:16 ratio to avoid inhibition during amplification caused by co-extracted humic substances. The efficiency of the qPCR was calculated as: Efficiency (%) = $[10^{(-1/slope)} - 1]$ and was always above 86% while the R^2 of the standard curve was above 0.99.

2.9. Calculations and statistical analyses

We calculated the total ^{33}P taken up by the plant for distinct points in time as follows.

$$Total\ 33P_{Plant}\ (Bq) = Total\ biomass_{Leaves}\ (g) \times 33P_{Leaves}\ 33P_{Stem}\left(Bq\ g^{-1}\right) + Total\ biomass_{Coase\ roots}\ (g) \times 33P_{Coarse\ root}\ 33P_{Fine\ roots}\left(Bq\ g^{-1}\right) + Total\ biomass_{\ Buds}\ (g)$$

The ^{33}P enrichment of the plant biomass was calculated as follows.

$$33P\ enrichment\ \left(Bq\ (g\ biomass)^{-1}\right) = \frac{Total\ 33P_{Plant}\ (Bq)}{Plant\ biomass\ (g)}$$

The total ^{33}P of EMF in one mesocosm was calculated based on the ^{33}P enrichment of the EMF biomass as follows.

$$Total\ 33P_{EMF}\ (Bq) = 33P_{EMF}\ \left(Bq(g\ biomass)^{-1}\right) \times Biomass\ o$$
$$Number\ mycorrhizal\ root\ tips$$

The ^{33}P enrichment of the SMB per gram dry soil was calculated separately for the mineral soil and the organic layer.

$$33P_{SMB}\left(Bq\ g^{-1}\ soil\right) = 33P_{Fumigated\ soil}\left(Bq\ g^{-1}\ soil\right) - 33P_{Non-fumigated\ soil}\left(Bq\ g^{-1}\ soil\right)$$

The total amount of ^{33}P taken up into the SMB in one mesocosm was calculated separately for the mineral soil and the organic layer as follows.

$$Total\ 33P_{SMB}\ (Bq) = 33P_{SMB}\left(Bq\ g^{-1}\ soil\right) \times Dry\ mass_{Soil\ mesocom}\ (g)$$

Total ^{33}P in the labile P fraction was calculated separately for the mineral soil and the organic layer for the harvest thirty days after ^{33}P application as follows.

$$Total\ 33P_{Labile\ P\ fraction}\ (Bq) = 33P_{Labile\ P\ fraction}\left(Bq\ g^{-1}\ soil\right) \times Dry\ mass_{Soil\ mesocom}\ (g)$$

Total P taken up by SMB, EMF and plants during thirty days was calculated taking into account the specific ^{33}P activity of the bioavailable soil P pool. This approach has been applied in many previous radiophosphorus labeling studies for calculating P mobilization by plants and soil microorganisms (Fardeau et al., 1995; Morel and Fardeau, 1989; Bünemann et al., 2007; Oberson et al., 2010; Schneider et al., 2017), and it corrects for the different dilutions of the added ^{33}P with P present in soil. The approach was applied separately for the organic layer and for the mineral soil.

The total amount of P taken by the SMB was calculated separately for the organic layer and for the mineral soil as follows:

$$P\ taken\ up\ by\ SMB\ (\mu g) = Total\ 33P\ in\ SMB\ (Bq)\ /\ Specific\ 33P\ activity\ \left(Bq\ \mu g\ P^{-1}\right)$$

Specific ^{33}P activity of a soil P pool is defined as follows

$$Specific\ 33P\ activity\ \left(Bq\ \mu g\ P^{-1}\right) = \frac{33P\ \left(Bq\ g^{-1}\ soil\right)}{P\left(\mu g\ g^{-1} soil\right)}$$

Total P taken up by *F. sylvatica* during thirty days was calculated based on the relative P availability and the relative root abundance in the mineral soil and the organic layer as follows.

$$\text{Total P taken up by plant } (\mu g\ P) = \text{Total } 33P_{Plant}\ (Bq) \times \Big(\big(\text{Specific activity in labile P fraction in } \ldots \text{(relative P availability in organic layer + relative root abuna} \Big(\text{Specific activity in labile P fraction in mineral so} \ldots \text{(relative P availability in mineral soil + relative root abunda}$$

where

$$\text{Relative P availability}_{Organic\ layer} = \frac{\text{Labile P}_{organic\ layer}}{\left(\text{Labile P}_{organic\ layer} + \text{Labile P}_{Mineral\ soil}\right)}$$

and

$$\text{Relative root abundace}_{Organic\ layer} = \frac{\text{DW of fine roots}_{Organic\ layer}}{\left(\text{DW of fine roots}_{Organic\ layer} + \text{DW of fine roots}_{Mineral\ soil}\right)}$$

The relative P availability in the mineral soil and the relative root distribution in the mineral soil were calculated accordingly. The total P taken up by EMF was calculated in the same way as the total P uptake by the plant, i.e. by taking into account the relative P availability and the root abundance both in the mineral soil and in the organic layer.

Homogeneity of variance was tested by the Levene-test. Differences between sites and dates or seasons were tested by ANOVA followed by Scheffé post-hoc test using R (R Core Team, 2013). We used the software package PAST 3.08 (http://folk.uio.no/ohammer/past/, Hammer et al., 2001) to calculate Shannon index (H), Simpson index, species richness and evenness of the EMF. To achieve species saturation, the data of the root systems of five plants sampled per time point, soil type and season were pooled. To compare the diversity of EMF in different soil types, all values obtained across the whole year were compared by a paired rank test. The similarity of the EMF community structures was analyzed by non-metric multidimensional scaling (NMDS) using Bray Curtis as the similarity measure.

3. Results

3.1. EMF, SMB and labile P

The number of root tips colonized by EMF was lower in the mesocosms from the P-poor site LUE than in the mesocosms from the P-rich site BBR throughout the year ($p < 0.001$; Fig. 1a). The community composition of EMF taxa showed seasonal fluctuations indicated by the separation along coordinate 1 of the NMDS (Fig. 2). The communities in spring and early summer formed one cluster, while a second clustered was formed by the communities in late summer, autumn, and winter (Fig. 2, Additional online material Table 2). The communities from LUE and BBR differed significantly in their structure shown by the separation along coordinate 2 (Fig. 2, Additional online material Table 2). This separation was due to higher EMF species richness on the roots from BBR than from LUE, and divergent composition of the assemblages (Additional online material Table 3). In the EMF assemblages of BBR, *Xerocomellus pruinatus*, *Tuber* sp, *Tomentellopsis* sp. and *Clavulina coralloides* were dominant, whereas in LUE *Russula ochroleuca*, *Lactarius blennius*, *Scleroderma citrinum*, and *Genea* cf. *anthracina* dominated. *Cenococcum geophilium* was the only species that formed a symbiosis with the trees from both sites throughout the year (Additional online material Table 1).

Concentrations of labile P were higher in the organic layer than in the mineral soil in the mesocosms from both forest sites ($p < 0.001$; Fig. 1b). While there was no significant difference in the labile P concentration in the mineral soil between the sites ($p > 0.05$), the labile P concentration in the organic layer was on average 2.4-times higher ($p < 0.01$) in the mesocosms from BBR than in the mesocosms from LUE throughout the year (Fig. 1b). Concentrations of labile P showed large variations in the organic layer between the mesocosms from one site, and no clear seasonal trend was observable neither in the organic layers nor in the mineral soils.

The SMB_C concentration was higher in the organic layer than in the mineral soil of both sites ($p < 0.01$). In the mineral soil, the SMB_C concentration at BBR was higher than at LUE throughout the year ($p < 0.01$; Fig. 1c). No significant seasonal variation in SMB_C in the mineral soil and in the organic layer of both sites was found ($p > 0.05$; Fig. 1c). In the organic layer, the SMB_C was significantly ($p < 0.05$) correlated with the gravimetric water content across seasons ($R^2 = 0.30$ and 0.25 in LUE and BBR, respectively).

3.2. ^{33}P enrichment and recovery

The young beech trees from the P-rich site BBR showed similar ^{33}P enrichment during all seasons, except in autumn when the highest ^{33}P enrichment was found ($p < 0.01$, Fig. 3a). In contrast, ^{33}P uptake by the young beech trees from the P-poor site LUE showed more pronounced seasonal dynamics (Fig. 3a). The plants from LUE exhibited increased ^{33}P enrichment in early summer compared to spring ($p < 0.01$), and they further increased the ^{33}P enrichment during late summer ($p < 0.01$). ^{33}P enrichment in EMF was fast during the first seven days after tracer addition and slowed strongly afterwards in the mesocosms from both sites (Fig. 3b). The same trend was observed for ^{33}P uptake by the SMB in the mineral soil and especially in the organic layer (Fig. 3c and d). The ^{33}P uptake per unit SMB was similar at both sites (data not shown). The dynamics in the ^{33}P uptake by plants and EMF observed in late summer, were confirmed in the second year. However, the ^{33}P uptake in late summer by SMB proceeded more evenly in the second year compared to the first year (Additional online material, Fig. 2).

Taking into account the soil dry mass in the mesocosms, in total more ^{33}P was recovered from the organic layer than from the mineral soil in LUE than in BBR, whereas the mineral soil sequestered more ^{33}P in BBR than in LUE (Fig. 4 and Additional online material Fig. 3). The total amount of ^{33}P recovered from the plant, the SMB and the labile P pool in the organic layer and in the mineral soil together, was quite stable across all five experiments (Fig. 4), and amounted on average to 44.4 and 23.4% of the initially added tracer in LUE and BBR, respectively. The largest proportion of added ^{33}P was stored in abiotic soil P fractions (63.6% in BBR and 76.6% in LUE), and was not recovered in the labile P fraction or in the biotic pools that were extracted (Fig. 4).

The specific ^{33}P activity of the labile P pool was slightly higher in LUE than in BBR both in the organic layer and in the mineral soil throughout the year (Additional online material Fig. 4). It was also larger in spring and in winter than in early and late summer (Additional online material Fig. 4), which is important for the calculation of the total P uptake.

3.3. Total P uptake

Total P taken up by the beech trees over thirty days in late summer amounted to 15.10 mg in the mesocosms from LUE and was significantly higher than P uptake by the young beech trees from BBR during the same time (1.75 mg in thirty days, Fig. 5). However, in autumn, total plant P uptake over thirty days was similar in LUE and BBR and amounted to 10.04 and 13.13 mg P per plant, respectively (Fig. 5). P uptake by EMF collected from the fine roots did not differ significantly

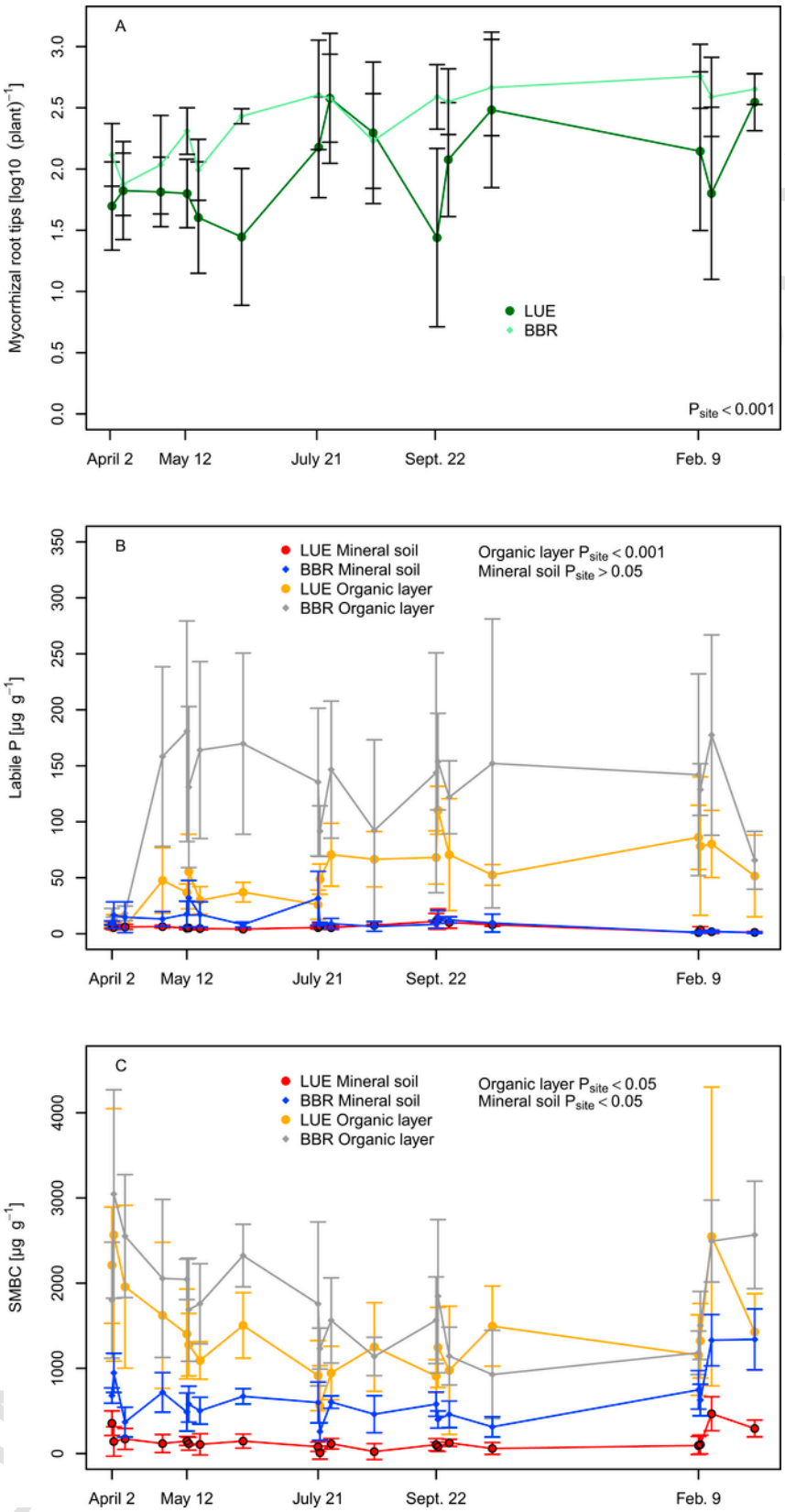

Fig. 1. Mycorrhizal root tips (A), labile inorganic P (B) and the soil microbial biomass C (C) from the P-poor (LUE) and the P-rich (BBR) site during all five labeling experiments. The number of mycorrhizal root tips was log10 transformed to meet the requirements of normal distribution. $P_{sites} < 0.05$ indicates significant differences between sites. Each data point was calculated based on five mesocosms (n = 5). Error bars show standard deviations.

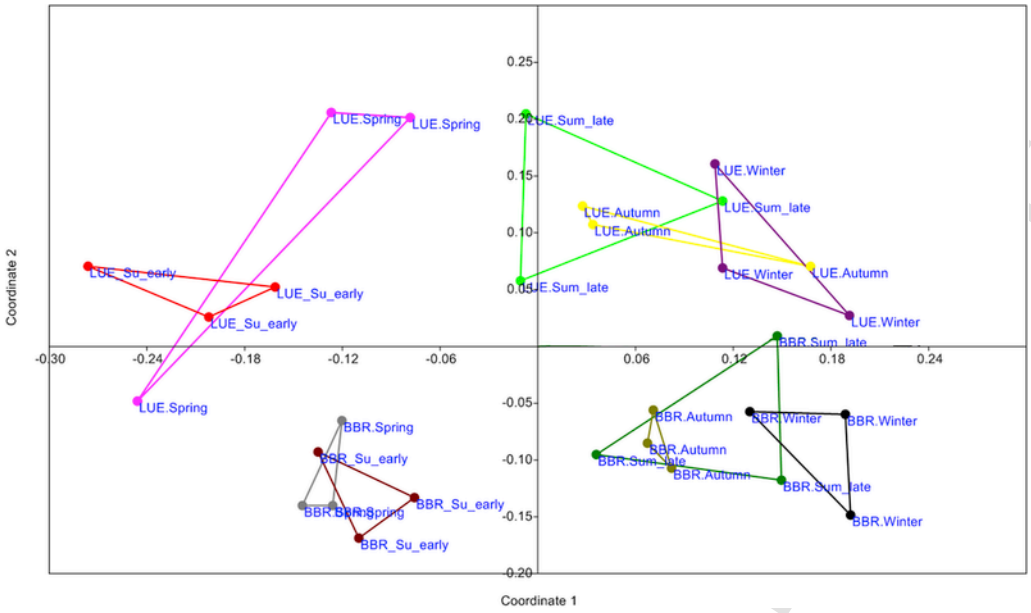

Fig. 2. Non-metric multidimensional scaling of the ectomycorrhizal fugal communities with Bray Curtis as similarity index. Su stands for summer.

between sites and seasons ($p > 0.05$). In autumn the uptake was increased at both sites, but this was not statistically significant due to variation between the mesocosms (Fig. 5). The net amount of new P taken up during thirty days by EMF collected at the fine roots did not exceed 0.02 mg P per mesocosm during most of the year, and only increased to 0.20 and 0.25 mg P per mesocosm, in LUE and BBR respectively, in autumn (Fig. 5). However, it has to be taken into account that we only measured P in EMF directly at the root, thus, the total amount of P taken up by the total EMF biomass is very likely much larger.

The total amount of P taken up over thirty days by the SMB in the organic layer in LUE was significantly larger ($p < 0.05$) than in BBR in early summer, late summer and autumn. The amount of P taken up by the SMB in the organic layer and in the mineral soil together was higher than the amount of P taken up by the plant throughout the year (Fig. 5). The amount of P taken up by the SMB in the mineral soil both in mesocosms from LUE and BBR did not differ significantly between the seasons ($p > 0.05$), whereas we found seasonal differences for the organic layer ($p < 0.01$). In LUE, P uptake by SMB in the organic layer was significantly larger in late summer than in winter ($p < 0.01$). In BBR, P uptake by SMB in the organic layer was significantly higher in spring than in winter ($p < 0.01$).

3.4. Acid phosphatases

In LUE, the potential acid phosphatase activity (APA) of the organic layer was higher in spring than late summer and winter, while in the mineral soil it was higher in spring than in early summer. In BBR, the APA of the organic layer was greater in spring than in late summer, autumn and winter, while in the mineral soil it was higher in spring and early summer compared to late summer (Table 2). APA in the organic layer was correlated with the gravimetric water content (Supplementary material Fig. 1B) across seasons ($R^2 = 0.30$ and 0.51 in BBR and LUE, respectively), while no significant correlation between water content and phosphatase activity was found for the mineral soil. APA was correlated with SMB_C and with the abundance of the bacterial *phoN* genes, especially in the mineral soil (Table 3).

4. Discussion

4.1. Methodological considerations

This is the first study to show that P uptake rates of a young tree, and of the SMB differ seasonally, and between contrasting forest sites. Since our study was conducted in a common garden, effects of low water availability were avoided, which are known to impact nutrient uptake under field conditions (Bimüller et al., 2014; Dannenmann et al., 2016; Leberecht et al., 2016).

We calculated total P uptake rates based on the ^{33}P uptake and the specific ^{33}P activity of the labile P pool, following previous ^{33}P labeling studies (Fardeau et al., 1995; Morel and Fardeau, 1989; Bünemann et al., 2007; Oberson et al., 2010; Schneider et al., 2017; Spohn and Widdig, 2017). This procedure corrects for the different dilutions of the added ^{33}P with P present in soil. The similar total recoveries of ^{33}P across all seasons (Fig. 4) indicate that additional P taken up by the plants in summer and/or autumn was derived from the labile P pool and was not mobilized from more stable soil P pools, supporting our approach to consider the Bray-1 pool as the bioavailable P pool. However, it has to be taken into account that the Bay-1 P pool contains more P than present in the soil solution. Hence, it gives a rather high estimate of plant available P and therefore also of the P taken up by plants and microorganisms. In all five labeling experiments, the ^{33}P recovery in the soils from LUE was higher than in the soils from BBR (Fig. 4). The reason for this is that the fine-textured soil from BBR was formed from basalt and has a higher P sorption capacity than the sandy soil from LUE, as previously described by Bünemann et al. (2016).

4.2. P uptake by plants and EMF and EMF community composition

We found that plant P uptake was increased in autumn in the mesocosms from the P-rich site BBR, and in late summer in the P-poor site LUE (Fig. 5). The temporal difference in plant P uptake between the sites might be due to lower P storage in the young trees from LUE, which required the trees to increase their uptake rates earlier in the year in LUE than in BBR. The high plant P uptake at the end of the growing season at both sites went along with strong growth of fine

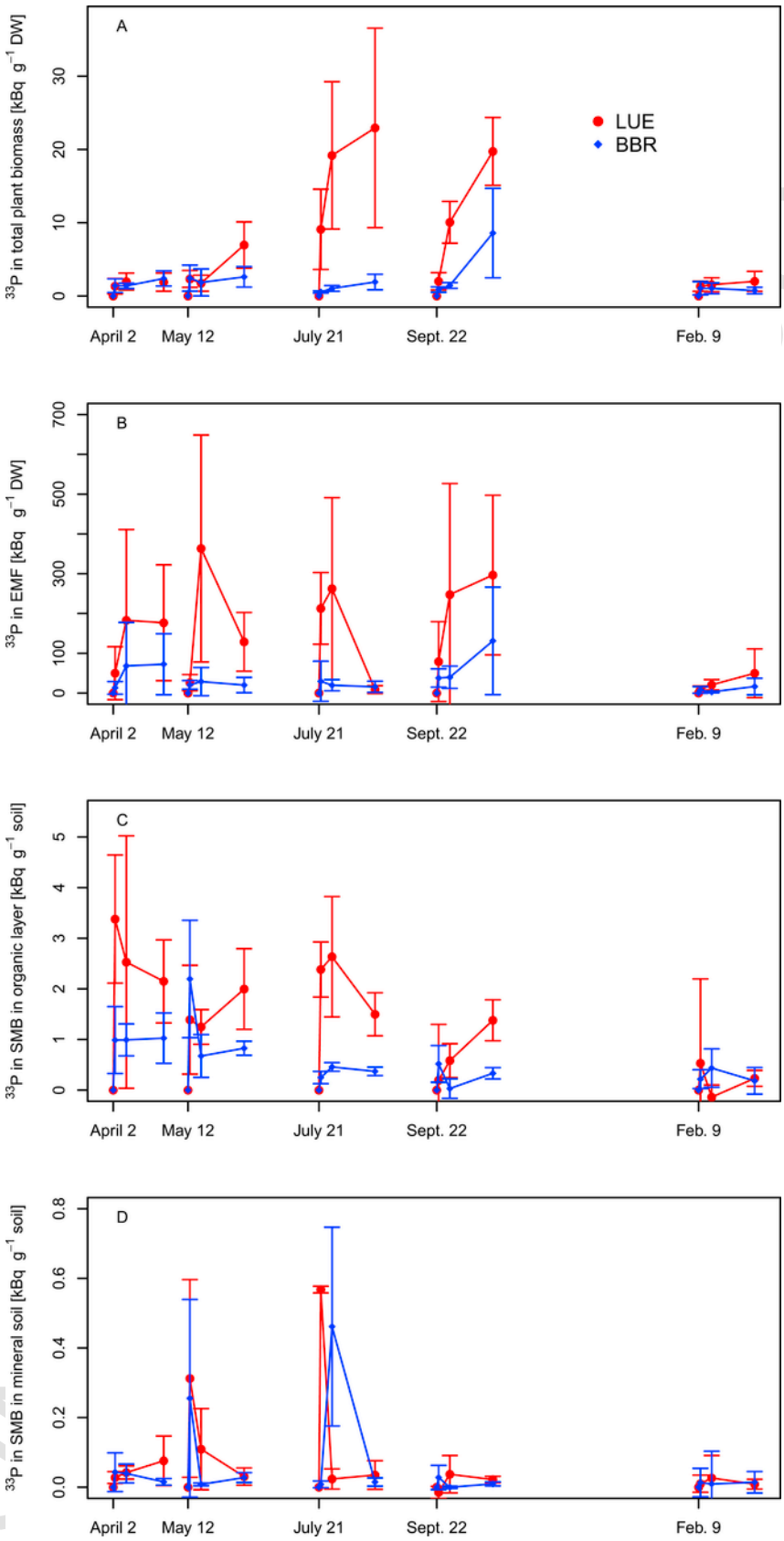

Fig. 3. ^{33}P enrichment of young beech trees (plant, A), ectomycorrhizal fungi (EMF, B), and soil microbial biomass (SMB) in the organic layer (C) and SMB in the mineral soil (D) in mesocosms from the P-poor site LUE and the P-rich site BBR during five consecutive labeling experiments. Each data point was calculated based on five mesocosms (n = 5). Error bars show standard deviations.

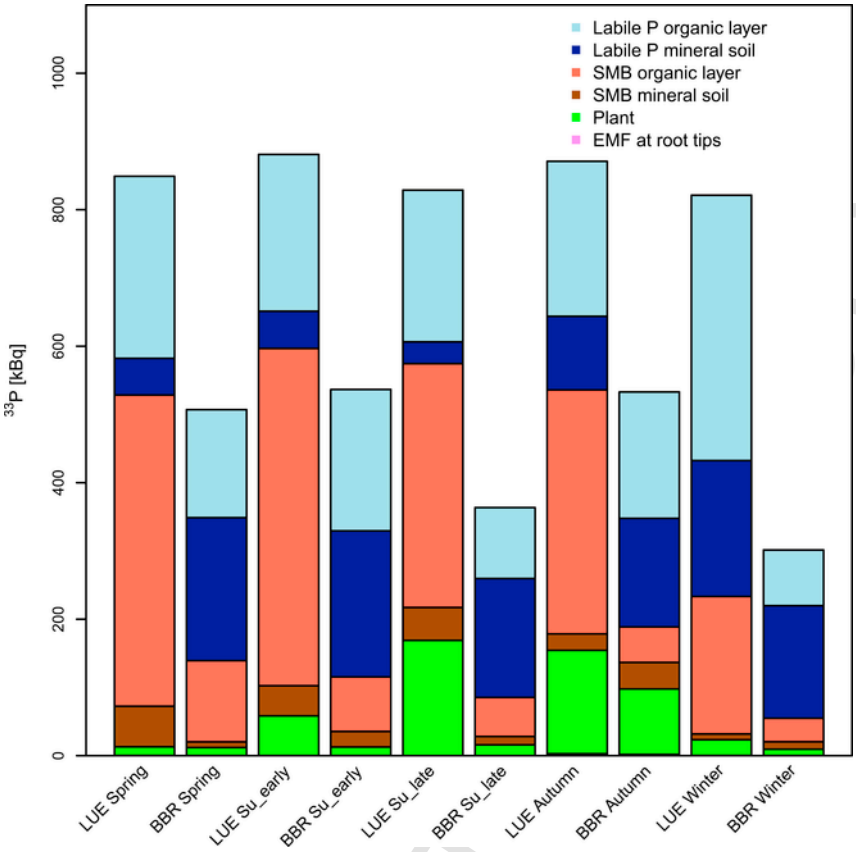

Fig. 4. Total amount of ^{33}P in young beech trees (plant), in ectomycorrhizal fungi (EMF) at the root tip, in the soil microbial biomass (SMB), and in the labile P pool in the mineral soil and in the organic layer in mesocosms from the P-poor site LUE and the P-rich site BBR thirty days after tracer application at five different times of the year. Each column was calculated based on five mesocosms (n = 5), taking into account the mass of the organic and the mineral soil in each mesocosm. Su stands for summer. Please note that the ^{33}P activity in the EMF at the roots tip was very low and can only be seen for autumn in this projection.

roots (data not shown), which might be the reason for the increased P uptake at this time of the year (details about plant P allocation are discussed in Zavišić and Polle, accepted). In LUE, the highest plant P uptake did not go along with significant increases in P accumulation in the ectomycorrhiza (Fig. 5). The reason for this is likely that the concentration of P in the EMF does not reflect the P flux to the plant, and the P concentration may be low even if the flux is high.

The EMF community was affected by season as well as by site and showed a large spatial and temporal variability (Fig. 2) similar as previously described for other forests (Van der Heijden et al., 2000; Koide et al., 2007; Walker et al., 2008; Pena et al., 2010). The finding that the number of root tips colonized by EMF was lower at LUE than at BBR indicates that P availability in the soils is only one out of many variables that affected EMF colonization of plant roots. Higher EMF species richness on the roots from BBR than from LUE is in accordance with a study on five beech forest sites in Germany that reported that variation in EMF species richness was unrelated to P concentrations in the soils (Zavišić et al., 2016). Our findings indicate that P nutrition of the young beech trees under study was not as critical as to be the main factor affecting EMF colonization. The EMF communities from LUE and BBR were different from each other during all seasons of the year. Despite the high species turnover in the EMF community, the potential APA was high throughout the year (Enowashu et al., 2009; Margalef et al., 2017), indicating functional redundancy of the microbial community with respect to P mineralization.

Scleroderma citrinum, which dominated the EMF community in late summer associated with the plants from LUE, was found to often coexist with biotite-weathering bacteria (Uroz et al., 2009). This is in agreement with a study on the microbiome of the soils from LUE and BBR, reporting a high number of Proteobacteria and Acidobacteria in LUE known to be able to mineralize biotite (Bergkemper et al., 2015). Russulaceae such as *Lactarius blennius* and *Russula ochroleuca* were also abundant in P poor soils (Zavišić et al., 2016; this study); these species are known to produce organic acids to mobilize inorganic P (Courty et al., 2010). In contrast, roots grown in soil from BBR were dominated by the EMF *Hydnum repandum* during late spring, which can produce high phytase activity (Goud and Suryam, 2009). During summer and autumn the EMF species *Tuber* sp. was found to be dominant on the root tips of plants from BBR. *Tuber* sp. produces a high amount of phosphatases, and has a high ability to mineralize N from N-containing biopolymers (Pena and Polle, 2014), along with being able to store large amounts of nutrients in its mantle (Walker et al., 2014). The different P availability of the sites was likely only one factor among others, such as stand age, water availability and the availability of N and of micronutrients that shaped the EMF community as indicated also by a recent study on the sites BBR and LUE (Zavišić et al., 2016).

4.3. P uptake by SMB and phosphatase activity

We found relatively stable SMB_C concentrations over the year (Fig. 1c), which is in accordance with previous studies on beech and spruce forests in Germany (Von Lützow et al., 1992; Bauhus and Barthel, 1995). P uptake by SMB was higher in the organic layer in LUE than in BBR throughout most of the year as hypothesized, which is in accordance with previous studies reporting that the SMB is a very important P pool in the organic layer of P-poor forests (Saggar et al., 1998; Chen et al., 2003; Achat et al., 2010; Zederer et al., 2017). The reason for the larger P uptake by the SMB in the organic layer in LUE than in BBR

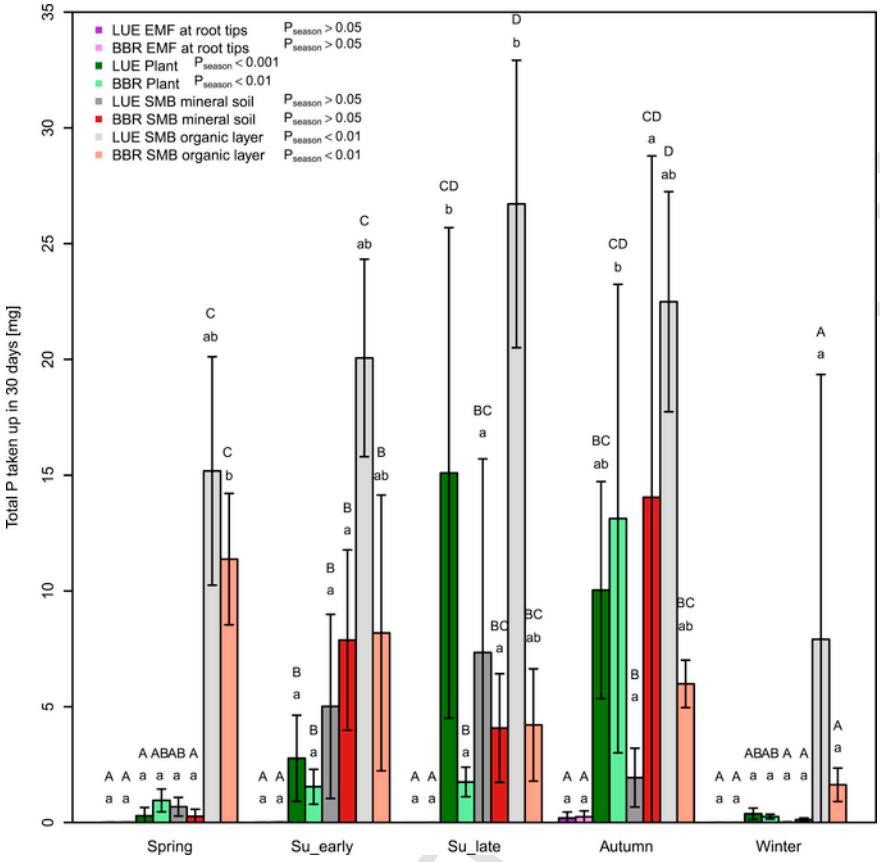

Fig. 5. Total P taken up during thirty days by ectomycorrhizal fungi (EMF) at the root tip, *F. sylvatica* (plant) and the soil microbial biomass (SMB) in mesocosms from the P-poor site LUE and the P-rich site BBR at five different times of the year. Each data column was calculated based on five mesocosms (n = 5), taking into account the mass of the organic and the mineral soil in each mesocosm. Different capital letters indicate significant differences ($p < 0.05$) between different variables in one season, while different lowercase letters indicate significant differences ($p < 0.05$) in one variable across seasons. Su stands for summer.

is mostly the larger mass of the organic layer in LUE (Table 1) that overcompensated for the lower SMB concentrations at this site (Fig. 3C) and together with a similar P uptake per mass SMB led to larger total P uptake by the SMB in the organic layer in LUE than in BBR. This finding shows that the SMB at both sites was able to immobilize available P very efficiently, leading to very low net P mineralization rates as shown previously (Heuck and Spohn, 2016). The efficient uptake is likely facilitated by the ubiquitous presence of microorganisms in soil. P uptake by the SMB in the organic layer in LUE was high in summer, coinciding with the highest plant P uptake in the mesocosms from this site. The reason for this might be that the organic layer in LUE was dominated by EMF as indicated by the similar ^{33}P uptake kinetics of EMF and SMB in the organic layer (Fig. 3b and c). A dominance of EMF over saprotrophs in the organic layer at LUE is also supported by a study on the microbial community of LUE and BBR (Zavišić et al., 2016) that reported higher EMF-derived ergosterol concentrations in the organic layer in LUE compared to BBR. In contrast to the organic layer, phosphatase activity in the mineral soil was dominated by bacteria as indicated by higher correlations between potential APA and copy number of bacterial *phoN* genes in the mineral soil than in the organic layer (Table 3). This is in agreement with Zavišić et al. (2016), reporting that the vast majority of mycorrhizal root tips in LUE and BBR were found in the organic layer.

In accordance with studies on N uptake by microorganisms and trees (Zak et al., 1990; Zogg et al., 2000; Cheng and Bledsoe, 2004; Grogan and Jonasson, 2003; Bloor et al., 2009; Dannenmann et al., 2016), we found that a larger proportion of phosphorus was immobilized in the SMB than in the plant during most seasons (Fig. 5). Similar as for N uptake, this was mostly due to the ubiquitous presence of microorganisms in soil and due to the relatively large mass of soil with respect to the plant biomass. In contrast to studies on N dynamics (Zak et al., 1990; Zogg et al., 2000; Cheng and Bledsoe, 2004; Grogan and Jonasson, 2003; Bloor et al., 2009; Dannenmann et al., 2016), we found that most of the added P tracer was sequestered in abiotic P pools, and was not recovered in the labile P pool (Fig. 4), showing that sorption plays a very important role for P availability in these soils.

Our finding that soil phosphatase activity underwent seasonal changes is in disagreement with a recent study that did not find seasonal differences in phosphatase activity in the rhizosphere of *F. sylvatica* and in the bulk soil in BBR and LUE during all four seasons of the year (Hofmann et al., 2016). The reason for the disagreement is likely that the soil water content was maintained constant in the previous experiment, while it was variable in the present study, and positively correlated with the phosphatase activity in the organic layer ($R^2 = 0.30$ and 0.51 in BBR and LUE, respectively, $p < 0.05$). Despite the elevated phosphatase activity in spring, labile P concentrations (Fig. 1b) were not increased during this time of the year.

4.4. Conclusions

Revisiting the hypotheses, we conclude, first, that in contrast to our expectations, we found an increased plant P uptake in summer exclusively in the P-poor site, whereas in the P-rich site, plant P uptake increased only in autumn. Second, P uptake by SMB was higher in the organic layer in LUE than in BBR throughout the year as hypothesized, underlining the importance of the microbial P pool in the organic layer

Table 2
Potential acid phosphatase activity and copy numbers of the bacterial acid phosphatase gene *phoN* in soil of mesocosms from the P-poor site LUE and the P-rich site BBR at five different times of the year. Each value was calculated based on five mesocosms (n = 5) at the first day after each labeling experiment. Significant seasonal differences ($p < 0.05$) in one variable tested separately for the organic layer and the mineral soil are indicated by different letters. P_{season} indicates the P value of the ANOVA testing difference between seasons. Su stands for summer.

		LUE						BBR					
		Spring	Su_early	Su_late	Autumn	Winter	P_{season}	Spring	Su_early	Su_late	Autumn	Winter	P_{season}
Organic layer	Phosphatase activity [mg Phenol $(3\ h)^{-1}\ g^{-1}$]	30.14^{b} (±15.96)	15.48^{ab} (±5.54)	8.07^{a} (±1.61)	19.07^{ab} (±5.35)	9.37^{a} (±3.05)	< 0.01	16.19^{b} (±2.83)	12.40^{ab} (±4.26)	7.71^{a} (±3.77)	8.40^{a} (±3.65)	7.28^{a} (±2.70)	< 0.01
	Number of *phoN* genes [×100,000 g^{-1}]	2.22 (±0.89)	0.58 (±0.49)	0.68 (±0.44)	0.76 (±0.29)	0.21 (±0.06)	> 0.05	1.46 (±0.72)	0.52 (±0.21)	0.57 (±0.31)	0.88 (±0.30)	0.25 (±0.11)	> 0.05
Mineral soil	Phosphatase activity [mg Phenol $(3\ h)^{-1}\ g^{-1}$]	1.49^{b} (±0.63)	0.59^{a} (±0.23)	1.01^{ab} (±0.13)	0.93^{ab} (±0.30)	0.73^{ab} (±0.04)	< 0.01	2.23^{b} (±0.81)	2.28^{b} (±0.52)	1.19^{a} (±0.22)	1.79^{ab} (±0.64)	1.73^{ab} (±0.38)	< 0.05
	Number of *phoN* genes [×100,000 g^{-1}]	0.27 (±0.11)	0.14 (±0.05)	0.08 (±0.09)	0.15 (±0.05)	0.05 (±0.04)	> 0.05	0.82 (±0.24)	0.64 (±0.23)	0.36 (±0.19)	0.64 (±0.23)	0.05 (±0.04)	> 0.05

Table 3
Correlation coefficients (R^2) among potential acid phosphatase activity, copy numbers of the bacterial acid phosphatase *phoN* genes, and soil microbial biomass carbon (SMB_C) in the mineral soil and the organic layer of the mesocosms across seasons. ***$p < 0.001$; *$p < 0.05$.

		Phosphatase activity [mg Phenol $(3\ h)^{-1}\ g^{-1}$]	SMB_C [$\mu g\ g^{-1}$]
Organic layer (LUE and BBR)	Number of *phoN* genes [g^{-1}]	0.21***	0.10*
	Phosphatase activity [mg Phenol $(3\ h)^{-1}\ g^{-1}$]	–	0.11***
Mineral soil (LUE and BBR)	Number of *phoN* genes [g^{-1}]	0.40***	0.41***
	Phosphatase activity [mg Phenol $(3\ h)^{-1}\ g^{-1}$]	–	0.35***
Organic layer and mineral soil (LUE and BBR)	Number of *phoN* genes [g^{-1}]	0.33***	0.26***
	Phosphatase activity [mg Phenol $(3\ h)^{-1}\ g^{-1}$]	–	0.41***

of P-poor forests. The finding shows that the SMB was able to compensate the lower P availability in the soil of the P-poor site LUE. Third, the EMF community composition was very variable over the year, and P uptake by *F. sylvatica* seemed to be independent of EMF community composition. Despite the high species turnover in the EMF community, the potential APA was high throughout the year, indicating functional redundancy of the microbial community with respect to P mineralization. Taken together, our study revealed important differences in temporal patterns of P uptake by *F. sylvatica* and the SMB as well as in the total partitioning of P between the SMB and *F. sylvatica* across the sites. The results indicate that decreasing P availability in forests would not only change the size of P stocks and of P cycling rates, but would also affect temporal dynamics of P uptake and the overall partitioning of P between different biotic compartments.

Author contributions

AP designed the experiments, analyzed data, wrote and commented on the manuscript. AZ conducted field work, analyzed samples, analyzed data, wrote and commented on the manuscript. MS analyzed samples, conducted data analysis and wrote the manuscript. PN, SM and EK analyzed APA and commented on the manuscript. SS, MSch and FB analyzed qPCR data and commented on the manuscript. All authors approved the final version of the manuscript.

Acknowledgements

We are grateful to the excellent technical assistance by M. Franke-Klein and the members of the Laboratory for Radio-Isotopes, B. Kopka, T. Klein and G. Lehmann. Financial support was provided by the Deutsche Forschungsgemeinschaft (DFG) to the Collaborative Research Program (Ecosystem Nutrition, SPP1685) by grants to AP (PO362/22-1, 22-2), EK (KA1590/12-1, 12-2), MS (SP1389/4-1, 4-2), and SS (SCHU 2907/3-1, 3-2).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2017.10.019.

Uncited references

Bücking and Heyser, 2003; Högberg et al., 2010; Marschner et al., 2011; Nehls et al., 2007.

References

Achat, D.L., Morel, C., Bakker, M.R., Augusto, L., Pellerin, S., Gallet-Budynek, A., Gonzalez, M., 2010. Assessing turnover of microbial biomass phosphorus: combination of an isotopic dilution method with a mass balance model. Soil Biology and Biochemistry 42, 2231–2240.

Agerer, R., 2006. Colour Atlas of Ectomycorrhizae. Einhorn-Verlag, Schwaebisch Gmuend, Germany.

Bauhus, J., Barthel, R., 1995. Mechanisms for carbon and nutrient release and retention in beech forest gaps: II the role of soil microbial biomass. Plant and Soil 168/169, 585–592.

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. Environmental Microbiology 18, 1462–2920.

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

Bimüller, C., Dannenmann, M., Tejedor, J., Von Lützow, M., Buegger, F., Meier, R., Haug, S., Schroll, R., Kögel-Knabner, I., 2014. Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions. Soil Biology and Biochemistry 68, 241–251.

Bloor, J.M., Niboyet, A., Leadley, P.W., Barthes, L., 2009. CO_2 and inorganic N supply modify competition for N between co-occurring grass plants, tree seedlings and soil microorganisms. Soil Biology and Biochemistry 41, 544–552.

Brandtberg, P.O., Bengtsson, J., Lundkvist, H., 2004. Distributions of the capacity to take up nutrients by Betula spp. and Picea abies in mixed stands. Forest Ecology and Management 198, 193–208.

Braun, S., Thomas, V.F., Quiring, R., Flückiger, W., 2010. Does nitrogen deposition increase forest production? The role of phosphorus. Environmental Pollution 158, 2043–2052.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 14, 319–329.

Bücking, H., Heyser, W., 2003. Uptake and transfer of nutrients in ectomycorrhizal associations: interactions between photosynthesis and phosphate nutrition. Mycorrhiza 13, 59–68.

Bünemann, E.K., Marschner, P., McNeill, A.M., McLaughlin, M.J., 2007. Measuring rates of gross and net mineralisation of organic phosphorus in soils. Soil Biology and Biochemistry 39, 900–913.

Bünemann, E.K., Augstburger, S., Frossard, E., 2016. Dominance of either physicochemical or biological phosphorus cycling processes in temperate forest soils of contrasting phosphate availability. Soil Biology and Biochemistry 101, 85–95.

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R., 2003. Seasonal changes in soil phosphorus and associated microbial properties under adjacent grassland and forest in New Zealand. Forest Ecology and Management 177, 539–557.

Cheng, X., Bledsoe, C.S., 2004. Competition for inorganic and organic N by blue oak (Quercus douglasii) seedlings, an annual grass, and soil microorganisms in a pot study. Soil Biology and Biochemistry 36, 135–144.

R Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Courty, P.E., Franc, A., Garbaye, J., 2010. Temporal and functional pattern of secreted enzyme activities in an ectomycorrhizal community. Soil Biology and Biochemistry 42, 2022–2025.

Crowley, K.F., McNeil, B.E., Lovett, G.M., Canham, C.D., Driscoll, C.T., Rustad, L.E., Weathers, K.C., 2012. Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the northeastern United States?. Ecosystems 15, 940–957.

Dannenmann, M., Bimüller, C., Gschwendtner, S., Leberecht, M., Tejedor, J., Bilela, S., Gasche, R., Hanewinkel, M., Baltensweiler, A., Kögel-Knabner, I., Polle, A., Schloter, M., Simon, J., Rennenberg, H., 2016. Climate change impairs nitrogen cycling in European beech forests. PLoS One 11 (7), e0158823. https://doi.org/10.1371/journal.pone.0158823.

Desai, S., Naik, D., Cumming, J.R., 2014. The influence of phosphorus availability and Laccaria bicolor symbiosis on phosphate acquisition, antioxidant enzyme activity, and rhizospheric carbon flux in Populus tremuloides. Mycorrhiza 24, 369–382.

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

Enowashu, E., Poll, C., Lamersdorf, N., Kandeler, E., 2009. Microbial biomass and enzyme activities under reduced nitrogen deposition in a spruce forest soil. Applied Soil Ecology 43, 11–21.
Fardeau, J.C., Guiraud, G., Marol, C., 1995. The role of isotopic techniques on the evaluation of the agronomic effectiveness of P fertilizers. Nutrient Cycling in Agroecosystems 45, 101–109.
Flückiger, W., Braun, S., 1998. Nitrogen deposition in Swiss forests and its possible relevance for leaf nutrient status, parasite attacks and soil acidification. Environmental Pollution 102, 69–76.
Giesler, R., Petersson, T., Högberg, P., 2002. Phosphorus limitation in boreal forests: effects of aluminum and iron accumulation in the humus layer. Ecosystems 5, 300–314.
Goud, M., Suryam, A., 2009. Extracellular hydrolytic enzyme profiles of certain South Indian basidiomycetes. African Journal of Biotechnology 8, 354–360.
Grogan, P., Jonasson, S., 2003. Controls on annual nitrogen cycling in the understory of a subarctic birch forest. Ecology 84, 202–218.
Hammer, , Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Paleontolology Electronica 4, 1–9.
Heinrichs, H., Brumsack, H.J., Loftfield, N., König, N., 1986. Verbessertes Druckaufschlusssystem für biologische und anorganische Materialien. Zeitschrift für Pflanzenernährung und Bodenkunde 149, 350–353.
Heuck, C., Spohn, M., 2016. Carbon, nitrogen and phosphorus net mineralization in organic horizons of temperate forests: stoichiometry and relations to organic matter quality. Biogeochemistry 131, 229–242.
Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil 237, 173–195.
Hoffmann, G., 1968. Eine photometrische Methode zur Bestimmung der Phosphatase Aktivität in Böden. Zeitschrift für Pflanzenernährung und Bodenkunde 118, 161–172.
Hofmann, K., Heuck, C., Spohn, M., 2016. Phosphorus resorption by young beech trees and soil phosphatase activity as dependent on phosphorus availability. Oecologia 181, 369–379.
Högberg, M.N., Briones, M.J., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J., Högberg, P., 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. New Phytologist 187, 485–493.
Ilg, K., Wellbrock, N., Lux, W., 2009. Phosphorus supply and cycling at long-term forest monitoring sites in Germany. European Journal of Forest Research 128, 483–492.
Jaeger, C.H., Monson, R.K., Fisk, M.C., Schmidt, S.K., 1999. Seasonal partitioning of nitrogen by plants and soil microorganisms in an alpine ecosystem. Ecology 80, 1883–1891.
Jansa, J., Finlay, R., Wallander, H., Smith, F.A., Smith, S.E., 2011. Role of mycorrhizal symbioses in phosphorus cycling. In: Bünemann, E.K., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action. Series Soil Biology, vol. 26, Springer Verlag, Berlin, Heidelberg, pp. 137–168.
Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the KEC value. Soil Biology Biochemistry 28, 25–31.
Jonard, M., Augusto, L., Morel, C., Achat, D.L., Saur, E., 2009. Forest floor contribution to phosphorus nutrition: experimental data. Annals of Forest Science 66, 1–9.
Jonard, M., Fürst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., Ponette, Q., 2015. Tree mineral nutrition is deteriorating in Europe. Global Change Biology 21, 418–430.
Jones, D.L., Oburger, E., 2011. Solubilization of phosphorus by soil microorganisms. In: Bünemann, E.K., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action. Series Soil Biology, vol. 26, Springer Verlag, Berlin, Heidelberg, pp. 169–198.
Kaiser, C., Fuchslueger, L., Koranda, M., Gorfer, M., Stange, C.F., Kitzler, B., Richter, A., 2011. Plants control the seasonal dynamics of microbial N cycling in a beech forest soil by belowground C allocation. Ecology 92, 1036–1051.
Kavka, M., Polle, A., 2016. Phosphate uptake kinetics and tissue-specific transporter expression profiles in poplar (Populus canescens) at different phosphorus availabilities. BMC Plant Biology 16, 206.
Koide, R.T., Shumway, D.L., Xu, B., Sharda, J.N., 2007. On temporal partitioning of a community of ectomycorrhizal fungi. New Phytologist 174, 420–429.
Lang, C., Polle, A., 2011. Ectomycorrhizal fungal diversity, tree diversity and root nutrient relations in a mixed Central European forest. Tree Physiology 31, 531–538.
Leberecht, M., Dannenmann, M., Gschwendtner, S., Bilela, S., Meier, R., Simon, J., Rennenberg, H., Schloter, M., Polle, A., 2015. Ectomycorrhizal communities on the roots of two beech (Fagus sylvatica) populations from contrasting climate differ in nitrogen acquisition in a common environment. Applied and Environmental Microbiology 81, 5957–5967.
Leberecht, M., Dannenmann, M., Tejedor, J., Simon, J., Rennenberg, H., Polle, A., 2016. Segregation of nitrogen use between ammonium and nitrate of ectomycorrhizas and beech trees. Plant Cell & Environment 39, 2691–2700.
Lipson, D.A., Schmidt, S.K., Monson, R.K., 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology 80, 1623–1631.
Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., et al., 2017. Global patterns of phosphatase activity in natural soils. Scientific Reports 7.
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 Biology and Biochemistry 43, 883–894.
Morel, C.H., Fardeau, J.C., 1989. Native soil and fresh fertilizer phosphorus uptake as affected by rate of application and P fertilizers. Plant and Soil 115, 123–128.
Nannipieri, P., Giagnoni, L., Landi, L., Renella, G., 2011. Role of phosphatase enzymes in soil. In: Bünemann, E.K., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action. Series Soil Biology, vol. 26, Springer Verlag, Berlin, Heidelberg, pp. 215–243.
Nehls, U., Grunze, N., Willmann, M., Reich, M., Kuester, H., 2007. Sugar for my honey: carbohydrate partitioning in ectomycorrhizal symbiosis. Phytochemistry 68, 82–91.
Oberson, A., Tagmann, H.U., Langmeier, M., Dubois, D., Mäder, P., Frossard, E., 2010. Fresh and residual phosphorus uptake by ryegrass from soils with different fertilization histories. Plant and Soil 334, 391–407.
Öhlinger, R., 1996. Phosphomonoesterase activity with the substrate phenylphosphate. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), Methods in Soil Biology. Springer-Verlag, Heidelberg, Germany, pp. 210–213.
Pena, R., Polle, A., 2014. Attributing functions to ectomycorrhizal fungal identities in assemblages for nitrogen acquisition under stress. The ISME Journal 8, 321–330.
Pena, R., Offermann, C., Simon, J., Naumann, P.S., Geßler, A., Holst, J., Dannenmann, M., Mayer, H., Kögel-Knabner, I., Rennenberg, H., Polle, A., 2010. Girdling affects ectomycorrhizal diversity and reveal functional differences of EM community composition in a mature beech forest. (Fagus sylvatica). Applied and Environmental Microbiology 76, 1831–1841.
Pena, R., Tejedor, J., Zeller, B., Dannenmann, M., Polle, A., 2013. Interspecific temporal and spatial differences for the acquisition of litter-derived nitrogen of ectomycorrhizal fungal assemblages. New Phytologist 199, 520–528.
Plassard, C., Dell, B., 2010. Phosphorus nutrition of mycorrhizal trees. Tree Physiology 30, 1129–1139.
Richardson, A., Barea, J.-M., McNeill, A., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and Soil 321, 305–339.
Saggar, S., Parfitt, R.L., Salt, G., Skinner, M.F., 1998. Carbon and phosphorus transformations during decomposition of pine forest floor with different phosphorus status. Biology and Fertility of Soils 27, 197–204.
Schneider, K.D., Voroney, R.P., Lynch, D.H., Oberson, A., Frossard, E., Bünemann, E.K., 2017. Microbially-mediated P fluxes in calcareous soils as a function of water-extractable phosphate. Soil Biology and Biochemistry 106, 51–60.
Smits, M.M., Bonneville, S., Benning, L.G., Banwart, S.A., Leake, J.R., 2012. Plant-driven weathering of apatite–the role of an ectomycorrhizal fungus. Geobiology 10, 445–456.
Spohn, M., Widdig, M., 2017. Turnover of carbon and phosphorus in the microbial biomass depending on phosphorus availability. Soil Biology and Biochemistry 113, 53–59.
Spohn, M., Ermak, A., Kuzyakov, Y., 2013. Microbial gross organic phosphorus mineralization can be stimulated by root exudates – a ^{33}P isotopic dilution study. Soil Biology and Biochemistry 65, 254–263.
Talkner, U., Meiwes, K.J., Potočić, N., Seletković, I., Cools, N., De Vos, B., Rautio, P., 2015. Phosphorus nutrition of beech (Fagus sylvatica L.) is decreasing in Europe. Annals of Forest Science 72, 919–928.
Uroz, S., Calvaruso, C., Turpault, M.P., Frey-Klett, P., 2009. Mineral weathering by bacteria: ecology, actors and mechanisms. Trends Microbiology 17, 378–387.
Valtanen, K., Eissfeller, V., Beyer, F., Hertel, D., Scheu, S., Polle, A., 2014. Carbon and nitrogen fluxes between beech and their ectomycorrhizal assemblage. Mycorrhiza 24, 645–650.
Van der Heijden, E.W., Vries, F.D., Kuyper, T.W., 2000. Mycorrhizal associations of Salix repens L communities in succession of dune ecosystems: above-ground and below-ground views of ectomycorrhizal fungi in relation to soil chemistry. Canadian Journal of Botany 77, 1821–1832.
Van Tichelen, K.K., Colpaert, J.V., 2000. Kinetics of phosphate absorption by mycorrhizal and non-mycorrhizal Scots pine seedlings. Physiologia Plantarum 110, 96–103.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707.
Vance, C.P., Uhde-Stone, C., Allan, D.L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytologist 157, 423–447.
Von Lützow, M., Zelles, L., Scheunert, I., Ottow, J.C.G., 1992. Seasonal effects of liming, irrigation, and acid precipitation on microbial biomass N in a spruce (Picea abies L.) forest soil. Biology and Fertility of Soils 13, 130–134.
Walker, J.F., Miller Jr., O.K., Horton, J.L., 2008. Seasonal dynamics of ectomycorrhizal fungus assemblages on oak seedlings in the southeastern Appalachian Mountains. Mycorrhiza 18, 123–132.
Walker, J.K.M., Cohen, H., Higgins, L.M., Kennedy, P.G., 2014. Testing the link between community structure and function for ectomycorrhizal fungi involved in a global tripartite symbiosis. New Phytologist 202, 287–296.
White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. Academic Press, New York, USA.
Xu, X., Thornton, P.E., Post, W.M., 2013. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Global Ecology and Biogeography 22, 737–749.
Yang, N., Zavišić, A., Pena, R., Polle, A., 2016. Phenology, photosynthesis and phosphorus in European beech (Fagus sylvatica L.) in two forest soils with contrasting P contents. Journal of Plant Nutrition and Soil Science 179, 151–158.
Zak, D.R., Groffman, P.M., Pregitzer, K.S., Christensen, S., Tiedje, J.M., 1990. The vernal dam: plant-microbe competition for nitrogen in northern hardwood forests. Ecology 71, 651–656.

Zavišić, A., Polle, A., accepted. Dynamics of phosphorus nutrition, allocation and growth of young beech (*Fagus sylvatica* L.) trees in P-rich and P-poor forest soil. Tree Physiology.

Zavišić, A., Nassal, P., Yang, N., Heuck, C., Spohn, M., Marhan, S., Polle, A., 2016. Phosphorus availabilities in beech (Fagus sylvatica L.) forests impose habitat filtering on ectomycorrhizal communities and impact tree nutrition. Soil Biology and Biochemistry 98, 127–137.

Zederer, D.P., Talkner, U., Spohn, M., Joergensen, R.G., 2017. Microbial biomass phosphorus and C/N/P stoichiometry in forest floor and A horizons as affected by tree species. Soil Biology Biochemistry(in press).

Zogg, G.P., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. Ecology 81, 1858–1866.