PROF. ANDREA POLLE (Orcid ID : 0000-0001-8697-6394)

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Carbohydrate depletion in roots impedes phosphorus nutrition in young forest trees

Simon Clausing[1] https://orcid.org/0000-0003-3640-5280,

Rodica Pena[1] https://orcid.org/0000-0002-7985-6906,

Bin Song[1],

Karolin Müller[2],

Paula Mayer-Gruner[2],

Sven Marhan[2], https://orcid.org/0000-0002-8954-5968

Martin Grafe[3],

Stefanie Schulz[3] https://orcid.org/0000-0001-5520-8106,

Jaane Krüger[4],

Friederike Lang[4],

Michael Schloter[3] https://orcid.org/0000-0003-1671-1125,

Ellen Kandeler[2], https://orcid.org/0000-0002-2854-0012,

Andrea Polle[1*] https://orcid.org/0000-0001-8697-6394

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[1]Forest Botany and Tree Physiology, University of Goettingen, Büsgenweg 2, 37077 Göttingen, Germany

[2]Soil Biology Department, Institute of Soil Science and Land Evaluation, University of Hohenheim, Emil-Wolff-Straße 27, 70593 Stuttgart, Germany

[3]Research Unit for Comparative Microbiome Analyses, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

[4]Soil Ecology, University of Freiburg, Bertoldstraße 17, 79085 Freiburg (i. Br.), Germany

*correspondence: A. Polle: apolle@gwdg.de, phone +49 551 3933480

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Summary

- Nutrient imbalances cause the deterioration of tree health in European forests, but the underlying physiological mechanisms are unknown. Here, we investigated the consequences of decreasing root carbohydrate reserves for phosphorus (P) mobilization and uptake by forest trees.
- In P-rich and P-poor beech (*Fagus sylvatica*) forests, naturally grown, young trees were girdled and used to determine root, ectomycorrhizal and microbial activities related to P mobilization in the organic layer and mineral topsoil in comparison with those in non-girdled trees.
- After girdling, root carbohydrate reserves decreased. Root phosphoenolpyruvate carboxylase activities linking carbon and P metabolism increased. Root and ectomycorrhizal phosphatase activities and the abundances of bacterial genes catalysing major steps in P turnover increased, but soil enzymes involved in P mobilization were unaffected. The physiological responses to girdling were stronger in P-poor than in P-rich forests. P uptake was decreased after girdling. The soluble and total P concentrations in roots were stable, but fine root biomass declined after girdling.
- Our results support that carbohydrate depletion results in reduced P uptake, enhanced internal P remobilization and root biomass trade-off to compensate for the P shortage. Since reductions in root biomass render trees more susceptible to drought, our results link tree deterioration with disturbances in the P supply as a consequence of decreased belowground carbohydrate allocation.

Key Words: European beech, carbohydrates, ectomycorrhiza, microbes, phosphorus deprivation, phosphatase, tree nutrition

Introduction

Large-scale surveys across the European continent detected declining forest productivity and deterioration of tree mineral nutrition in the past two decades (Wardle, 2004; Ilg et al., 2009; Trichet et al., 2009; Jonard et al., 2015; Talkner et al., 2015). Changes in tree nutrition were particularly pronounced for phosphorus (P) (Duquesnay et al., 2000; Ilg et al., 2009; Talkner et al, 2015). At the ecosystem level, increasing constraints on forest P nutrition have been related to anthropogenic pollution and climate change (Duquesnay et al., 2000; Prietzel & Stetter, 2010; Lang et al., 2016; Augusto et al., 2017), via low P mineralization and mobility in dry soil (Schachtman et al., 1998; Schimel et al., 2007; Kreuzwieser & Gessler, 2010), soil acidification, and N deposition (Vitousek & Howarth, 1991; Penuelas et al., 2013). However, the physiological processes that regulate tree P supply along the soil-root continuum of forest trees are not fully understood.

In forest soil, bioavailable P (inorganic P, P_i) is scarce because P_i has low solubility, is bound by soil minerals and is replenished slowly from recalcitrant P pools (Holford, 1997). P mobilization can be achieved by ion exchange and by the recycling of organically bound P (Lambers et al., 2015; Lang et al., 2017). Common physiological mechanisms used to increase P bioavailability are the exudation of organic acids and extracellular acid phosphatases by plant roots, root-associated mycorrhizal fungi, and soil microbes (Kandeler, 1990; Schneider et al., 2001; Uroz et al., 2007; Kluber et al., 2010; Nannipieri et al., 2011; Pritsch & Garbaye, 2011; Spohn et al., 2013).

Trees engage two processes to cope with P shortage: they enhance soil P mobilization and uptake capacity (Desai et al., 2014; Kavka & Polle, 2016), and they tighten internal P cycling by growth adjustment and internal P mobilization (Netzer et al., 2018; Zavišić & Polle, 2018). At the molecular level, P deprivation results in the increased expression of P-related enzymes and of enzymes involved in carbohydrate and energy metabolism (Misson et al., 2004; Misson et al., 2005; Gan et al., 2016; Kavka & Polle, 2017; Png et al., 2017). The enzyme PEPC (phosphoenolpyruvate carboxylase) is a hub for P and carbon metabolism, catalysing the release of P and the production of oxaloacetate from phosphoenolpyruvate and bicarbonate (López-Arredondo et al., 2014). Oxaloacetate is the precursor of malate, a main compound in root exudates used for P mobilization (Richardson et al., 2011; Meier et al., 2020). PEPC activity is strongly induced by P starvation (Peñaloza et al., 2004; Shane et al., 2013), thereby driving internal P recycling and the production of organic acids.

The activities of soil microbes, which are key to nutrient mobilization for plants (Bucher, 2007; Jacoby et al., 2017; Bargaz et al., 2018; Nehls & Plassard, 2018), crucially depend on their supply of photoassimilates via root exudates (Heinonsalo et al., 2004; Cairney, 2011; Becquer et al., 2014; Johri et al., 2015; Kaiser et al., 2015; Nehls & Plassard, 2018). A shortage of labile carbon in the soil caused by the girdling of trees, for example, results in decreased soil respiration, the altered composition of mycorrhizal and microbial communities, and changed soil enzyme activities (Heinonsalo et al., 2004; Pena et al., 2010; Kaiser et al., 2015). In contrast, enhanced carbon availability after the addition of glucose to soil increases microbial phosphatase activities (Högberg et al., 2003; Spohn et al., 2013). Despite the tight links between the belowground allocation of plant assimilates and the activities of soil microbiota, on the one hand, and the importance of microbes for P mobilization, on the other hand, it is unknown whether the P acquisition abilities of trees depend on their carbohydrate resources. Since ectomycorrhizal fungi are crucial to the plant P supply (Lambers et al., 2008; Nehls & Plassard, 2018) and thrive on plant-derived carbohydrates, we expected to observe a relationship between carbohydrate availability and P nutrition.

Here, we investigated whether plant carbohydrate resources are important for P nutrition in temperate beech (*Fagus sylvatica*) forests. We interrupted the belowground allocation of photoassimilates by girdling. By comparing the processes in non-girdled controls and girdled trees, we disentangled the effects of belowground plant-derived carbohydrates on P uptake, P concentrations and enzyme activities related to P mobilization in roots, ectomycorrhizas and soil as well as the abundance of bacterial functional genes important for P cycling. Root soil exploration as well as microbial and mycorrhizal activities are vertically stratified, with strong differences between P-rich and P-poor forest ecosystems (e.g., lower root biomass and lower ectomycorrhizal activities in the organic layer than in the mineral layer in P-rich forests compared with those in P-poor forests, Jonard et al., 2009; Zavišić et al., 2016; Lang et al., 2017; Clausing & Polle, 2020). Furthermore, young beech trees growing in P-poor forest soils show lower photosynthesis rates than those growing in P-rich forest soils (Yang et al., 2016). Therefore, we expected stronger negative effects of carbon starvation on root metabolism and associated soil processes in P-poor than in P-rich soil. Here, we studied the consequences of carbon starvation on P metabolism and root biomass in the forest floor and in the mineral topsoil in two well-characterized forest ecosystems that differ strongly in P stocks (Lang et al. 2017). We addressed the following specific hypotheses. (i) Carbohydrate depletion leads to a decrease in root P concentrations and an increase in the enzyme activities required for internal P mobilization in roots (PEPC, phosphatase) as well as for P mobilization from soil (mycorrhizal phosphatases). (ii)

Since soil microbes are not directly reliant on root carbohydrates (Kaiser et al., 2010), microbial phosphatases are unaffected by girdling. (iii) The consequences of root carbon starvation on P nutrition are stronger in soils with low P availabilities than in soils with high P availabilities because the mobilization of scarce P requires higher resource investment than that of sufficient P.

Materials and Methods

Site characteristics and study plots

The study was conducted in two beech (*F. sylvatica* L.) forests, both stocking on silicate rock but differing in total P stocks (160 and 900 g P m^{-2} in P-poor and P-rich forests, respectively, down to 1 m soil depth). The P-rich (HP) site Bad Brückenau (BBR) is located in the biosphere reserve 'Bayerische Rhön' (50°21'7.2"N 9°55'44.5"E, 801 to 850m a.s.l.). The mean long-term sum of annual precipitation is 950mm, and the long-term mean annual temperature (1981-2010) is 6.1°C. The average tree age at this beech stand is 137 years (LWF 2016). The soil is a Dystric Skeletic Cambisol (Hyperhumic, Loamic) (WRB 2015) derived from basalt. The P-poor (LP) forest site is situated in the district Celle in Lower Saxony (52°50'21.7"N 10°1.6'2.3"E, 115 m a.s.l.) and is stocked with approximately 120-year-old beech trees (BMEL 2016). The mean annual temperature (1981-2010) at this site is 8.6°C, and the mean annual sum of precipitation is 899mm (BMEL 2016). The soil is a Hyperdystric Folic Cambisol (Areni, Loamic, Nechic, Protospodic) derived from sandy till substrate. Further details are reported by Lang et al. (2017).

For this study, three girdling plots were installed in the HP and four in the LP forest (HP: 12.05.2017, LP: 05.05.2017) in gaps with a minimum distance of 5 m between large trees. Each plot had an area of 4 m² and was separated from the surrounding soil by a 0.25-m-deep trench to prevent the roots of the mature beech trees from affecting the study. Each plot was divided into two equally-sized subplots by inserting a lawn edge into the soil. The understory was removed. The HP plots contained 0.9 mg P_{tot} g^{-1} dry mass in both the organic layer and the mineral topsoil and had pH values ranging from 3.9 to 4.3 (Table 1). The LP plots contained 0.2 mg and 0.02 mg P_{tot} g^{-1} dry mass in the organic layer and mineral topsoil, respectively, and had pH values ranging from 3.3 to 3.5 (Table 1). Additional information on soil and plant nutrient concentrations is provided in Supplemental Table S1.

The young, naturally regenerated beech trees stocking on the plots had an average height of 2 m and an average diameter of 24 mm in HP and a height of approximately 4 m and a diameter of 16 mm in LP. The mean number of trees per plot ranged from 15 to 25. In July 2017, all trees on one half of the plots were girdled by removal of a 20-mm-wide strip of bark at a height of 0.4 m from the ground (HP: 18.07, LP: 17.07). The other half of each plot was used as an untreated control.

Harvest and processing of soil cores

In each subplot, 8 soil cores (diameter 55 mm, depth 0.21 m) were sampled one week (HP: 25.07.2017, LP: 24.07.2017) and eight weeks (HP: 27.09.2017, LP: 20.09.2017) after girdling. A schematic overview on the sampling procedure is provided in Supplemental Fig. S1. The soil cores were separated into organic and mineral topsoil layers. The average depth of the organic layer was 60 mm in the LP and 30 mm in the HP forest. Each sample was further fractionated into bulk soil, rhizosphere soil, fine roots (<2 mm) and coarse roots (>2 mm) in the field. We defined rhizosphere soil as soil adhering to roots. The rhizosphere soil was collected by streaking the adhering soil from the roots with a paintbrush. All fractions were immediately weighed. Bulk soil, rhizosphere soil and roots were divided into three aliquots directly in the field: a fresh aliquot that was kept cool at 4°C until use, an aliquot that was immediately frozen in liquid nitrogen and stored at -80°C (roots) or -20°C (soil), and an aliquot that was dried (40°C, 14 days). Bulk soil was sieved (mesh width: 4 mm) before the aliquots were prepared.

Ectomycorrhizal (EMF) morphotyping, species identification, and extracellular phosphatase activities

The beech roots were gently washed using 4°C precooled tap water, spread in water in a glass dish, and examined under a stereomicroscope (Leica M205 FA, Wetzlar, Germany). The root tips were classified as either vital EMF, vital nonmycorrhizal or dead root tips.

The EMF root tips were categorized into morphotypes using the identification keys of Agerer (1987-2012). We collected the morphotypes, which were present on at least three root tips per sample. Mycorrhizal species identities were determined after DNA extraction and ITS sequencing (Pena et al. 2017). The sequences were analysed with the Staden package (http://staden.sourceforge.net), BLASTed against the NCBI GenBank (www.ncbi.nlm.nih.gov) and UNITE (unite.ut.ee) databases and deposited in the NCBI GenBank MN970515 to MN970525 (Supplemental Fig. S2). Species richness, Shannon index, and evenness were determined with PAST 4.03 (https://folk.uio.no/ohammer/past/) (Hammer et al., 2001).

Individual EMF root tips, each assigned to a morphotype, were collected, and the extracellular acid phosphatase (EC 3.1.3.2) activity was determined with fluorescent 4-methylumbelliferone (MUF) phosphate at pH 4.5 using a high-throughput microplate fluorometric assay (Pritsch et al. 2011). Afterwards, the root tips were scanned, and the activity was related to the tip surface. The root tip collection and enzyme activity measurements were performed within 48 h of sampling time. A detailed description of fungal species identification and enzyme activity measurements can be found in Supplemental Methods S1.

Quantitative real-time PCR assays of P cycle-related genes in bulk soil

For nucleic acid extraction, a phenol-chloroform-based protocol, modified according to Stempfhuber et al. (2017), was used to extract total genomic DNA from 0.5 g frozen bulk soil. The extracts were used to determine the abundance of seven bacterial genes that code for enzymes catalysing important steps in P turnover, including *pitA*, a constitutively expressed P transporter, *pstS*, a P transporter involved in the P starvation response, four genes [*phoD, phoN, phnX, appA*], which encode enzymes with phosphatase activities, and *gcd*, which solubilizes P by the oxidation of glucose and aldose sugars (Supplemental Table S2) using the primers described by Bergkemper et al. (2016). The genes *phoD, phoN, appA* and *phnX* encode extracellular enzymes, while the transporters *pitA*, *pstS* and *gcd* are periplasmic. The 16S rRNA gene served as a proxy for the overall bacterial biomass (Bach et al., 2002). A detailed description of the methods, including the thermal profiles of the PCR, the source of the standard, and the primers used, is shown in Supplemental Methods S1.

Enzymatic activities in fine roots, bulk soil and rhizosphere soil

Frozen fine roots were milled and used for the preparation of protein extracts and the analysis of potential enzymes (Supplemental Methods S1). Acid phosphatase (EC 3.1.3.2) and phosphoenolpyruvate carboxylase (PEPC) (EC 4.1.1.31) activities in root extracts were measured spectrophotometrically (Bergmeyer, 2014). A detailed description can be found in Supplemental Methods S1.

The rhizosphere and soil enzymes were extracted from fresh soil that had been stored frozen at -20 °C (Supplemental Methods S1). Enzyme activities in the soil and rhizosphere (using MES buffer at pH 6.1 for L-leucine peptidase, α-D-glucosidase, β-D-glucosidase, xylosidase, N-acetyl-glucosaminidase, acid phosphomonoesterase and phosphodiesterase; MUB buffer at pH 6.1 for acid phosphomonoesterase; and MUB buffer at pH 11 for alkaline phosphomonoesterase) were determined with fluorescent 4-methylumbelliferone (4-MUF) and L-leucine peptidase with 7-amino-4-methylcoumarin-linked substrates (Sigma Aldrich, St. Louis, USA) (Marx et al., 2001) in soil suspensions (for details, see Supplement Methods S1). The phosphomonoesterases activities were determined in both MES buffer at pH 6.1 and MUB buffer at pH 6.1 to account for potential differences caused by the buffer system, but no effects were observed. Phosphomonoesterases (MES pH 6.1) are, further on, called acid phosphatases.

Phenoloxidase and peroxidase activities were determined using 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) with MUB buffer at pH 3 in soil and rhizosphere suspensions (Floch et al., 2007; Bach et al., 2013). A detailed description can be found in Supplemental Methods S1. Using the dry-to-mass ratio of soil, the enzyme activities were expressed on the basis of the dry mass.

Total and soluble phosphorus in roots and soil

Dry soil and root samples were milled in a ball mill (Retsch) to a fine powder. For determination of total P (P_{tot}), approximately 50 mg powder was weighed and extracted in 25 ml 65% HNO_3 at 160°C for 12 h (Heinrichs et al. 1986). For the determination of soluble P (P_{sol}), approximately 100 mg of powder was extracted in 150 ml Bray-1 solution (0.03 N NH_4F, 0.025 N HCl) for 60 min on a shaker at 180 rpm (Bray & Kurtz, 1945). The extracts were filtered using phosphate-free filter paper (MN 280 ¼, Macherey-Nagel, Düren, Germany) and used for elemental analysis by inductively coupled plasma–optical emission spectroscopy (ICP-OES) (iCAP 7000 Series ICP–OES, Thermo Fisher Scientific, Dreieich, Germany) (Clausing & Polle, 2020).

The P stocks in the soils (depth 0.21 m) were determined by multiplying the soil P concentrations with the total soil dry mass of the soil cores. The P stocks in the fine roots in the soil cores were calculated by multiplying the P root concentrations with the total fine root dry mass in the soil cores. To derive P stocks per area, cross sections of the soil cores (0.152 m^{-2} for 8 soil cores) were used.

Microbial phosphorus

For microbial P (P_{mic}) determination, the soil samples were divided into three subsamples. Two subsamples were extracted by hexanol fumigation (with and without spiking with a P standard). The third subsample was extracted by deionized water to obtain soluble P_i (Kouno et al., 1995). P_{mic} was obtained by the subtraction of water-soluble P_i and correction for the recovery, as described in detail in Supplemental Methods S1.

Carbohydrate concentrations in roots

Frozen fine root powder (described above) was extracted in dimethylsulfoxide 25% HCl (80%:20%) and then used for enzymatic carbohydrate analyses (Bergmeyer, 2014), in a spectrophotometer at 340nm and 25°C. The analysis is based on the subsequent enzymatic conversions of fructose, sucrose and starch into glucose, the concentration of which is determined by the formation of NADPH. The details are described in Supplemental Methods S1.

Soil pH and water-extractable organic carbon (WEOC)

The pH values were measured by suspending field-moist, sieved soil in 0.01 M $CaCl_2$ (1:5 soil-to-solution ratio) after 16 h of equilibration (ISO10390, 2005). For the determination of water-extractable organic carbon (WEOC) field-moist, sieved samples were suspended in deionized water (EC <0.06 µS cm^{-1}) at a soil-to-solution ratio of 1:5. After 16 h of equilibration, the suspensions were membrane-filtered at 0.45 µm (cellulose-nitrate, Sartorius, Göttingen, Germany), and WEOC was measured using a TOC analyser (multi N/C® 2100S, Analytik Jena, Jena, Germany).

Microbial biomass by fatty acid determination

Fatty acid methyl esters (FAMEs) were extracted by using the protocol of Frostegård et al. (1993). We used the following PLFAs as specific biomarkers for microbial groups: i15:0, a15:0, i16:0 and i17:0 for gram-positive bacteria; cy17:0 and cy19:0 for gram-negative bacteria (Frostegård et al., 1993); and 18:2ω6,9 for fungi (Frostegård & Bååth, 1996). The sum of these markers plus 16:1ω7 was used as a proxy for the total microbial biomass (Frostegård & Bååth, 1996). A detailed description can be found in Supplemental Methods S1.

Phosphorus uptake of fine roots determined by radioactive labelling

To determine the P uptake of fine roots, a radioactive labelling experiment with $H_3{}^{33}PO_4$ (Hartmann Analytic GmbH, Braunschweig, Germany) was conducted under laboratory conditions. To test our hypothesis with an independent experiment, we collected 20 young beech trees (height: 0.5 m, stem diameter 5 mm, measured 0.1 m above ground) with an intact soil core (tube: height: 0.2 m x diameter: 0.12 m) from another beech forest (Billingshäuser Schlucht, coordinates: 51°34'43.8"N 9°59'04.8"E, 308 m a.s.l, Göttingen, Germany). The mineral topsoil texture consisted of 59% silt, 38% clay and 3% sand (Brumme & Khana, 2009). The average pH_{KCl} of the organic layer was 5.16 and that of the mineral topsoil was 5.54, with a P concentration of 0.53 mg g^{-1} in the organic layer and 0.63 mg g^{-1} in the mineral topsoil (Brumme & Khana, 2009). The plants were left in the tubes with intact soil cores and were transported to the experimental garden at the University of Goettingen. The plants were acclimated for one month under field conditions and watered regularly before the uptake experiments started (Collection: 13.05.2019, labelling experiments from 13.06.2019 to 25.6.2019). Half of the trees were girdled as described above; the other half remained untreated. The trees were used for uptake experiments one week after girdling. For this purpose, a beech tree with an intact root system was cautiously removed from its pot and washed carefully to rinse off all soil particles. Then, a selected root was exposed for 3 h in 2 ml artificial soil solution (after Gessler et al., 2009) containing 1 KBq $^{33}PO_4$ (Hartmann Analytic GmbH, Braunschweig, Germany). Thereafter, the submersed part of the root (exposure part) and the subsequent root segment of approximately 10 mm (transport part), which was not in contact with the uptake solution, were separately cut off, washed with artificial soil solution, dried, combusted, mixed with scintillation cocktail (Rotiszint eco plus, Roth, Karlsruhe, Germany), and used to measure the radioactivity of the ^{33}P. A total of seven girdled and seven untreated trees were analysed using three fine roots per tree. The details of the exposure experiments are described in Supplemental Methods S1.

Statistical analyses

The statistical analyses were performed with R version 3.6.0 (R Core Team 2012). The normal distribution and homogeneity of variances were tested by analysing the residuals of the models and performing a Shapiro-Wilk test. Data were logarithmically or square-root-transformed where necessary to meet the criteria of the normal distribution and homogeneity of variances. Since the plots were separated into girdled and non-girdled subplots, we used a paired test to determine the girdling effect. The test was conducted with the originally measured data. The graphs show response ratios calculated as the means of $plot_{x(girdled)}$ / $plot_{x(non\text{-}girdled)}$. To determine the effects of the forest type, soil layer, sampling dates, and treatment, linear mixed effect models (´*lmer*´, R package lme4) were used with plot as random factor. Pairwise comparisons of the sample means were conducted using Tukey's HSD (package: '*multcomp*'). Means were considered to be significantly different from each other when $p \leq 0.05$, and differences with $p \leq 0.1$ were considered to indicate a trend. Data are shown as the means (HP: n = 3; LP: n = 4) and standard errors (±SE), if not indicated otherwise. The function ´*anosim*´ from the vegan package (Oksanen et al., 2019) was used to test differences among the community composition of mycorrhizal fungi for the following factors: forest type, treatment, and harvest time point.

Results

Girdling decreases root carbohydrate status and activates PEPC

The non-structural carbohydrate concentrations (determined as sum of starch, glucose, fructose and sucrose) in fine roots declined after girdling (Fig. 1). Overall, roots in the organic layer already showed significant decreases in carbohydrate concentrations one week after girdling ($F = 48.19$, $p < 0.001$); this decline was particularly strong in the HP forest (Interaction $F = 15.99$, $p = 0.002$, Fig. 1a). The decreases were less pronounced in the mineral layer ($F = 4.74$, $p = 0.064$, Fig. 1b). Eight weeks after girdling, roots in both soil layers from both the HP and LP forests contained significantly lower carbohydrate concentrations than did the roots of non-girdled trees (Fig. 1a, b).

In general, the fine root carbohydrate concentrations were higher in roots from the organic layer of the HP than in those from the LP forest (F = 70.20, p < 0.001), whereas no significant differences were found between HP and LP roots in the mineral topsoil (F = 0.05, p = 0.822). The carbohydrate concentrations were not affected by season (organic layer: F = 3.14, p = 0.088, mineral topsoil: F = 0.12, p = 0.730, i.e., sampling one and eight weeks after girdling corresponding to summer (July) and early fall (September), respectively) with the exception of the mineral layer in the HP forest.

Since girdling showed the strongest effects after eight weeks, we used this time point to test the effect of carbon depletion on PEPC activity in roots. We found significant increases in PEPC activities in roots of girdled trees compared with control trees in both soil layers and at both study sites (means across all site conditions +26 ± 4%, F = 37.36, p < 0.001, Fig. 2).

Girdling stimulates acid phosphatase activities in roots and EM fungi but has a moderate impact on microbial P mobilization in soil

To test whether girdling affected P mobilization in beech roots or from soil, we determined the intrinsic acid phosphatase activities in fine roots, the extracellular phosphatase activities on the mycorrhizal hyphal mantle surfaces and the soil-residing acid phosphatase activities. We also determined the gene abundances of P-related enzymes in soil microbes. Since most of these variables differed between the HP and LP forests and between different soil layers and time points of harvest, we focused on the girdling effects by investigating the response ratios of girdled/control treatments (the means of the original data and statistical information can be seen in Supplemental Table S3).

After girdling, the response ratios of root phosphatase activities were consistently enhanced at the LP site, regardless of soil layer or time point, whereas the HP roots from the organic layer showed a strong enhancement only one week after girdling and the HP roots from the mineral topsoil showed a moderate enhancement eight weeks after girdling (Fig. 3).

Girdling further caused the strong enhancement of the extracellular phosphatase response ratio for ectomycorrhizal root tips in both soil layers in the LP forest (Fig. 3b, d) but not in the HP forest (Fig. 3a, c). The ectomycorrhizal colonization of root tips and the community composition of the ectomycorrhizal fungi were unaffected by girdling (ANOSIM: R^2 = 0.418, p > 0.05, Supplemental Fig. S2), with the exception of the LP mineral topsoil, where mycorrhizal species richness significantly decreased from an average of 10 to 5 species eight weeks after girdling (Supplemental Table S4). The mycorrhizal fungal species composition differed between the HP and LP forests (ANOSIM: $R^2 = 0.135$ $p \leq 0.05$) and between the two harvest time points (ANOSIM: $R^2 = 0.988$, $p < 0.05$, Supplemental Fig. S2).

Unlike phosphatases in roots and mycorrhizas, the response ratios of acid phosphatase activities in the rhizosphere and bulk soil were unaffected by girdling (Fig. 3). Similarly, acid phosphodiesterase (pH 6.1) and alkaline phosphatase activities (monophosphoesterase pH 11) did not increase in response to girdling (Supplemental Fig. S3). In agreement with these results, no changes in the abundance of genes for microbial P mobilization were detected in the HP organic layer or in the mineral topsoil (Fig. 4a, c). Only for *gcd* and for *pitA* were late responses to girdling in the HP soil observed (Fig. 4a). In contrast to the results of the HP soils, the response ratio of gene copy numbers for P transporters and P mineralization of soil microbes (*pitA, pstS, phoD, phoN, phnX*) showed a transient increase one week after girdling in the LP forest (Fig. 4b). This response was confined to the organic layer, whereas none of those genes in the mineral soil was significantly affected by girdling (Fig. 4d). In general, the HP and LP forest soils differed strongly in the abundance of the analysed genes (*pitA, pstS, appA, phnX, phoD, phoN, gcd*) with higher copy numbers in both soil layers (organic layer: $F = 58.84$, $p < 0.001$, mineral topsoil: $F = 9.93$, $p = 0.005$) of the HP than the LP forest.

We did not find any significant effects of girdling on PLFA biomarkers for bacterial and fungal biomass (Supplemental Fig. S4); but, in agreement with the higher copy numbers for genes driving P turnover in HP than in LP soils, the microbial biomass was also higher in HP than LP soils (Supplemental Table 3).

We also measured soil enzyme activities involved in carbon or nitrogen mineralization (Supplemental Table S5). We found no significant increases in response to girdling (Supplemental Fig. S3). However, many of the carbon-related enzymes in the rhizosphere (organic layer) showed trends towards increased activities eight weeks after girdling (Supplemental Fig. S3e).

P concentrations are stable in roots and soil, while root biomass and P uptake decline

Girdling did not affect the P concentrations (P_{tot}, P_{sol}) in bulk soil, in the rhizosphere or in microbes (P_{mic}) (Table 1). The root P concentrations were also unaffected by girdling, with the exception of soluble P in fine roots in the organic layer of the HP forest (Table 1). One week after girdling, an approximately two-fold decline occurred ($F = 25.26$, $p = 0.037$), but the resulting P concentration was still higher than that of the fine roots in the LP forest (0.18 ± 0.02 mg P_{sol} g^{-1} dry mass, Table 1) and recovered after eight weeks.

We found that root biomass decreased in response to girdling, especially in the LP forest (Supplemental Table S4). Consequently, the stock of P present in roots was strongly reduced by girdling (Fig. 5). In the HP forest, the initial decline was moderate and significant after eight weeks, whereas in the LP forest, a strong decline was already apparent one week after girdling (Fig. 5). While the fine root biomass decreased in response to girdling, the fraction of vital root tips of the remaining roots was unaffected at the early time point and only slightly decreased (-7%) eight weeks after girdling (Supplemental Table S4). In contrast to roots, the stock of P in soil and the stock of P_{mic} were unaffected by girdling (Supplemental Table S6).

To test whether root P uptake was affected by girdling, we conducted an independent labelling experiment with young beech trees under controlled conditions. One week after girdling, roots attached to beech trees were exposed to $^{33}P_i$ in artificial soil solution. The $^{33}P_i$ uptake of girdled plants was only half of that of the roots of the non-girdled trees (Fig. 6). In both girdled and non-girdled plants, approximately 20% of the total measured ^{33}P uptake was present in the transport segment (not in contact with the labelling solution), showing that girdling reduces P uptake but not translocation (Fig. 6).

Discussion

Carbohydrate depletion affects P mobilization and the plant P supply

In this study, we investigated the links between root carbohydrate resources, P uptake and P mobilization in forest soils. In agreement with previous studies (Druebert et al., 2009; Pena et al., 2010; Krause et al., 2013; Jing et al., 2015), girdling caused a strong reduction in soluble sugars, particularly starch, in fine roots. Girdling blocks the carbon supply from the canopy almost completely; therefore, fine root metabolism must rely on stored compounds. An important novel result of our study was that these responses occurred relatively fast, within one week after girdling in the upper soil layer, and were stronger in roots with higher starch contents than in those containing less starch. This finding suggests compensatory resource use in trees from the HP forests, which was precluded in the LP forest due to low resource availability. Consequently, the young trees at LP, which contained lower carbohydrate reserves and P stocks in their root systems, suffered from greater root biomass loss than did those in the HP forest. In our earlier studies, we found that the photosynthesis rates of trees in LP soil is suppressed (Yang et al., 2016) and can be rescued by P fertilization (Zavišić et al., 2018), supporting that the lower availability of photoassimilates in LP than in HP trees is caused by P limitation. To cope with low P availability, LP beech trees rely on internal P recycling and adjust their growth accordingly (Netzer et al., 2018; Zavišić & Polle, 2018). Therefore, the decline of sugars along with the drastic loss of root biomass observed in our girdling study emphasizes the critical situation of young trees grown under P-limiting conditions. Environmental stresses such as drought and defoliation impede the allocation of photoassimilates to the roots and cause decreases in carbohydrate reserves and losses in root biomass (Ruehr et al., 2009; Jing et al., 2015; Hesse et al., 2019); these effects are similar to those of girdling (Jordan et al., 1998; Kaiser et al., 2010; Krause et al., 2013). In light of these observations, our results support that P shortage is likely to aggravate other environmental stresses.

According to our initial hypothesis, we expected that the depletion of carbohydrates in response to girdling would lead to a decrease in root P concentrations in beech trees because molecular studies with model plants such as *Populus* or *Arabidopsis* identified sucrose as a central regulator of P starvation responses, orchestrating the expression of P-related genes (Lei et al., 2011). At the onset of P starvation, phloem loading and sucrose translocation to roots is enhanced (Hermans et al., 2006). In addition to increasing P transport and intracellular acid phosphatase activities, P starvation increases the transcription of genes encoding enzymes for anaplerotic reactions (Wang et al., 2002; Müller et al., 2007; Kavka & Polle, 2016; Kavka & Polle, 2017). For example, in poplar trees suffering from P shortage, PEPC is strongly enhanced at the levels of transcript abundances (Kavka & Polle, 2017) and enzyme activities (Gan et al., 2016). PEPC is a tightly regulated enzyme of primary carbon metabolism that replenishes the tricarboxylic acid (TCA) cycle. In *Arabidopsis thaliana*, PEPC upregulation results in starch depletion (Rademacher et al., 2002), whereas knockdown mutants accumulate starch (Shi et al., 2015). In our girdling study, the decrease in starch and the increase in PEPC, together with increases in acid phosphatases, suggest energy depletion and metabolic P shortage signals, similar to the P starvation response described by Plaxton and Tran (2011). This notion is also supported by the strong decrease in P uptake seen after girdling. The uptake of P_i is achieved by H^+:P_i symporters, requiring a pH gradient across the plasma membrane, which is generated by ATP-dependent proton pumps (Plassard et al., 2019). Similar to plants, Basidiomycota, which form the major clade of fungi that colonized the beech roots in our study, depend on pH-driven H^+:P_i symporters (Plassard et al., 2019). Therefore, it is likely that carbohydrate depletion of the roots caused an energy limitation of P uptake and might have interrupted sucrose signalling that is required for regulation of P uptake.

It was puzzling that the P concentrations in root tissues were relatively stable despite decreased P uptake. Tissue nutrient concentrations are the result of import and export. Translocation to aboveground tissues requires the functioning of photosynthesis and transpiration. Previous girdling studies showed that these processes decline very slowly over months (Druebert et al., 2009; López et al., 2015). For instance, Druebert et al. (2009) found no difference in photosynthesis ten weeks after girdling compared with non-girdled beech trees. Therefore, it is reasonable to assume that photosynthesis was still unaffected in the current study. Moreover, we demonstrated that similar fractions of the newly taken-up P were translocated upstream in girdled and non-girdled young trees. Therefore, it is unlikely that the stability of the root P concentrations was the result of lower export from below- to aboveground tissues. Our results suggest that P homeostasis was achieved by a combination of biomass trade-off and P resorption from declining roots.

Girdling has little effect on the soil P availability of beech

The interruption of carbohydrate transport to roots affects soil processes by decreasing rhizodeposition (Zeller et al., 2008). Labile carbon in soil and microbial activities fluctuate strongly with changing environmental conditions, seasons, distances from the root and durations of girdling (Giesler et al., 2007; Dannenmann et al., 2009; Kaiser et al., 2010; Koranda et al., 2011) and are therefore difficult to compare among different studies. Some studies observed a decline in labile carbon shortly after girdling or found transient changes (Giesler et al., 2007; Dannenmann et al., 2009; Koranda et al., 2011). Kaiser et al. (2010) reported enhanced activities of biomass-degrading enzymes in the second year after girdling but not in the first year. Therefore, it may also not be surprising that we found only marginal or no effects on labile P, on water-extractable carbon in soil nor on enzymes related to litter degradation.

A notable result was that P_{tot} and P_{sol} were consistently higher in the rhizosphere than in the bulk soil and may have precluded responses of microbial phosphatase activities to girdling. However, in the organic layer of the LP forest, where the P_{sol} concentration was lower by almost a factor of ten than in the HP forest, girdling transiently affected bacterial P mobilization, indicated by increased abundances of bacteria, which catalyse major steps in P transformation. We speculate that this activation might be related to a strong competition with roots and to high mycorrhizal P uptake efficiency present in the organic layer under P-limiting conditions (Clausing & Polle, 2020). Since gene abundances depend on the composition of microbial communities, which are strongly influenced by plant carbon (Koranda et al., 2011; Rasche et al., 2011), it is conceivable that, initially, the resident microbes responded to girdling, and that, subsequently, the community composition changed to adapt to girdling conditions.

In our study, we focused on the structure of the mycorrhizal fungal community composition associated with roots. In agreement with previous investigations (Zavišić et al., 2016; Clausing & Polle, 2020), we found a strong difference between the HP and the LP forest but not between the fungal assemblages in the organic and mineral topsoil layers per forest ecosystem. The mycorrhizal fungal community structures showed seasonal turnover, as in previous studies (Buée et al., 2005; Courty et al., 2008; Pena et al., 2010). Girdling resulted in mycorrhizal fungal species loss in the early fall in the mineral soil of the LP forest, which contained the lowest root tip density and contributed least to the plant P supply (Hauenstein et al., 2018; Clausing & Polle, 2020). Pena et al. (2010) demonstrated that abundant mycorrhizal fungal species were retained after girdling of mature beech trees, whereas mainly the rare species colonizing only a small portion of the root tips were lost. However, very rare species were excluded by our sampling design since we included only mycorrhizal species that colonized more than three root tips

Ectomycorrhizal fungi are important producers of enzymes in soils (Courty et al., 2005; Pritsch & Garbaye, 2011). The high abundance of ectomycorrhizal fungi in temperate forest soils (Awad et al., 2019; Müller et al., 2020) and their stable composition during the early phase after girdling may be a reason for the relatively stable enzyme activities found here. These results agree with previous studies, showing little or no change initially but significant increases in enzyme activities for the degradation of organic matter with a delay of approximately one year after girdling (Weintraub et al., 2007; Kaiser et al., 2010). Significant decreases in root carbohydrates were, however, already observed within the first year after the girdling of mature beech trees (Pena et al., 2010). Therefore, it is likely that despite a large buffer of carbohydrate reserves in the root system, extended periods of drought that restrict production and belowground allocation of carbohydrates (Hartmann et al., 2013; Klein et al., 2014; Chuste et al., 2020 Ji et al., 2020) will also decrease the P uptake of mature trees. In conclusion, our study emphasizes that P uptake and metabolism in young forest trees and associated ectomycorrhizas are more vulnerable to a shortage of carbohydrates than the associated soil-residing processes. Neither soil, microbial, rhizosphere nor root P levels changed. However, girdling, which caused carbohydrate depletion, resulted in a decrease in P uptake into roots, implying that stable root P levels were maintained by P recycling from the degradation of root biomass. The negative consequences of carbohydrate depletion were massive under P limitation. These results are important because they highlight the higher susceptibility of P-deficient trees than well-nourished trees to stress. Consequently, our results have critical implications for forest carbon and P cycling in future climates that will be warmer and drier than the current climate and suggest the aggravation of nutrient imbalances imposed by high nitrogen deposition (Vitousek et al., 2010; Peñuelas et al., 2013; Huang et al., 2016).

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Data availability

The data are available in the dryad repository at https://doi.org/10.5061/dryad.cvdncjt2t

Author contributions

SC and AP designed the study. SC, RP, BS, KM, PMG, SM, MG, SS, and JK conducted laboratory measurements and provided primary data. AP, MS, SS, FL, and EK supervised data analyses. SC wrote the first draft of the manuscript. AP revised the draft. All authors commented on the manuscript and approved the submitted version.

Supplementary data

Supplement Figure S1: Harvest and sampling scheme

Supplement Figure S2: Ectomycorrhizal fungal community on beech (*Fagus sylvatica* L.) roots after girdling (G) and of untreated control plants (C) analysed in summer (a) and early fall (b).

Supplement Figure S3: Response ratios of enzyme activities after girdling in relation to non-girdled controls in the bulk soil (a-d) and the rhizosphere (e-f).

Supplement Figure S4: Response ratio of microbial biomass (based on total PLFAs) as well as of group-specific PLFAs after girdling in relation to non-girdled controls.

Supplement Table S1: Nitrogen and carbon contents in trees and soil in the organic layer and mineral topsoil of P-rich (HP) and P-poor (LP) forests.

Supplement Table S2: Functions, primers, standards and thermal profiles for the targeted microbial genes in soil.

Supplement Table S3: Acid phosphatase activities, gene abundances of soil microbes for P mobilization and microbial biomass on non-girdled control plots in the organic layer and mineral topsoil of P-rich (HP) and P-poor (LP) forests.

Supplement Table S4: Fine root biomass, root tip vitality, ectomycorrhizal colonization rate, species richness, Shannon diversity and Evenness of the ectomycorrhizal communities in P-rich (HP) and a P-poor (LP) forest after girdling (G) and of non-girdled beech trees (C).

Supplement Table S5: Soil enzyme activities (nmol g-1 h-1) in non-girdled control plots in the organic layer and mineral topsoil of P-rich (HP) and P-poor (LP) forests.

Supplement Table S6: Phosphorus stocks (g m^{-2}) in bulk soil and microbes after girdling (G) and in non-girdled control plots (C) in P-rich (HP) and P-poor (LP) forests.

Supplement Methods S1: Detailed description of the laboratory methods.

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Table 1: Soil pH, water-extractable organic carbon (WEOC) and phosphorus concentration (P) in different fractions of soil and roots in P-rich (HP) and P-poor (LP) forests.

Forest		**HP**								**LP**															
Harvest		**1wk**				**8wk**				**1wk**				**8wk**				Forest site		Harvest		Treatment		SxT	
Treatment		**C**		**G**		**C**		**G**		**C**		**G**		**C**		**G**		F	*p*	F	*p*	F	*p*	F	*p*
Organic layer																									
	pH	3.98	± 0.24	3.97	± 0.25	4.32	± 0.29	4.30	± 0.33	3.26	± 0.04	3.26	± 0.03	3.41	± 0.04	3.41	± 0.04	12.5	**<0.001**	47.9	**<0.001**	0.1	0.826	0.0	0.880
	WEOC	1.23	± 0.25	1.23	± 0.25	1.33	± 0.08	1.43	± 0.07	0.49	± 0.04	0.51	± 00.07	0.74	± 0.14	0.58	± 0.08	23.2	**0.005**	7.6	**0.015**	0.0	0.828	1.2	0.286
Bulk soil	P_{tot}	0.91	± 0.02	0.93	± 0.02	1.44	± 0.14	1.42	± 0.21	0.17	± 0.01	0.16	± 0.01	0.22	± 0.04	0.18	± 0.03	336.0	**<0.001**	36.7	**<0.001**	0.6	0.469	0.4	0.560
	P_{sol}	0.31	± 0.04	0.33	± 0.04	0.32	± 0.08	0.33	± 0.08	0.037	± 0.002	0.034	± 0.003	0.05	± 0.01	0.042	± 0.004	33.0	**0.002**	1.8	0.205	1.1	0.306	3.9	0.068
	P_{mic}	0.046	± 0.007	0.045	± 0.005	0.070	± 0.006	0.078	± 0.008	0.012	± 0.001	0.012	± 0.002	0.038	± 0.010	0.025	± 0.004	38.3	**<0.001**	54.8	**<0.001**	0.2	0.668	2.4	0.141
Rhizo	P_{tot}	2.38	± 0.39	2.39	± 0.78	3.28	± 0.55	2.81	± 0.93	0.35	± 0.03	0.47	± 0.14	0.59	± 0.17	0.53	± 0.09	31.5	**0.004**	2.3	0.150	0.6	0.464	0.8	0.380
sphere	P_{sol}	0.30	± 0.06	0.33	± 0.03	0.34	± 0.06	0.41	± 0.09	0.06	± 0.00	0.06	± 0.01	0.08	± 0.01	0.07	± 0.01	43.3	**0.001**	17.9	**<0.001**	4.0	0.066	7.3	**0.017**
Fine	P_{tot}	1.29	± 0.33	1.24	± 0.04	0.93	± 0.06	1.12	± 0.01	0.61	± 0.04	0.56	± 0.03	0.47	± 0.02	0.46	± 0.05	43.4	**0.002**	5.6	**0.035**	0.0	0.879	0.3	0.601
roots	P_{sol}	0.56	± 0.03	0.72	± 0.04	0.88	± 0.07	0.39	± 0.04	0.26	± 0.02	0.26	± 0.02	0.18	± 0.01	0.18	± 0.02	201.1	**<0.001**	5.8	**0.032**	17.2	**0.001**	16.4	**0.001**
Coarse	P_{tot}	0.49		0.88	± 0.10	0.87	± 0.06	1.33		0.31	± 0.03	0.31	± 0.04	0.31	± 0.02	0.30	± 0.02	140.4	**<0.001**	25.6	**<0.001**	23.6	**<0.001**	24.8	**<0.001**
roots	P_{sol}	0.21		0.50	± 0.03	0.53		na		0.13	± 0.02	0.13	± 0.03	0.15	± 0.02	0.16	± 0.02	146.6	**<0.001**	25.9	**<0.001**	22.6	**<0.001**	21.5	**<0.001**
Mineral topsoil																									
	pH	3.98	± 0.12	3.97	± 0.11	3.96	± 0.16	3.92	± 0.18	3.53	± 0.05	3.51	± 0.06	3.42	± 0.07	3.45	± 0.05	15.5	**0.011**	3.7	0.074	0.3	0.605	0.7	0.402
	WEOC	0.34	± 0.04	0.34	± 0.01	0.36	± 0.03	0.56	± 0.18	0.23	± 0.02	0.27	± 0.02	0.31	± 0.04	0.30	± 0.04	6.3	0.054	4.8	**0.044**	2.0	0.175	1.3	0.277
Bulk soil	P_{tot}	0.88	± 0.02	0.90	± 0.02	1.16	± 0.05	1.29	± 0.02	0.08	± 0.01	0.08	± 0.01	0.12	± 0.03	0.14	± 0.05	1125.6	**<0.001**	45.8	**<0.001**	11.5	**0.004**	5.9	**0.028**
	P_{sol}	0.20	± 0.05	0.23	± 0.08	0.24	± 0.04	0.24	± 0.03	0.03	± 0.00	0.04	± 0.00	0.04	± 0.01	0.04	± 0.01	23.9	**0.005**	4.6	**0.049**	2.8	0.115	1.4	0.248
	P_{mic}	0.014	± 0.005	0.013	± 0.008	0.030	± 0.006	0.034	± 0.004	0.001	± 0.000	0.001	± 0.000	0.005	± 0.001	0.004	± 0.001	26.1	**0.004**	31.5	**<0.001**	0.1	0.726	0.3	0.570

Rhizo	P_{tot}	2.32	± 0.46	2.08	± 0.43	3.14	± 0.57	3.28	± 0.24	0.14	± 0.03	0.13	± 0.02	0.20	± 0.02	0.14	± 0.03	189.8	**<0.001**	8.0	**0.010**	0.0	0.833	0.0	0.964
sphere	P_{sol}	0.17	± 0.03	0.20	± 0.06	0.25	± 0.03	0.20	± 0.03	0.03	± 0.00	0.04	± 0.00	0.04	± 0.00	0.04	± 0.00	30.1	**0.003**	10.7	**0.005**	0.1	0.757	0.1	0.705
Fine	P_{tot}	1.39	± 0.11	1.23	± 0.15	1.13	± 0.12	0.96	± 0.15	0.47	± 0.01	0.42	± 0.01	0.37	± 0.01	0.38	± 0.02	69.3	**<0.001**	19.8	**<0.001**	6.4	**0.023**	3.6	0.076
roots	P_{sol}	0.81	± 0.11	0.66	± 0.11	0.64	± 0.05	0.55	± 0.12	0.17	± 0.01	0.19	± 0.02	0.16	± 0.01	0.16	± 0.01	42.8	**0.001**	10.9	**0.005**	6.9	**0.019**	5.2	**0.038**
Coarse	P_{tot}	0.90	± 0.25	1.04	± 0.25	1.08	± 0.20	0.99	± 0.29	0.30	± 0.01	0.30	± 0.02	0.27	± 0.03	0.26	± 0.02	15.4	**0.011**	0.1	0.771	0.0	0.890	0.1	0.766
roots	P_{sol}	0.57	± 0.05	0.57	± 0.19	0.70	± 0.24	0.79	± 0.33	0.13	± 0.01	0.14	± 0.01	0.15	± 0.02	0.14	± 0.02	13.5	**0.017**	0.6	0.448	0.6	0.444	0.6	0.444

Young beech (*Fagus sylvatica*) trees were girdled (G) or kept as untreated controls (C). Samples were harvested in summer (1wk) and in early fall (8wk) after girdling. Bulk soil, rhizosphere, microbes and roots from the organic layer and the mineral topsoil were analyzed separately. Data show means for pH, WEOC (mg g^{-1} dry weight), total phosphorus (P_{tot}) (mg g^{-1} dry weight), soluble phosphorus (P_{sol}) (mg g^{-1} dry weight) and microbial phosphorus (P_{mic}) (µg g^{-1} dry weight) (HP: n = 3, LP: n = 4) ± SE. When only one coarse root sample was available, SE is missing. To determine the effects of forest, sampling date, treatment and the interaction of Forest site x treatment (SxT) linear mixed effect models (´lmer´) were used with plot as random factor. Bold letters indicate significant differences at $p \leq 0.05$. na = not available

Figure legends

Fig. 1: Carbohydrate concentrations (mg g^{-1} dw) in fine roots of beech trees (*Fagus sylvatica*) after girdling (G, light colors) and of untreated control plants (C, dark colors) in P-rich (HP) and P-poor (LP) forests. Roots from the organic layer (a) and the mineral topsoil (b) were analyzed separately one week (1wk) and eight weeks (8wk) after girdling. Data indicate means (HP: n = 3, LP: n = 4) ± SE. To determine the effects of forest type, sampling date, treatment their interaction linear mixed effect models (´lmer´) were used with plot as random factor and a posthoc Tukey HSD was performed to detect differences between means. Different letters indicate significant differences at $p \leq 0.05$. Colors of bars refer to starch (turquoise), glucose (red), fructose (green) and sucrose (orange).

Fig. 2: PEPC activity (µmol g^{-1} fw h^{-1}) of fine roots of beech trees (*Fagus sylvatica*) after girdling (G, light color) and of untreated controls (C, dark color) in P-rich (HP) and P-poor (LP) forests. Roots from the organic layer (a) and the mineral topsoil (b) were analyzed separately. Data indicate means (HP: n = 3, LP: n = 4) ± SE. To determine the effects of forest type, treatment and their interaction linear mixed effect models (´lmer´) were used with plot as random factor and a posthoc Tukey HSD was performed to detect differences between means. Different letters indicate significant differences of the means at $p \leq 0.05$.

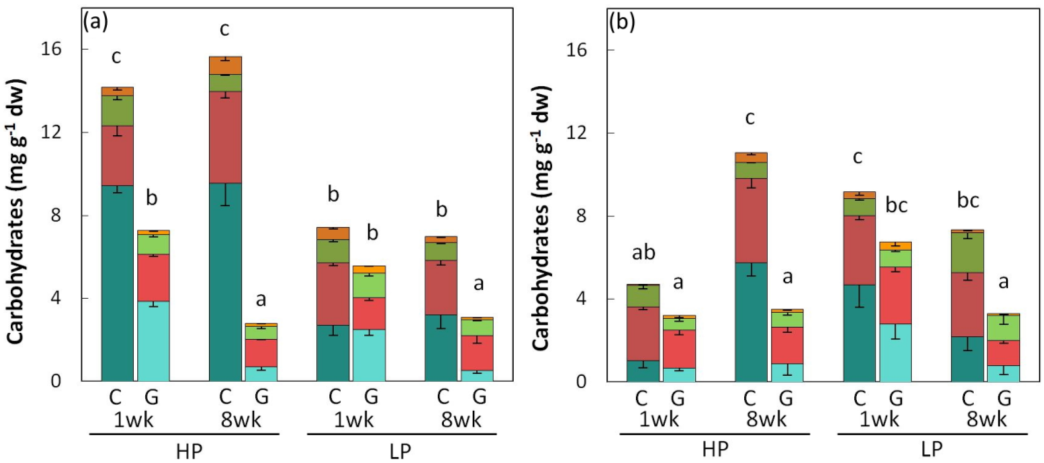
Fig. 3: Response ratio of acid phosphatase activities after girdling in relation to non-girdled controls. Bars indicate the response ratio of phosphatase activities (µmol g^{-1} fresh weight * h^{-1}) for girdled/control determined in fine roots (Root) of *Fagus sylvatica*, mycorrhiza (EMF), rhizosphere (Rhizo) and bulk soil (Bulk) one week (black) and eight weeks after girdling (white bars). The response ratios were determined for phosphatase activities in the organic layer of a P-rich (a) and a P-poor forest (b) and in the mineral topsoil of a P-rich (c) and a P-poor forest (d). Data indicate means of the response ratios (HP: n = 3, LP: n = 4) ± SE. Differences between means of girdled and non-girdled treatments were tested by Student's paired t-test and indicated by

stars (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). Black squares above bars indicate a marginal difference (trend with $p \leq 0.10$). Controls are marked with the dashed line. nd = not determined.

Fig. 4: Response ratios of gene abundances for P transporters (pitA, pstS), P mineralization (phoD, phoN, phnX, appA) and P_i solubilization (gcd) of soil microbes after girdling in relation to non-girdled controls. Bars indicate the response ratio for girdled/control of young *Fagus sylvatica* trees determined one week (black) and eight weeks after girdling (white bars). The response ratios were determined in the organic layer of a P-rich (a) and a P-poor forest (b) and in the mineral topsoil of a P-rich (c) and a P-poor forest (d). Data indicate means (HP: n = 3, LP: n = 4, ± SE). Differences between means of girdled and non-girdled treatments were tested by Student's paired t-test and indicated by stars (* $p \leq 0.05$). Black squares above bars indicate a marginal difference ($p \leq 0.10$). Controls are marked with the dashed line. bt = below threshold

Fig. 5: Phosphorus stocks in fine roots (mg m^{-2}) in the soil beneath girdled (G, light colors) and non-girdled control (C, dark colors) trees (*Fagus sylvatica*). Trees were investigated in P-rich (HP) and P-poor (LP) forests. Fine roots from the organic layer (turquoise bars) and the mineral topsoil (orange bars) were analyzed separately one week (1wk) and eight weeks (8wk) after girdling. Data indicate means (HP: n = 3, LP: n = 4) ± SE. To determine the effects of forest type, harvest time point, treatment and their interaction linear mixed effect models (´lmer´) were used with plot as random factor and a posthoc Tukey HSD was performed to detect differences between means. Different letters above the bars indicate significant differences at $p \leq 0.05$.

Fig. 6: ^{33}P uptake (Bq mg^{-1} dw h^{-1}) of beech (*Fagus sylvatica*) roots one week after girdling (G, light color) and of non-girdled control plants (C, dark color). Uptake was determined for the root part which was exposed to the labeling solution (exposure) and the upstream part of the roots to which P was transported (transport). Data indicate means (n = 7) ± SE. To determine the effects of treatment and root fraction, linear mixed effect models (´lmer´) were used with root number as random factor. A posthoc Tukey HSD was performed to detect differences between means. Different letters indicate significant differences at $p \leq 0.05$.



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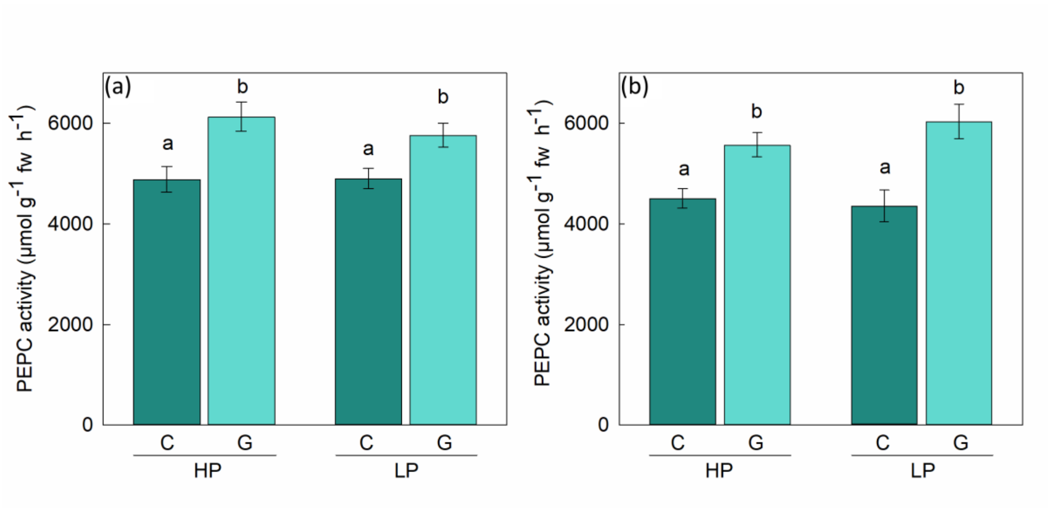

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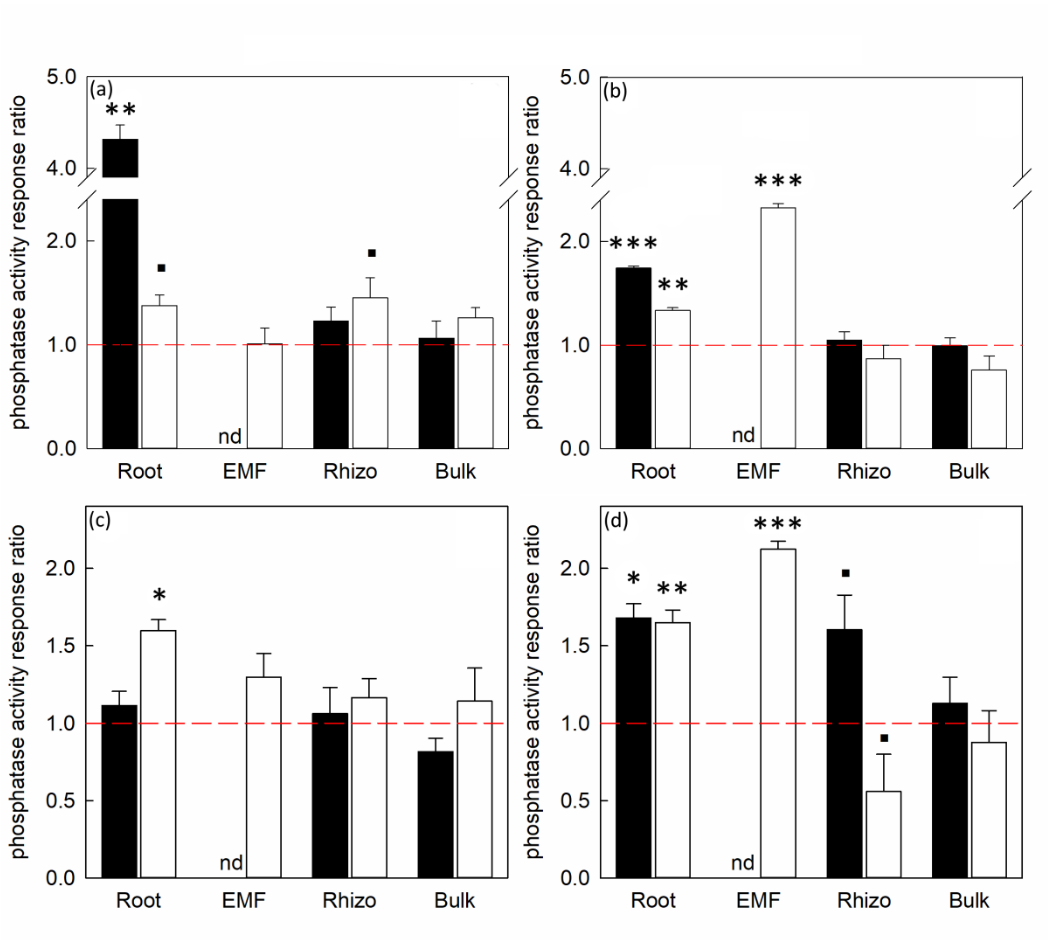

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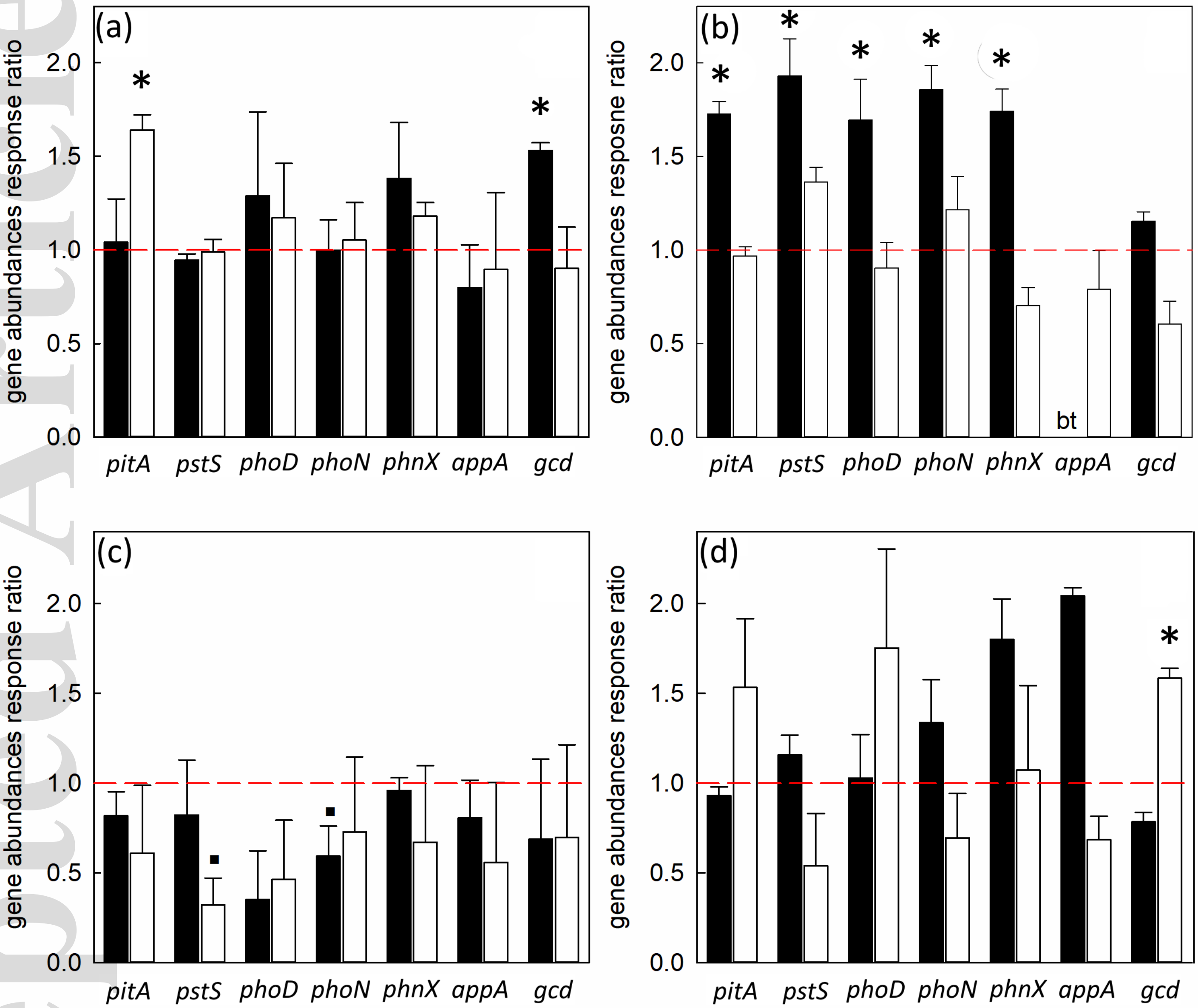

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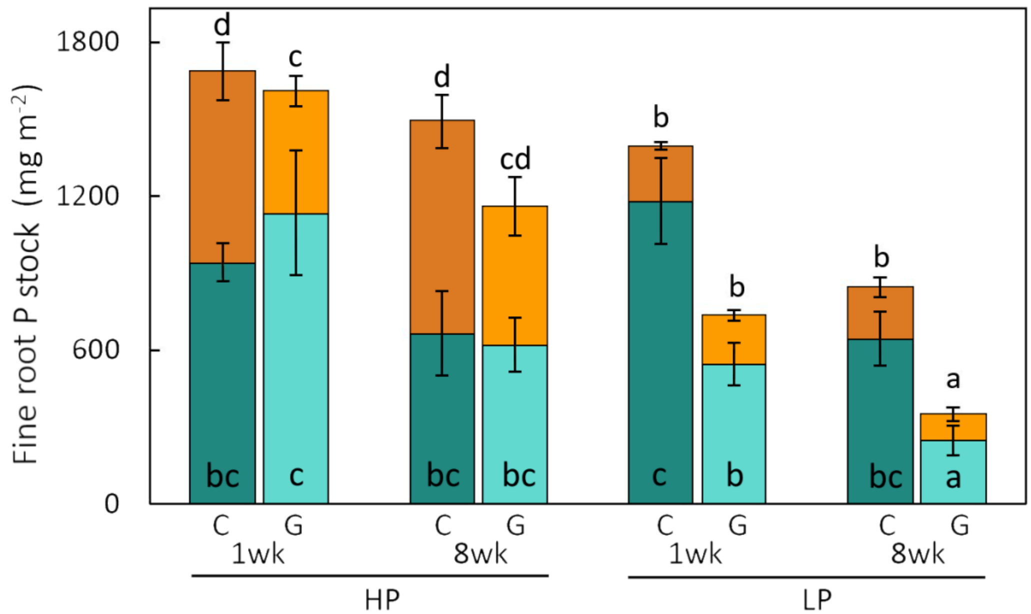

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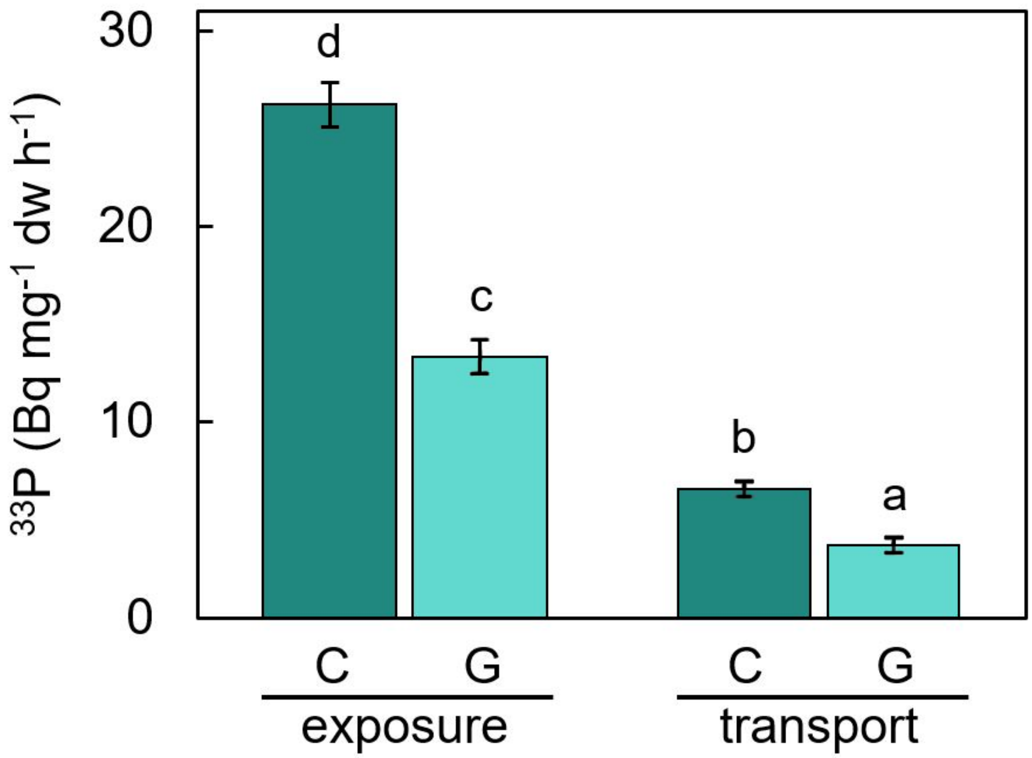

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