



# Mycorrhizal Phosphorus Efficiencies and Microbial Competition Drive Root P Uptake

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Phosphorus (P) availability shows large differences among different soil types, affecting P nutrition of forest trees. Chemical binding of P to soil moieties affects partitioning of P between soil particles and solution, influencing soluble P concentrations upon which plants, their associated mycorrhizal symbionts, and microbes feed. The goal of this study was to characterize root P uptake by mycorrhizal and non-mycorrhizal root tips in competition with microbes *in situ* in the organic and mineral layer of a P-rich and a P-poor forest. We used intact soil cores (0.2 m depth) from beech (*Fagus sylvatica*) forests to tracing the fate of  $^{33}\text{P}$  in soil, plant and microbial fractions. We used the dilution of  $^{33}\text{P}$  in the rhizosphere of each soil layer to estimate the enrichment with new P in mycorrhizal and non-mycorrhizal root tips and root P uptake. In soil cores from P-rich conditions, 25 and 75% of root P uptake occurred in the organic and mineral layer, respectively, whereas in the P-poor forest, 60% occurred in the organic and 40% in the mineral layer. Mycorrhizal P efficiency, determined as enrichment of new P in mycorrhizal root tips, differed between soil layers. Root P uptake was correlated with mycorrhizal P efficiency and root tip abundance but not with root tip abundance as a single factor. This finding underpins the importance of the regulation of mycorrhizal P acquisition for root P supply. The composition of mycorrhizal assemblages differed between forests but not between soil layers. Therefore, differences in P efficiencies resulted from physiological adjustments of the symbionts. Non-mycorrhizal root tips were rare and exhibited lower enrichment with new P than mycorrhizal root tips. Their contribution to root P supply was negligible. Microbes were strong competitors for P in P-poor but not in P-rich soil. Understorey roots were present in the P-rich soil but did not compete for P. Our results uncover regulation of mycorrhizal P efficiencies and highlight the complexity of biotic and abiotic factors that govern P supply to trees in forest ecosystems.

**Keywords:** ectomycorrhiza, rhizosphere, organic soil, mineral soil, phosphorus, radioactive labeling

## INTRODUCTION

Plants take up phosphorus (P) as inorganic phosphate ( $\text{P}_i$ ,  $\text{HPO}_4^{2-}$ , or  $\text{PO}_4^{3-}$ ) from the soil solution (Plassard and Dell, 2010). However, this P source is scarce because  $\text{P}_i$  has low diffusion rates in soil (Shen et al., 2011), is sequestered by soil minerals such as Fe and Al hydroxides (Lambers et al., 2015b; Prietzel et al., 2016) or is bound to organic matter (Ilg et al., 2008; Lambers et al., 2008).

Besides, P input into soil by P deposition is extremely low (Peñuelas et al., 2013). Consequently, weathering and P utilization lead to a shift in P availability for plants (Walker and Syers, 1976; Callaway and Nadkarni, 1991; Vitousek et al., 2010; Vincent et al., 2013) and drive P reductions during soil geological aging (Turner and Condron, 2013). As a result, different soils vary drastically in P availability (Chadwick et al., 1999; Lang et al., 2016).

Since P is an essential element for plant nutrition, plants have to adjust their acquisition strategies to cope with different P availabilities (Vance et al., 2003; Lambers et al., 2008, 2015a,b). This requirement is especially important for tree species such as beech (*Fagus sylvatica* L.), which occur on a broad range of different soil types (Leuschner et al., 2006). It has been shown that beech ecosystems along a geosequence of different P availabilities adjust their nutritional strategies from P acquisition under high P availability to P recycling when P is scarce (Lang et al., 2017). Due to differences in soil structure and nutrient availability, beech trees in P-poor soil modify their root systems to explore the organic soil layer more intensely than trees in P-rich soil (Lang et al., 2017). They further exhibit extended seasonal P acquisition (Spohn et al., 2018), stronger recycling of P resources from internal storage tissues (Netzer et al., 2017; Zavišić and Polle, 2018), and reductions in growth and photosynthesis (Yang et al., 2016), which are released upon P-fertilization (Zavišić et al., 2018). In acidic, P-poor soils, forests build up a thick organic layer from which P can be re-mobilized by decomposition (Heuck and Spohn, 2016; George et al., 2018; Zederer and Talkner, 2018). In mesocosms with reconstructed soil layers composed of homogenized organic and mineral soil, the organic layer was important for the P supply to young beech saplings (Hauenstein et al., 2018). Under field conditions, understory vegetation is present and more abundant in P-rich than P-poor beech forests (Rieger et al., 2019). To assess beech P nutrition, the potential competition of understory for nutritional resources needs to be taken into account (Nambiar and Sands, 1993). Furthermore, soil microbes are important competitors for mineral nutrients (Kuznyakov and Xu, 2013; Zhu et al., 2017).

It is well-known that ectomycorrhizal fungi play a critical role for nutrition of forest trees in temperate ecosystems (Cairney, 2011; Becquer et al., 2014; Johri et al., 2015; Nehls and Plassard, 2018). Ectomycorrhizas produce hyphal networks that overcome zones of P depletion by hyphal tunneling (Jongmans et al., 1997; Hoffland et al., 2003; Jansa et al., 2011). Mycorrhizal fungi further contribute to bioweathering by secretion of organic acids (Wallander, 2000; Balogh-Brunstad et al., 2008; Jansa et al., 2011) and to the recycling of organic-bound P by production of acidic phosphatases (Hinsinger et al., 2015; Smith et al., 2015). Already early tracer studies with radioactive  $P_i$  showed that mycorrhizal roots of pine and beech accumulate more P than non-mycorrhizal roots (Kramer and Wilbur, 1949; Harley et al., 1954). In beech forests, the fraction of non-mycorrhizal root tips is generally small (Pena et al., 2010; Lang et al., 2017) but whether they play a role for P acquisition in natural forest soils, for example, in deeper soil layers, has not yet been studied. P acquisition strategies of distinct mycorrhizal communities in natural soils have mainly been studied empirically and not directly by tracing P uptake. It is

therefore unknown if mycorrhizal fungal assemblages at different soil depth show physiological differences that adjust P acquisition to P availability in soil. It is further unclear whether differences in fungal assemblages and soil structures affect competition for P of beech with roots of understory plants and soil microbes. The present study was undertaken to investigate these open points.

The aims of this study were to quantify the contributions of mycorrhizal and non-mycorrhizal root tips to  $P_i$  acquisition in the organic layer and mineral top soil of a P-poor and a P-rich beech forest and to elucidate the competition between beech roots, microbes and other plants roots for P uptake. To address these goals, we excised intact soil cores from two well-characterized beech forests, which constitute the high-P (HP) and low-P (LP) ends of a geosequence in Central Europe (Germany), previously characterized by Lang et al. (2017). We irrigated the soil cores with  $^{33}P$ , traced the distribution of the label and measured the P contents in the soluble and insoluble fractions of bulk soil, rhizosphere, beech roots, mycorrhizal and non-mycorrhizal root tips, other roots, and microbes in the organic layer and mineral topsoil. As a methodological innovation, we determined the bioavailability of  $P_i$  not only in bulk but also in rhizosphere soil. We tested the following hypotheses: (i) the main P source for beech P supply is the mineral topsoil under P-rich and the organic layer under P-poor conditions. (ii) Mycorrhizal root tips exhibit different P efficiencies, thereby counteracting low P availability by relatively higher P uptake than under high P availability; the contribution of non-mycorrhizal root tips to P uptake is low. (iii) Under high P availability, beech roots do not compete with understory or microbes for  $P_i$ , whereas the competition is strong under low P availability.

## MATERIALS AND METHODS

### Site Characteristics

Two beech (*F. sylvatica* L.) forests, which differ strongly in soil properties and P stocks (**Supplementary Table S1**) were selected for this study; a low-P (LP) and Bad Brückenau, a high-P (HP) site (Zavišić et al., 2016; Lang et al., 2017). Briefly, the LP forest is located in the district Celle in Lower Saxony (R: 3585473 E, H: 5857057 N; 115 m above sea level) and stocked with about 132-year-old beech trees (Haußmann and Lux, 1997). The soil type is sandy-loam from silicate substrate, containing 164 g P  $m^{-2}$  to a depth of 1 m (Lang et al., 2017). The mean annual temperature was 8.0°C and the mean annual sum of precipitation 730 mm. The HP forest is situated in the biosphere reservation 'Bayerische Rhön' (R: 3566195 E, H: 5579975 N; 801 to 850 m above sea level). The mean long-term sum of annual precipitation was 1000 mm and the mean annual temperature 5.8°C. The soil type is basalt of volcanic origin, containing 904 g P  $m^{-2}$  to a depth of 1 m (Lang et al., 2017). The average age of the beech stand is 137 years (Haußmann and Lux, 1997). Additional information on the research sites has been compiled in **Supplementary Table S1**.

### Collection of Soil Cores

We collected intact soil cores in the HP (27.6.2017) and the LP (19.6.2017) forest, respectively, using PVC pipes of 120 mm

diameter and a length of 200 mm. Each pipe was hammered completely into the soil at a distance of about 0.2 m to a beech tree and was pulled up containing the soil and forest floor. The soil cores remained in the PVC pipes. They were immediately transported to laboratory, kept at room temperature, weighed, watered with 100 ml of tap water, and used the next day for labeling ( $n = 5$  per forest). Soil cores of the same dimensions ( $n = 5$  per forest) for mycorrhizal inspection were harvested on the same plots about 2–3 weeks later in the same manner.

## Radioactive Labeling and Harvest

We used the soil cores for uptake studies of  $^{33}\text{P}$  into roots. Pretests with soil cores collected in the same manner in 2015 showed that the roots were still active exhibiting similar uptake of the label as roots of intact plants (Spohn et al., 2018). We labeled five soil cores per forest by addition of 1850 kBq of  $\text{H}_3^{33}\text{PO}_4$  (Hartmann Analytic GmbH, Brunswick, Germany) in 40 ml of tap water to each soil core. The added  $^{33}\text{P}$  corresponded to 1.66 ng P per soil core. Each soil core was subsequently watered with 40 ml tap water to distribute the radioactive marker throughout the soil core. To avoid loss of label by through-flow a plastic saucer was placed underneath each soil core. Any through-flow was returned to the soil core. After 24 h incubation in the laboratory at  $20^\circ\text{C}$ , the soil cores were pushed out of the pipe and were immediately separated in organic and mineral topsoil. Each sample was further fractionated in bulk soil, rhizosphere soil, beech roots (fine roots:  $< 2$  mm, coarse roots:  $> 2$  mm), other roots (distinguished by color and surface structure, Hölscher et al., 2002), and residual materials (litter, fruits, twigs, and stones).

We defined rhizosphere soil as the soil adhering to roots. Our rhizosphere effectively represents the mycorrhizosphere since fungal hyphae also contribute to soil adherence (York et al., 2016) but for consistency, the term rhizosphere was used throughout this study. The rhizosphere soil was collected by streaking the adhering soil from the roots with a paintbrush. Afterward, fresh fine roots were cut into pieces of similar lengths and yielded one mixed sample per soil layer and soil core, which was immediately weighed. All other fractions were also immediately weighed. Well-mixed aliquots of soil and plant fractions were dried at  $40^\circ\text{C}$  for 1 week, weighed, and used for analyses of the dry mass per soil layer and plant fraction.

## Analyses of Root Tips and Mycorrhizal Species

Approximately half of the fresh beech fine roots, extracted from each soil core, were used to determine the number of root tips and mycorrhizal colonization. The sample was weighed and the number of root tips in this sample was counted under a stereomicroscope (Leica M205 FA, Wetzlar, Germany). The total number of root tips per soil layer was calculated as:

### Equation 1

Total number<sub>Root tips</sub>

$$= \frac{\text{Aliquot}_{\text{root tips}}}{\text{Aliquot}_{\text{fine root biomass}} (\text{g})} \times \text{total biomass of FR} (\text{g}) \quad (1)$$

Root tips were categorized according to their visual appearance as vital ectomycorrhizal, vital non-mycorrhizal or dry (Winkler et al., 2010). Mycorrhizal root tips and non-mycorrhizal root tips were collected separately from each sample. The mean weight of mycorrhizal and non-mycorrhizal root tips was determined by collecting 100 and 200 root tips, respectively, and weighing the sample after drying ( $40^\circ\text{C}$  for 1 week, followed by freeze-drying for 4 days and equilibration at room temperature in a desiccator with silica gel). For the analyses of P and  $^{33}\text{P}$ , mycorrhizal root tips were collected for each soil layer and forest type separately. The mycorrhizal samples represented a mixture of morphotypes. Mycorrhizal colonization and root vitality were calculated as:

Mycorrhizal colonization (%)

$$= \frac{\text{number of mycorrhizal root tips}}{\text{number of vital root tips}} \times 100 \quad (2)$$

Nonmycorrhizal root tips (%)

$$= \frac{\text{number of nonmycorrhizal root tips}}{\text{number of vital root tips}} \times 100 \quad (3)$$

$$\text{Root vitality} (\%) = \frac{\text{number of vital root tips}}{\text{number of all counted root tips}} \times 100 \quad (4)$$

The abundance of different morphotypes was determined under the stereomicroscope (Leica MF205) using a simplified identification key (after Agerer, 1987–2012) as described by Pena et al. (2010). For mycorrhizal species identification, morphotypes of all species were collected, which comprised at least three root tips per sample. Mycorrhizal species identities were determined after DNA extraction and ITS sequencing using the protocol of Pena et al. (2017).

## Determination of Total and Soluble Phosphorus in Roots and Soil

Dry soil and coarse and fine root samples were milled in a ball mill (Retsch MN 400, Haan, Germany) to a fine powder. For determination of total P ( $P_{\text{tot}}$ ), about 50 mg of the powder was weighed and extracted in 25 ml of 65%  $\text{HNO}_3$  at  $160^\circ\text{C}$  for 12 h according to Heinrichs et al. (1986). For  $P_{\text{tot}}$  determination of root tips, dry root tips, either mycorrhizal or non-mycorrhizal, with weights ranging from 0.2 to 30 mg were weighed and extracted in 2 ml of 65%  $\text{HNO}_3$  at  $160^\circ\text{C}$  for 12 h according to Heinrichs et al. (1986). For determination of soluble P ( $P_{\text{sol}}$ ) about 100 mg of plant or soil powder was extracted in 150 ml of Bray-1 solution (0.03 N  $\text{NH}_4\text{F}$ , 0.025 N  $\text{HCl}$ ) for 60 min on a shaker at 180 rpm (Bray and Kurtz, 1945). Microbial P was determined using the chloroform fumigation extraction method according to Brookes et al. (1985). About 5 g organic layer or 10 g mineral topsoil were fumigated for 24 h with chloroform ( $\text{CHCl}_3$ ). The fumigated samples were extracted for 60 min on a shaker at 180 rpm in 150 ml Bray-1 solution. After P

measurement (described below),  $P_{mic}$  was calculated according to Brookes et al. (1982):

$$P_{mic} \text{ (mg g}^{-1}\text{)} = 2.5 \times (P_{\text{Chloroform extract}} - P_{\text{sol}}) \quad (5)$$

All extracts were filtered through phosphate free filter paper (MN 280 1/4, Macherey-Nagel, Düren, Germany). The  $P_{\text{tot}}$  and Bray-1 extracts were used for elemental analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (iCAP 7000 Series ICP-OES, Thermo Fisher Scientific, Dreieich, Germany). P was measured at the wavelength of 185.942 nm (axial) and calibrated with a series of dilutions (0.1 to 20 mg l<sup>-1</sup>) using the element standard for P (Einzelstandards, Bernd Kraft, Duisburg, Germany).

## Determination of <sup>33</sup>P by Scintillation Counting

The  $P_{\text{tot}}$  and Bray-1 extracts of all soil, plant and root tip samples were used to measure <sup>33</sup>P. For this purpose, 3 ml of plant or soil extract or 1.5 ml of root tip extracts were mixed with 10 ml of scintillation cocktail (Rotiszint eco plus, Roth, Karlsruhe, Germany). The mixture was used for detection of <sup>33</sup>P signals (DPM, disintegrations per minute) in a PerkinElmer scintillation counter (Tri-Carb TR/SL 3180, Waltham, MA, United States). The signal was corrected for the <sup>33</sup>P half-life of 25.34 days using QuantSmart (version 4.00, PerkinElmer) to take the decay time between harvest and measurements into account. A background correction value was subtracted from all samples, which was obtained by measuring only scintillation cocktail and extraction solution. <sup>33</sup>P concentrations in different fractions were expressed as <sup>33</sup>P (Bq g<sup>-1</sup> dry mass):

$$^{33}\text{P (Bq g}^{-1}\text{)} = \frac{\text{DPM}}{60 \times \text{dilution} \times \text{dry mass of the sample (g)}} \quad (6)$$

To determine the whole amount of <sup>33</sup>P in a distinct soil or plant compartment (whole amount = WA), we multiplied the <sup>33</sup>P concentration (Eq. 6) with the total mass of that compartment, for example for fine roots (FR):

$$\text{WA}_{\text{FR}} \text{ } ^{33}\text{P (Bq)} = \text{ } ^{33}\text{P (Bq g}^{-1}\text{ FR)} \times \text{total biomass of FR (g)} \quad (7)$$

The WA of <sup>33</sup>P in coarse roots (CR), other roots, rhizosphere soil (Rhizo) in the organic layer (OL), rhizosphere soil in the mineral layer (ML), in bulk soil (Bulk) in the organic layer and in bulk soil in the mineral layer were determined correspondingly.

## Calculation of P Uptake

Irrigation with <sup>33</sup>P solution ( $^{33}\text{P}_{\text{tot}}$  = total <sup>33</sup>P) leads to distribution of <sup>33</sup>P between soil solution (soluble <sup>33</sup>P =  $^{33}\text{P}_{\text{sol}}$ ) and absorption to soil particles (bound <sup>33</sup>P =  $^{33}\text{P}_{\text{bound}}$ ). Furthermore, only a fraction of <sup>33</sup>P will percolate through the organic layer into the mineral top soil, where it can be expected to result in another distribution factor between soil solution and soil particles than in the organic layer. As the consequence,  $^{33}\text{P}_{\text{sol}}$  will vary

depending on soil layer and forest type. Similarly, we expect that soluble P ( $P_{\text{sol}}$ ) present in our soil cores will also vary between soil layers and forest type. Therefore, the dilution of <sup>33</sup>P in the P concentration of the soil will vary among different soil layer and forest types, resulting in different specific activities. We determined the specific activity of  $^{33}\text{P}_{\text{sol}}$  (spec  $^{33}\text{P}_{\text{sol}}$ ) for each soil compartment separately using the P and <sup>33</sup>P concentrations of the Bray-1 solution (**Supplementary Table S2**):

$$\text{spec } ^{33}\text{P}_{\text{sol}} \text{ (Bq mg}^{-1}\text{P}_{\text{sol}}\text{)} = \frac{^{33}\text{P}_{\text{sol}} \text{ (Bq g}^{-1}\text{ soil)}}{\text{P}_{\text{sol}} \text{ (mg g}^{-1}\text{ soil)}} \quad (8)$$

We used the specific activities to determine P uptake and enrichment in plant roots, microbes and mycorrhizal and non-mycorrhizal tips after 1 day using the whole amounts of these fractions in different soil layers (cv. Eq. 7) and the corresponding specific activities (cv. Eq. 8). The specific activity of  $^{33}\text{P}_{\text{sol}}$  in the bulk soil was used to calculate P uptake of microbes separately for the organic and the mineral layer:

$$\text{P uptake}_{\text{microbes}} \text{ (mg)} = \frac{\text{WA}_{\text{microbes}} \text{ } ^{33}\text{P}}{\text{spec } ^{33}\text{P}_{\text{sol}} \text{ (bulk)}} \quad (9)$$

We used the specific activity of the rhizosphere to calculate P uptake of beech roots because roots absorb nutrients mainly from the rhizosphere (York et al., 2016):

$$\text{P uptake}_{\text{beech}} \text{ (mg)} = \frac{(\text{WA}_{\text{FR}} \text{ } ^{33}\text{P} + \text{WA}_{\text{CR}} \text{ } ^{33}\text{P})}{\text{spec } ^{33}\text{P}_{\text{sol}} \text{ (rhizo)}} \quad (10)$$

We calculated P uptake<sub>beech</sub> separately for the mineral (ML) and organic layer (OL). With P uptake<sub>beech</sub> in OL and in ML, we calculated the relative contribution of each soil layer to P uptake of beech roots:

Relative P uptake in OL

$$= \frac{\text{P uptake}_{\text{beech}} \text{ in OL}}{(\text{P uptake}_{\text{beech}} \text{ in OL} + \text{P uptake}_{\text{beech}} \text{ in ML)}} \quad (11)$$

The relative P uptake in the mineral layer was determined correspondingly.

Mycorrhizal (EM) and non-mycorrhizal (NM) root tips take up nutrients for their own metabolism and translocation to the host. The <sup>33</sup>P label in the tips should therefore not be termed “uptake” but represents enrichment of new P (ENP) as the result of P metabolism in the mycorrhiza and host allocation at a distinct time point, as exemplified previously for nitrogen (Pena and Polle, 2014; Leberecht et al., 2016). To calculate ENP in EM root tips, we used the <sup>33</sup>P concentration in root tips (Eq. 6), the specific activity of  $^{33}\text{P}_{\text{sol}}$  in the rhizosphere (Eq. 8) and the mass of an individual EM root tip:

$$\text{ENP}_{\text{EM}} \text{ (mg root tip}^{-1}\text{)} = \frac{^{33}\text{P}_{\text{tot}} \text{ of EM} \times \text{mass of 1 root tip}}{\text{spec } ^{33}\text{P}_{\text{sol}} \text{ (rhizo)}} \quad (12)$$



ENP of non-mycorrhizal root tips was calculated accordingly. We determined and used the following masses for roots tips: 1 EM root tip in HP = 18.35  $\mu\text{g}$ , in LP = 15.68  $\mu\text{g}$ , and 1 NM root tip = 8.84  $\mu\text{g}$  in both HP and LP.

To determine the whole amount of new P (WA ENP) in all mycorrhizal root tips of a distinct layer, we used EMP (Eq. 12) and the total number of root tips in that layer (Eq. 1):

$$\begin{aligned} \text{WA ENP}_{\text{EM}} \text{ in OL (mg)} \\ = \text{ENP}_{\text{EM}} \times \text{number of all EM root tips in OL} \end{aligned} \quad (13)$$

WA ENP for EM in the mineral layer and for the non-mycorrhizal root tips in those layers were calculated correspondingly. With these values we calculated WA ENP in all root tips of the whole soil core:

$$\begin{aligned} \text{WA ENP}_{\text{soil core}} \text{ (mg)} = \text{WA ENP}_{\text{EM}} \text{ in OL} + \text{WA ENP}_{\text{EM}} \text{ in ML} \\ + \text{WA ENP}_{\text{NM}} \text{ in OL} + \text{WA ENP}_{\text{NM}} \text{ in ML} \end{aligned} \quad (14)$$

Using the enrichment of new P of a distinct fraction of root tips (according to Eq. 12) and the total enrichment of new P in all root tips of the soil core (Eq. 13), we determined the relative enrichment of new P in distinct soil layer and types of root tips:

$$\text{Relative ENP}_{\text{EM}} \text{ in OL} = \frac{\text{WA ENP}_{\text{EM}} \text{ in OL}}{\text{WA ENP}_{\text{soil core}}} \quad (15)$$

The relative enrichments of new P in EM of the ML and in NM root tips per soil layer were determined correspondingly.

## Calculations of Relative Competition Intensities (RCI)

According to Grace (1995) the dynamics of mixtures can be accurately determined by the relative competition intensity. We adapted the concept originally used to compare the performance of plants in different ecological settings (Grace, 1995) to calculate the competition intensities for P between beech roots and microbes, or beech roots and other roots in our soil cores or beech roots and all competitors (= microbes + other roots). In line with Grace (1995), we reasoned that new P could end in any of these fractions and the relative amount of new P in beech roots compared to other fractions indicates their competitive strengths:

$$\text{RCI}_{\text{beech vs microbes}} = \frac{\text{P uptake}_{\text{beech}} - \text{P uptake}_{\text{microbes}}}{\text{P uptake}_{\text{beech}}} \quad (16)$$

$\text{RCI}_{\text{beech versus other roots}}$  and  $\text{RCI}_{\text{beech versus all competitors}}$  were calculated accordingly. Negative values of RCI indicate competition; positive values or values that do not deviate from zero indicate no competition. To test whether the values deviated from zero, we used a non-parametric signed rank test. We considered  $p$ -values < 0.05 to indicate significant effects.

## Statistical Analyses

Data are shown as means ( $n = 5$  to 8 per soil layer and forest type  $\pm$  SE), if not indicated otherwise. Statistical analyses were conducted with R version 3.6.0 (R Core Development Team, 2012). Normal distribution and homogeneity of variances were tested by analyzing residuals of the models and performing a Shapiro–Wilk test. Data were logarithmically transformed to meet the criteria of normal distribution and homogeneity of variances, where necessary. General linear models were used to test the factor “Forest type” with the two layers (OL and ML) as random factors; otherwise analysis of variance (ANOVA) was used. Differences between means were tested by a *post hoc* test Tukey HSD (package: ‘multcomp’). Means between the two forest types were compared with Student’s  $t$ -test. Means were considered to be significantly different from each other when  $p \leq 0.05$ . For relative values, a generalized linear model using Poisson distribution was used and data were considered to be significantly different from each other when  $p \leq 0.05$ . To detect linear relationships between variables, Pearson’s correlation coefficient was determined.

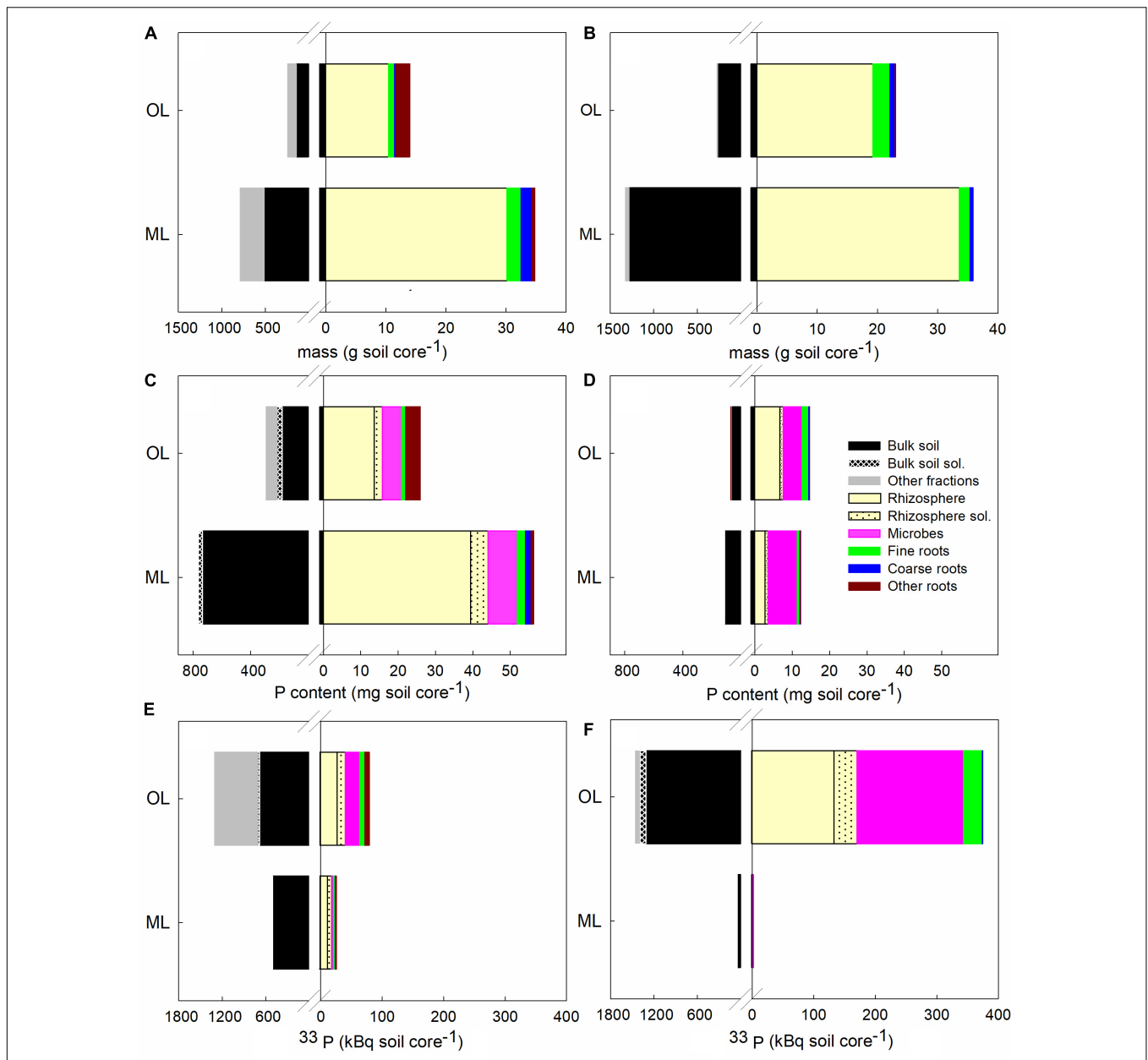
For the analyses of diversity data (count data for mycorrhizal species), we used PAST version 3.22 (Hammer et al., 2001) to determine mean species abundance and to test differences in the fungal assemblages by a two-way PERMANOVA for the factors: forest type and soil layer. Bray–Curtis was used as the similarity measure.

## RESULTS

### Biomass, Total P and $^{33}\text{P}$ Distribution in Soil, Roots, and Microbes Differ Between Soil Layers of P-Rich and P-Poor Soils

One day after irrigation of intact soil cores from a HP and an LP forest with  $^{33}\text{P}$ , we determined the masses of soil and root fractions (Figures 1A,B), the amounts of bound and soluble P (Figures 1C,D), and the amounts of  $^{33}\text{P}$  present in the different fractions (Figures 1E,F, complete statistical information: Supplementary Tables S2, S3). Bulk soil was always the largest mass fraction, followed by residual materials (leaf litter and twigs in the organic layer and a large stone fraction in the HP mineral soil), and rhizosphere soil (Figures 1A,B). LP contained more rhizosphere soil in the organic layer than HP ( $p = 0.028$ ), probably because the biomass of beech fine roots, from which the adhering soil was collected, was higher in the organic layer at LP than at HP ( $p < 0.001$ ) (Figures 1A,B and Supplementary Table S2). Beside beech roots, the soil cores also contained roots from other plant species in both layers (Figures 1A,B). Other roots were more abundant than beech roots in the organic layer at HP ( $p = 0.014$ ), while they constituted a smaller fraction to that of beech roots in the organic layer at LP ( $p < 0.001$ ) (Figures 1A,B). In the mineral layer, beech roots strongly dominated over other roots at HP ( $p = 0.002$ ) and were mostly absent at LP ( $p < 0.001$ ) (Figures 1A,B).

As expected (Lang et al., 2017), the vast majority of total P was sequestered in bulk soil and the amounts of P present in



**FIGURE 1** | Distribution of biomass, P content and <sup>33</sup>P activity in intact soil cores 24 h after label application. Soil cores were obtained from P-rich (**A,C,E**) and P-poor (**B,D,F**) forests. Soils from the organic layer (OL) and the mineral topsoil (ML) were analyzed separately. Data indicate means (*n* = 5). Standard errors and statistical information is shown in **Supplementary Tables S2, S3**. Colors of bars refer to bulk soil (black) with potential plant available *P<sub>sol</sub>* (white hatched), residual materials (gray), rhizosphere soil (light yellow) with potential plant available *P<sub>sol</sub>* (dotted), microbes (purple), fine roots (green), coarse roots (blue), and other roots (dark red).

most of the analyzed fractions were higher at HP than at LP (**Figures 1C,D** and **Supplementary Table S3**). Still, we noted some exceptions: the total amounts of P bound by beech fine roots (*p* = 0.231), beech coarse roots (*p* = 0.522), and microbes (*p* = 0.114) in the organic layer of LP were similar to those at HP. In the mineral layer of LP, the amount of P in microbes was even higher than that of HP (*p* = 0.003). The *P<sub>sol</sub>* content of the rhizosphere, which we considered as the potentially plant-available P pool, was higher at HP than at LP in both soil

layers (organic layer: *p* < 0.001, mineral layer: *p* < 0.001) (**Figures 1C,D**).

To investigate the fate of newly added *P<sub>i</sub>*, we studied the distribution of <sup>33</sup>P 1 day after labeling. The majority of the label was sequestered in bulk soil and was not soluble in mild acid at HP (*p* < 0.001) and LP (*p* < 0.012), suggesting that it was immediately removed from the easily available bioactive pool (**Figures 1E,F**). Furthermore, the distribution of the added <sup>33</sup>P showed interesting differences between the organic layer and

**TABLE 1** | Specific activity  $^{33}\text{P}$  in rhizosphere and bulk soil of beech forests.

Site	HP		LP		Forest		OL		ML	
Layer	OL	ML	OL	ML	F	p	t	p	t	p
Bulk soil	0.86 ± 0.15	0.27 ± 0.04	26.69 ± 3.60	0.30 ± 0.17	13.012	<b>0.002</b>	7.160	<b>&lt;0.001</b>	0.638	0.541
Rhizosphere	6.39 ± 0.97	1.44 ± 0.37	46.17 ± 8.70	0.08 ± 0.04	8.593	<b>0.009</b>	4.542	<b>0.002</b>	3.638	<b>0.007</b>

Soils were collected in a P-rich (HP) and P-poor (LP) forest and separated into organic layer (OL) and the mineral topsoil (ML) for analyses. Data indicate means ( $n = 5 \pm \text{SE}$ ). To compare means we used general linear models for the overall comparisons between HP and LP (OL and ML as random factor), otherwise Student's *t*-test. Bold letters indicate significant differences at  $p \leq 0.05$ .

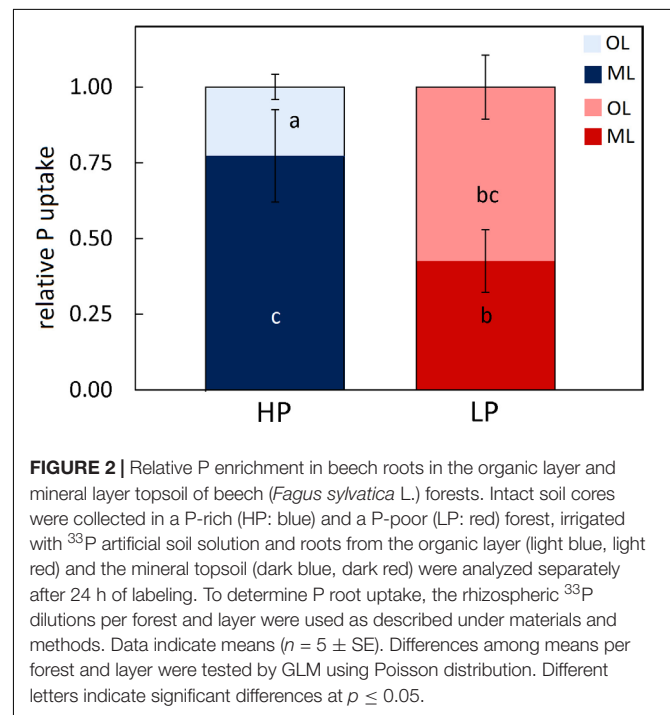
the mineral top soil at HP and LP (Figures 1E,F). At LP, only a relatively small fraction of the added  $^{33}\text{P}$  percolated into the mineral soil (Figure 1F,  $p < 0.001$ ), while at HP, the organic layer including microbes and rhizosphere retained less  $^{33}\text{P}$ , resulting in a higher through flux of  $^{33}\text{P}$  into the mineral layer (Figure 1E). These distribution patterns also influenced the specific activities of  $^{33}\text{P}_{\text{sol}}$  in the rhizosphere (Table 1), resulting in the highest specific activity in the organic layer and the lowest in mineral soil at LP, while HP assumed intermediate specific  $^{33}\text{P}$  activities (Table 1). Overall, the recovery of applied  $^{33}\text{P}$  was at  $84.7\% \pm 5.1$  at HP and  $99.2\% \pm 0.4$  at LP (calculated with the data in Supplementary Table S3). It is likely that the lower recovery at HP was caused by the stone fraction in the mineral layer, which could not be measured, but probably also absorbed a portion of the added  $^{33}\text{P}$ .

## P Uptake and P Distribution in Beech Roots Differ Between Soil Layers in P-Rich and P-Poor Forests

The specific activities of  $^{33}\text{P}_{\text{sol}}$  in rhizosphere per forest type and soil layer (Table 1) were used to estimate P uptake of beech roots in each layer separately. To compare the importance of soil layers for P supply at HP and LP, we normalized the data relative to P uptake of beech roots (Eq. 11 under materials and methods). We found that beech roots in our soil cores acquired about 75% of P from the mineral soil and 25% from the organic layer at HP, whereas beech roots at LP took up about 60% P from the organic and 40% from the mineral soil (Figure 2). These figures corresponded well to the distribution of total P in beech roots between the organic layer and mineral soil at HP (25% and 75%, respectively) and at LP (69% and 30%, respectively), calculated with the data in Supplementary Table S3).

## Mycorrhizal P Efficiencies Differ Between Soil Layers in P-Rich and P-Poor Soil

Regardless of forest type and soil layer, 96% of the vital root tips were colonized by ectomycorrhizal fungi (Table 2). In soil cores from HP about 50% of the root tips were present in the organic layer and the other 50% in the mineral soil, whereas at LP 75% of the root tips were present in the organic layer and 25% in the mineral soil (Table 2). In HP soil, the P concentrations of mycorrhizal root tips were similar to those of non-mycorrhizal root tips in HP soil and did not differ between soil layers (Figure 3A). In LP soil, the P concentrations of mycorrhizal root



**FIGURE 2** | Relative P enrichment in beech roots in the organic layer and mineral layer topsoil of beech (*Fagus sylvatica* L.) forests. Intact soil cores were collected in a P-rich (HP: blue) and a P-poor (LP: red) forest, irrigated with  $^{33}\text{P}$  artificial soil solution and roots from the organic layer (light blue, light red) and the mineral topsoil (dark blue, dark red) were analyzed separately after 24 h of labeling. To determine P root uptake, the rhizospheric  $^{33}\text{P}$  dilutions per forest and layer were used as described under materials and methods. Data indicate means ( $n = 5 \pm \text{SE}$ ). Differences among means per forest and layer were tested by GLM using Poisson distribution. Different letters indicate significant differences at  $p \leq 0.05$ .

tips were higher than those in HP soil ( $p = 0.002$ ), whereas those of non-mycorrhizal root tips were by far lower than those of non-mycorrhizal root tips in HP soil ( $p = 0.015$ , Figure 3A).

To test whether these differences were caused by differences in the abilities for P acquisition in different soil layers, we determined the  $^{33}\text{P}$  enrichment of mycorrhizal and non-mycorrhizal root tips. We calculated the relative enrichment of new  $P_{\text{roottip}}$  with the specific activities of  $^{33}\text{P}$  in the rhizosphere of each soil layer and forest type (Table 1) as described under materials and methods (Eq. 12). The relative enrichment of new P per root tip indicates a mean value for the ability of the mycorrhizal root tips in that soil layer to acquire P (Eq. 15). We define this ability as mycorrhizal P efficiency. P accumulation in non-mycorrhizal root tips was treated correspondingly. The relative accumulation of new P per non-mycorrhizal root tip was low and did not differ between HP and LP (Figure 3B). In the mineral soil of LP, where only very little of the total added  $^{33}\text{P}$  was present (Figure 1F), non-mycorrhizal root tips showed no measurable  $^{33}\text{P}$  enrichment (Figure 3B). In contrast to non-mycorrhizal root tips, the relative enrichment per mycorrhizal root tip showed significant differences between soil layers and

**TABLE 2** | Vitality, ectomycorrhizal (EM) colonization and number of beech root tips (soil core<sup>-1</sup>) in the organic layer (OL) and mineral topsoil (ML) from a P-rich (HP) and P-poor beech (*Fagus sylvatica*) forest.

Site	HP		LP		Forest		OL		ML	
Layer	OL	ML	OL	ML	F	p	t	p	t	p
Vital root tips (%)	91.08 ± 1.44	84.72 ± 1.76	89.95 ± 1.37	84.76 ± 1.81	0.106	0.748	0.899	0.395	0.032	0.98
EM root tips (%)	96.39 ± 1.31	95.19 ± 1.77	97.89 ± 1.25	96.43 ± 1.32	2.271	0.149	1.308	0.227	0.887	0.401
EM (number)	2701.1 ± 1095.2	3287.1 ± 562.5	5324.9 ± 1150.0	1783.0 ± 393.1	0.33	0.575	1.651	0.137	2.388	<b>0.044</b>
Dry (number)	301.8 ± 136.8	615.2 ± 102.5	635.3 ± 164.5	317.1 ± 52.1	0.16	0.899	1.559	0.158	2.593	<b>0.032</b>
Non-EM (number)	11.3 ± 68.0	160.7 ± 39.7	119.2 ± 55.2	56.6 ± 12.3	1.28	0.272	0.533	0.609	2.501	<b>0.037</b>

Data indicate means ( $n = 5 \pm SE$ ). To compare means we used general linear models (GLM) for the overall comparisons between HP and LP (OL and ML as random factor), otherwise Student's *t*-test. Relative data were tested by a GLM using the Poisson distribution. Bold letters indicate significant differences at  $p \leq 0.05$ . Number = number of root tips per layer and soil core with the following dimensions, height: 0.2 m and diameter: 0.12 m.

forest types (**Figure 3B**). In the organic layer, mycorrhizal P efficiency (per root tip) was higher at LP than at HP (**Figure 3B**). In the mineral layer, mycorrhizal P efficiency (per root tip) was lower at LP than at HP (**Figure 3B**). These differences were even more pronounced when the P enrichment in root tips was up-scaled to the whole soil core (**Figure 3C**). At HP, mycorrhizal root tips of the whole root system in the mineral layer exhibited 70% of P enrichment, followed by mycorrhizal root tips of the organic layer (26%), whereas non-mycorrhizal root tips of the mineral layer accumulated only about 4% and of the organic layer less than 0.1% ( $p < 0.001$ ) of the total new P in root tips. At LP, mycorrhizal root tips of the whole root system in the organic layer contributed most to relative P accumulation in root tips (78%), followed by mycorrhizal root tips in the mineral layer (22%), whereas non-mycorrhizal root tips contributed less than 0.1% to P accumulation in root tips in the organic layer and nothing in the mineral layer ( $p = 0.003$ , **Figure 3C**).

If the enrichment of mycorrhizal root tips with new P was an indicator for root P supply, we expected that beech root P uptake is correlated with the abundance of mycorrhizal root tips and their specific enrichment with new P. In accordance with this suggestion, we found a strong relationship between the total amount of new P accumulated in all root tips of a soil layer and the estimated amount of new P taken up by beech roots in that layer (**Figure 4**). We did not find a correlation between the number of root tips and P uptake ( $R = 0.332$ ,  $p = 0.164$ ) indicating that the P efficiencies of mycorrhizal root tips are decisive for beech P acquisition.

### Mycorrhizal Community Structures Differ Between the HP and LP Forests but Not Between the Soil Layers

We investigated whether the observed differences in P acquisition were accompanied by differences in the mycorrhizal communities. Roots in HP and LP forests were colonized by different fungal assemblages (PERMANOVA,  $p_{(forest)} = 0.012$ ). Ascomycota (Hyaloscyphaceae, *Cenococcum geophilum*) were more abundant in LP soil, whereas Basidiomycota of the orders of Russulales and Boletales (several *Lactarius* sp., *Xerocomellus* sp.) were more abundant in the HP soils (**Figure 5**). However, there were some exceptions, *Lactarius blenni* and *Lactarius*

*tabidus* occurred only in the LP forests, whereas *Genea hispidula* was only found in the HP forest (**Figure 5**).

We further analyzed mycorrhizal community structures in different soil layers. *Clavulina cristata* and *Rhizodermea veluwensis* occurred only in LP mineral (**Figure 5**) but these species were rare. Overall, no significant differences between the fungal assemblages in the organic layer and mineral top soil were detected (PERMANOVA,  $p_{(soilayer)} = 0.747$ ).

### Competition of Beech Roots, Understory Roots, and Microbes for P Resources in Different Layers in P-Rich and P-Poor Forests

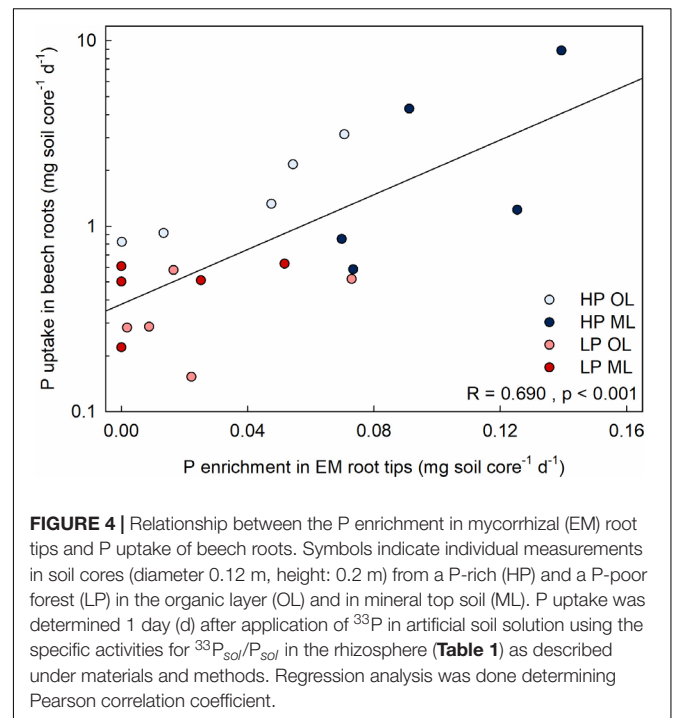
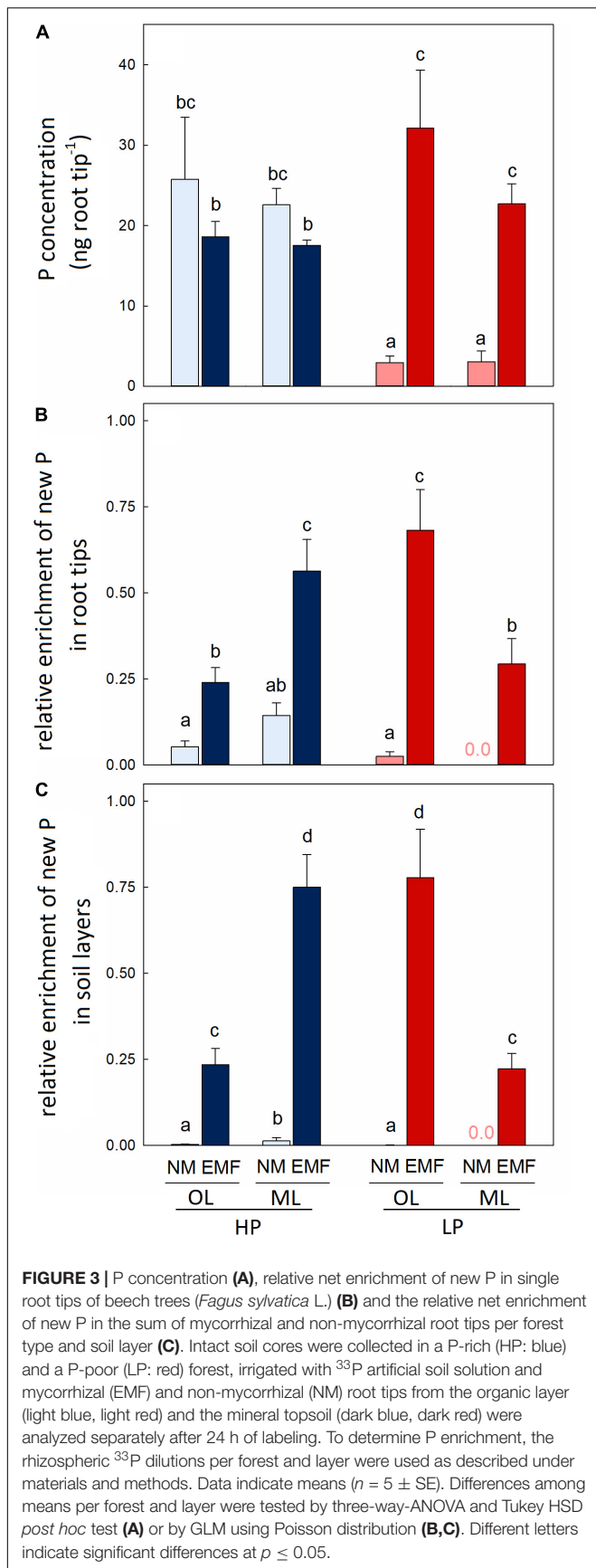
We expected that low P availability would result in strong competition of microbes and other roots with beech roots (i.e., negative values for RCI) and that high P availability would prevent competition. In line with this assumption, we found that the RCI for the competition of beech with microbes together with other roots at HP were not smaller than zero and higher than those at LP (**Figure 6**). Other roots, despite their relatively high abundance in the organic layer at HP (**Figure 1A**), did not significantly affect the competition of beech roots for  $P_i$  (vertical lines in the bars for HP in **Figure 6**). At LP, other roots were too rare to affect the competition between beech roots and microbes. At LP, microbes outcompeted beech roots in both soil layers (**Figure 6**).

## DISCUSSION

### Soil Structure and $P_i$ Availability Impact Beech Root P Uptake

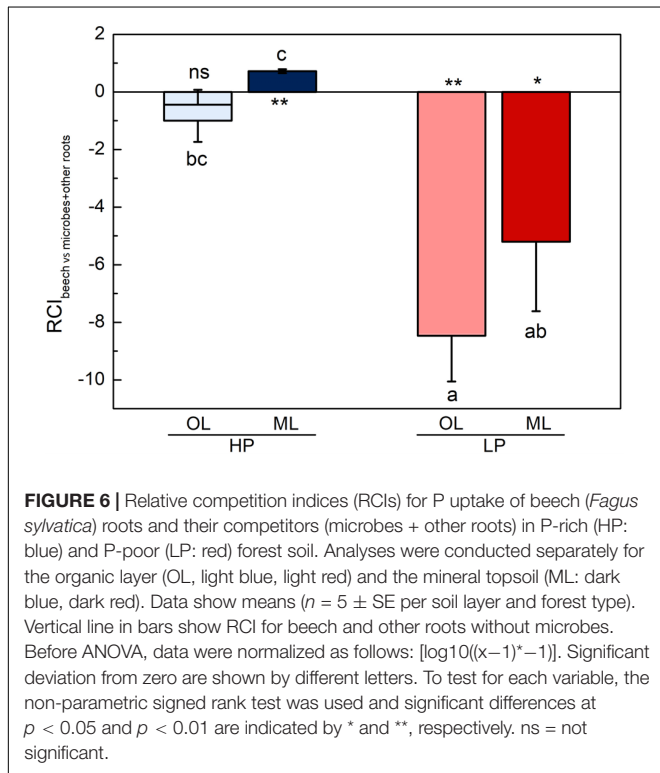
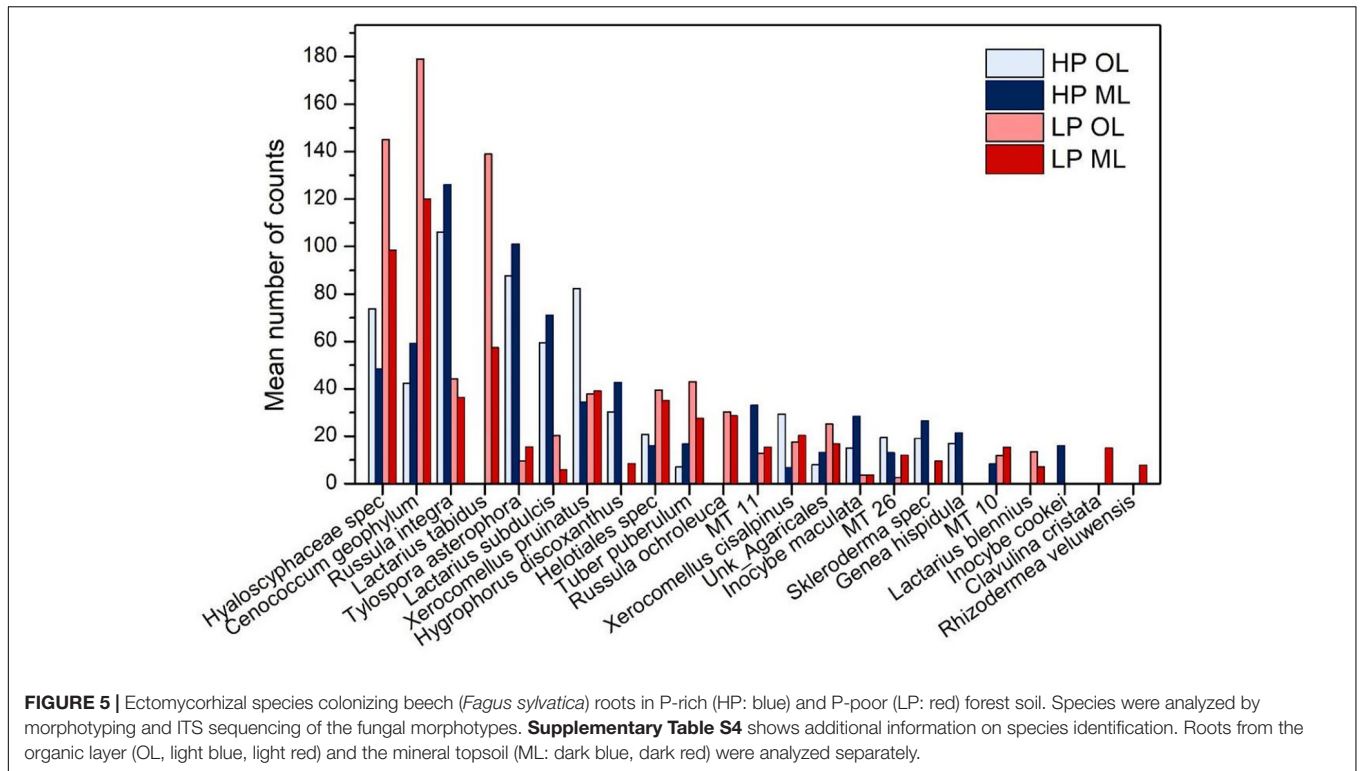
In recent years, the concept of ecosystem nutrition has gained support, which proposes a gradual shift in nutritional strategies from P acquisition to P recycling when P soil stocks are decreasing from P-rich to P-poor forest sites (Lang et al., 2016). According to this model, we expected that bio-available P varied among soil layers of P-rich and P-poor forests, resulting in differences for root P supply. Using undisturbed soil horizons for P tracing, our results support our first hypothesis and comply with previous experiments in mesocosms with young beech





plants (Brandtberg et al., 2004; Jonard et al., 2009; Hauenstein et al., 2018; Spohn et al., 2018; Zavišić and Polle, 2018). It should be noted that our approach might have some limitations because (i) the roots were severed at the edge of the soil cylinders and (ii) only the upper portion of the whole-rooted soil profile was included. Firstly, excised fresh roots are still highly active in P uptake and translocation (Noggle and Fried, 1960; Franklin, 1969; Mejstrik and Benecke, 1969; Rubio et al., 1997). Furthermore, P root uptake in the present study corresponded well to aboveground P translocation in young intact beech trees (Yang et al., 2016; Hauenstein et al., 2018; Zavišić and Polle, 2018). Therefore, we have no evidence for confounding effects of root damage in our experimental set-up. Secondly, based on our experimental unit “soil core,” we estimated a contribution of 25-to-75% of the organic layer-to-mineral layer to P root supply in the P-rich forest. Since a larger root fraction is present below the forest floor and the A horizon in HP (about 80%) than in LP soil (30%, Lang et al., 2017), the ratios for the contribution of soil layers to beech P supply may further diverge in favor of the mineral layer at HP. Our results, thus, underpin an at least twice higher contribution of the organic layer to root P acquisition (60-to-40%) under P-poor conditions. This result strongly supports that the organic layer is critical for P nutrition under P-poor conditions (Lang et al., 2017; Hauenstein et al., 2018) and underpins that our comparison of two sites concurs with field studies along a larger P gradient (Lang et al., 2017).

We demonstrated that the function of the organic layer for root P supply occurred already at a short time scale of 1 day. One reason for this finding is probably that the sorption capacity for different P species is lower in the organic layer in P-poor than in P-rich soil (Lang et al., 2017), thus, resulting in relatively higher P<sub>i</sub>



bioavailability. Our results show that microbes benefited strongly but roots also obtained a higher share of P than one would expected on the basis of the low P stock in the P-poor forest. As

the consequence of high plant and microbial activities (this study; Bergkemper et al., 2016; Hofmann et al., 2016) the P retention of the organic layer was high, despite its lower sorption capacity (Lang et al., 2017).

Our estimation of the relative P partitioning in roots in different layers and soil types was based on the premise that P uptake into roots occurs from the rhizosphere and therefore, we used the rhizospheric dilution of the P tracer for the estimation of root P uptake. This is a novel approach since previous studies considered either the dilution of added P in pooled soil samples (Jonard et al., 2015) or used more specifically tracer dilution in the organic layer and the mineral soil, respectively (Di et al., 2000; Hauenstein et al., 2018; Spohn et al., 2018; Zavišić and Polle, 2018). Our results indicate strong differences for soluble P and consequently for the specific dilution of the tracer ( $^{33}\text{P}/\text{mg } P_{\text{sol}}$ ) between rhizosphere and bulk soil from the organic and mineral layers, respectively. The differences between bulk soil and rhizosphere might have been expected because plant roots and their associated mycorrhizal fungal symbionts exude organic acids, metal-chelating compounds and acid phosphatases that enhance solubilization of P (Leyval and Berthelin, 1993; Wallander, 2000; Casarin et al., 2004; Liu et al., 2007). These biotic activities result in an altered rhizosphere chemistry, in turn leading to higher P availability for mycorrhizal and root uptake (Wallander, 2000; Casarin et al., 2004; Liu et al., 2007). Consequently, we recommend that future studies should consider rhizospheric P availability when estimating root P uptake. Overall, our short-term tracer experiment with forest-grown roots in naturally stratified soil horizons highlight the

prominent role of the organic layer for beech P nutrition under P poor conditions and uncovered heterogeneity for bioavailable P between rhizosphere and bulk soil in different soil layers and forest types.

## **Mycorrhizal P Efficiencies Regulate Beech P Uptake in Different Soil Layers but Do Not Prevent Competition With Microbes in P-Poor Soil**

Roots of beech trees in temperate forests are usually almost completely colonized by a diverse spectrum of different mycorrhizal fungi (Courty et al., 2010; Pena et al., 2010, 2017). The taxonomic composition of ectomycorrhizal fungi differs between P-rich and P-poor beech forests (this study; Zavišić et al., 2016). Notably, the dominant ectomycorrhizal species present in both forest types were characterized by short hyphae of a few micrometers (short-distance exploration types) or by the absence of hyphae (contact types) (Weigt et al., 2012). Among the fungi in our study, only the Boletales, which were not very abundant (approximately 8% of the mycorrhizal root tips), produce long distance hyphae (Agerer, 1987) and may explore regions beyond the rhizosphere. Therefore, mycorrhizal P mobilization in P-rich and P-poor forests was mainly confined to the vicinity of roots. These considerations further underpin that for the assessment of P uptake by mycorrhizal root tips and plant roots, the specific activities of rhizospheric rather than those of bulk soil should be taken into account.

In our study forests, non-mycorrhizal root tips were rarely found and therefore were unimportant for gross host P provision. However, they provided insights into the benefits of P handling by mycorrhizal root tips in naturally composed fungal community. Drastically lower P concentrations in non-mycorrhizal root tips under low than under high P in soil highlight the superiority of mycorrhizas for P acquisition under P limitation. Although the beneficial influence of mycorrhiza for P provision is well-established (Nehls and Plassard, 2018), studies demonstrating mycorrhizal P facilitation in natural soils are scarce. An enhanced P enrichment of mycorrhizal root tips can be regarded as a measure for P efficiency. A relatively stronger enrichment with new P in mycorrhizal compared to non-mycorrhizal root tips, regardless of P availability in the soil, also indicated the predominance for P acquisition of mycorrhizal root tips. Despite lower P efficiency of non-mycorrhizal root tips in P-rich soil, their P concentrations, which are the result of more long-term processes, were similar to mycorrhizal root tips. This suggests that in contrast to P-limited conditions, mycorrhizal and non-mycorrhizal root tips do not compete for P under P-rich conditions.

An obvious question is whether differences in fungal community structures or differences in the regulation of P-uptake in the P-rich and P-poor forest were responsible for differences in plant supply. An ultimate answer to that question is not possible but our finding that similar fungal community structures in organic and mineral soil layers

exhibited different P efficiencies suggests a strong impact of regulatory processes on P uptake. P-limitation leads to an increase in high-affinity P transporters to ensure P uptake, whereas under high P, low affinity transporters maintain P uptake (Wu et al., 2003; Desai et al., 2014; Kavka and Polle, 2016, 2017). Therefore, our initial hypothesis was that low P availability is counteracted by relatively higher P uptake efficiencies than under high P availability. However, in contrast to that assumption relatively higher P efficiencies of mycorrhizal root tips occurred in those soil layers that contributed most to P provision to roots and not in those with lower P availability. The most likely explanation for these findings is that both adaptedness of fungal assemblages to certain habitat conditions together with regulation of P supply in response to plant demand (Zavišić and Polle, 2018) were responsible for the observed root P supply. Since the abundance of root tips alone cannot predict root P uptake, it is obvious that the properties of the symbionts are essential for P acquisition. Here, any contribution of distinct taxa to P provision to the roots remained enigmatic because we used pooled samples. Previous studies have shown large differences among different fungal species for P translocation (Cumming, 1996; Brandes et al., 1998; Colpaert et al., 1999; Jentschke et al., 2001). Field studies in beech forests demonstrated high phosphatase activities in *Lactarius* sp., *Russula* sp., and *Cenococcum* sp. (Courty et al., 2005). These genera were also abundant in our investigation, suggesting a high potential to release  $P_i$  from organic material. In conclusion, the significant relationship between root P uptake and mycorrhizal P enrichment corroborates that mycorrhizal P efficiency governs P provision to the host.

Plant P supply is not only affected by the complex interactions of mycorrhizal properties and geochemical soil properties but usually also by competition with other biota (Curt et al., 2005; Zhu et al., 2016). When nutrients such as P are inadequately available in the soil of natural ecosystems to suffice plant and microorganism requirements, plants are thought to be inferior in the competition (Woodmansee et al., 1981) but some studies have found that plants compete successfully with soil microorganisms (Schimel and Bennett, 2004) or are even superior under specific circumstances (Wang and Lars, 1997). Our study did neither reveal competition between beech roots and microbes nor with other understory roots in P-rich soil, even though the biomass of other roots was considerable and the biomass of microbes about 10-fold higher in the P-rich than in the P-poor forest (Bergkemper et al., 2016; Lang et al., 2017). The absence of P competition in P rich soil underpins P sufficiency for biological processes. By contrast, the competition between beech roots and microbes was strong in P-poor soil and might have precluded the establishment of further understory species. An unexpected result was that the competition strength did not differ between organic and mineral soil layers. This observation was surprising since we anticipated lower competition in the organic layer with higher P availability than in the mineral soil where P availability was lower. Possibly, the regulation of P uptake into roots by different

mycorrhizal efficiencies balances nutrient competition across different soil layers. This idea should be followed up in future studies.

## CONCLUSION

This study reveals that P uptake by plant roots is not only based on soil P stocks but differs between different layers, underlies regulation by mycorrhizal efficiencies, and faces competition with soil microbes under P-limiting conditions. Revisiting our hypotheses, we confirmed that (i) the main P source for beech P supply is the mineral topsoil under P-rich and the organic layer under P-poor conditions. Our results pinpoint different mycorrhizal efficiencies as drivers for root P uptake. As expected non-mycorrhizal root tips did not contribute markedly to root P supply in natural forest soils. However, the simplistic idea (hypothesis ii) that the mycorrhizal P enrichment is relatively higher to compensate for lower P bioavailability can be refuted because the relative mycorrhizal P enrichments were highest in those layers that contributed most to root P uptake. In agreement with our hypothesis (iii) no competition for P was detected in soil of the P-rich forest, whereas microbes were strong competitors under P-poor conditions.

In accordance with previous studies (Lang et al., 2017; Hauenstein et al., 2018), we conclude that an intact forest floor is of tremendous importance under nutrient-poor conditions for uptake and recycling of P. Our study provides insights into the mechanisms that contribute to the adaptation of ecosystems to low P availability. Obviously, forests that rely on P recycling with the majority of roots in the upper layer are at risk under climate change. When the duration and intensity of summer droughts increase, the top layer roots and microbes will be the first to be negatively affected by water shortage. Further depletion of P is expected due to lower P mineralization rates (Schimel et al., 2007) and lower P mobility in drier soil (Schachtman et al., 1998; Kreuzwieser and Gessler, 2010). This study envisages decreasing P retention as the result of declining microbial biomass and declining mycorrhizal activities in future climate scenarios.

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## DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the Dryad digital repository (<https://doi.org/10.5061/dryad.4j0zpc87g>). The sequences for identified mycorrhizal fungi are available in GenBank (accession numbers in **Supplementary Table S4**).

## AUTHOR CONTRIBUTIONS

SC and AP contributed to the conception and design of the study and contributed to manuscript revision and approved the final version. SC conducted the measurements, analyzed the data, and wrote the first draft of the manuscript. AP revised the draft.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2020.00054/full#supplementary-material>



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**Conflict of Interest:** 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.

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