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Research

Carbohydrate depletion in roots impedes phosphorus nutrition in young forest trees

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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) mobilisation 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 mobilisation in the organic layer and mineral topsoil in comparison with those in nongirdled 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 mobilisation 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 remobilisation and root biomass trade-off to compensate for the P shortage. As 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.

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 mineralisation and mobility in dry soil (Schachtman *et al.*, 1998; Schimel *et al.*, 2007; Kreuzwieser & Gessler, 2010), soil acidification, and N deposition (Vitousek & Howarth, 1991; Peñuelas *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 mobilisation 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 mobilisation and uptake capacity (Desai *et al.*, 2014; Kavka & Polle, 2016), and they tighten internal P cycling by growth adjustment and internal P mobilisation (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, 2005; Gan *et al.*, 2016; Kavka & Polle, 2017; Png *et al.*, 2017). The enzyme phosphoenolpyruvate carboxylase (PEPC) 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 mobilisation (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 mobilisation 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). By 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 mobilisation, on the other hand, it is unknown whether the P acquisition abilities of trees depend on their carbohydrate resources. As 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 nongirdled controls and girdled trees, we disentangled the effects of belowground plant-derived carbohydrates on P uptake, P concentrations and enzyme activities related to P mobilisation 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 compared with 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 characterised forest ecosystems that differed strongly in P stocks (Lang *et al.*, 2017). We addressed the following specific hypotheses:

(**1**) Carbohydrate depletion leads to a decrease in root P concentrations and an increase in the enzyme activities required for internal P mobilisation in roots (PEPC, phosphatase) as well as for P mobilisation from soil (mycorrhizal phosphatases).

(**2**) As soil microbes are not directly reliant on root carbohydrates (Kaiser *et al.*, 2010), microbial phosphatases are unaffected by girdling.

(**3**) 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 mobilisation 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 is located in the biosphere reserve 'Bayerische Rhön' (50°21′7.2″N 9°55′44.5″E, 801–850 m above sea level (asl)). The mean long-term sum of annual precipitation is 950 mm, and the long-term mean annual temperature (1981–2010) is 6.1°C. The average tree age at this beech stand is 137 yr (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°16′2.3″E, 115 m asl) and is stocked with *c.* 120-yr-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 899 mm (BMEL, 2016). The soil is a Hyperdystric Folic Cambisol (Areni, Loamic, Nechic, Protospodic) derived from sandy till substrate. Further details have been 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 May 2017, LP: 5 May 2017) in gaps with a minimum distance of 5 m between large trees. Each plot had an area of 4 m^2 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 Supporting Information 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 *c.* 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

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		1 wk		8 wk		1 wk		8 wk		Forest site		Harvest		Treatment		S×T	
Treatment		C	G	C	G	C	G	C	G	F	*P*	F	*P*	F	*P*	F	*P*
Organic layer																	
Bulk soil	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
	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
Rhizosphere	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
	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 roots	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
	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 roots	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**
	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																	
Bulk soil	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
	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 sphere	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
	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 roots	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
	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 roots	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
	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	0.44

Young beech (*Fagus sylvatica*) trees were girdled (G) or kept as untreated controls (C). Samples were harvested in summer (1 wk) and in early autumn (8 wk) after girdling. Bulk soil, rhizosphere, microbes and roots from the organic layer and the mineral topsoil were analysed separately. Data show means for pH, WEOC (mg g^{-1} DW), total phosphorus (P_{tot}) (mg g^{-1} DW), soluble phosphorus (P_{sol}) (mg g^{-1} DW) and microbial phosphorus (P_{mic}) (μg g^{-1} DW) (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 × treatment (S×T) 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.

removal of a 20-mm-wide strip of bark at a height of 0.4 m from the ground (HP: 18 July, LP: 17 July). The other half of each plot was used as an untreated control.

Harvest and processing of soil cores

In each subplot, eight soil cores (diameter 55 mm, depth 0.21 m) were sampled 1 wk (HP: 25 July 2017, LP: 24 July 2017) and 8 wk (HP: 27 September 2017, LP: 20 September 2017) after girdling. A schematic overview on the sampling procedure is provided in 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 d). 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 categorised 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 under numbers MN970515 to MN970525 (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 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*) that encode enzymes with phosphatase activities, and *gcd*, which solubilises P by the oxidation of glucose and aldose sugars (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 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 (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 Methods S1.

The rhizosphere and soil enzymes were extracted from fresh soil that had been stored frozen at −20°C (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, MO, USA) (Marx *et al.*, 2001) in soil suspensions (for details, see 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, from this point forward, 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 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}), 50 mg powder was weighed and extracted in 25 ml 65% HNO_3 at 160°C for 12 h (Heinrichs *et al.*, 1986). For determination of soluble P (P_{sol}), 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 280¼, 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 eight 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 deionised 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 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 340 nm 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 Methods S1.

Soil pH and water-extractable organic carbon

The pH values were measured by suspending field-moist, sieved soil in 0.01 M $CaCl_2$ (1 : 5 soil : solution ratio) after 16 h of equilibration (ISO10390, 2005). For determination of water-extractable organic carbon (WEOC) field-moist, sieved samples were suspended in deionised water (EC < 0.06 µS cm^{-1}) at a soil : solution ratio of 1 : 5. After 16 h of equilibration, the suspensions were filtered through a 0.45 µm membrane (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 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 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 × diameter: 0.12 m) from another beech forest (Billingshäuser Schlucht, coordinates: 51°34′43.8″N 9°59′04.8″ E, 308 m asl, 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 1 month under field conditions and watered regularly before the uptake experiments started (collection: 13 May 2019, labelling experiments from 13 June 2019 to 25 June 2019). Half of the trees were girdled as described above; the other half remained untreated. The trees were used for uptake experiments 1 wk 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.*, 2005) 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 *c.* 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. In total, seven girdled and seven untreated trees were analysed using three fine roots per tree. The details of the exposure experiments are described in Methods S1.

Statistical analyses

Statistical analyses were performed with R v.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 when necessary to meet the criteria of the normal distribution and homogeneity of variances. As the plots were separated into girdled and nongirdled 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 $plot_{x(girdled)}$/ $plot_{x(nongirdled)}$. To determine the effects of the forest type, soil layer, sampling dates and treatment, linear mixed effects models ('lmer', R package LME4) were used with plot as random factor. Pairwise comparisons of the sample means were conducted using Tukey's honest significant difference (HSD) test (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

Nonstructural 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 1 wk 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). At 8 wk after girdling, roots in both soil layers from both the HP and LP forests contained significantly lower carbohydrate concentrations than did the roots of nongirdled 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: $F = 0.12$, $P = 0.730$, that is sampling 1 wk and 8 wk after girdling corresponding to summer (July) and early autumn (September), respectively, with the exception of the mineral layer in the HP forest.

As girdling showed the strongest effects after 8 wk, 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 ectomycorrhizal fungi but has a moderate impact on microbial P mobilisation in soil

To test whether girdling affected P mobilisation 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. As 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 Table S3).

After girdling, the response ratios of root phosphatase activities were consistently enhanced at the LP site, regardless of soil layer

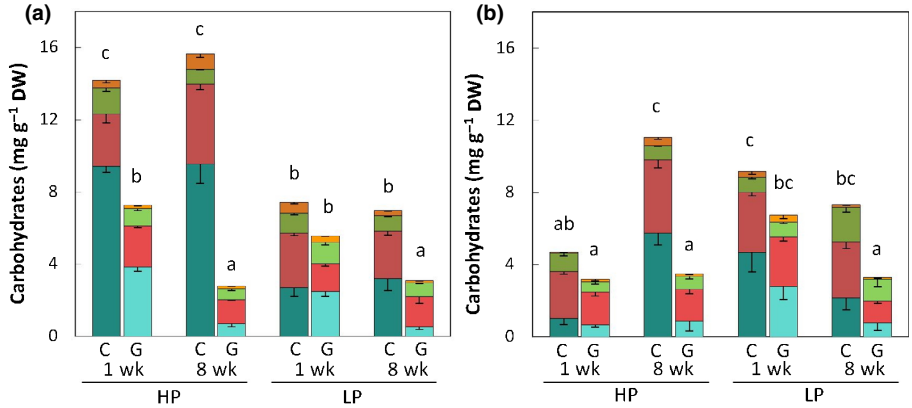

Fig. 1 Carbohydrate concentrations (mg g^{-1} DW) in fine roots of beech trees (*Fagus sylvatica*) after girdling (G, light colours) and of untreated control plants (C, dark colours) in phosphorus (P)-rich (HP) and P-poor (LP) forests. Roots from the organic layer (a) and the mineral topsoil (b) were analysed separately 1 wk and 8 wk 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 effects models ('lmer') were used with a plot as random factor and a post hoc Tukey HSD was performed to detect differences between means. Different letters indicate significant differences at $P \leq 0.05$. Colours of bars refer to starch (turquoise), glucose (red), fructose (green) and sucrose (orange).

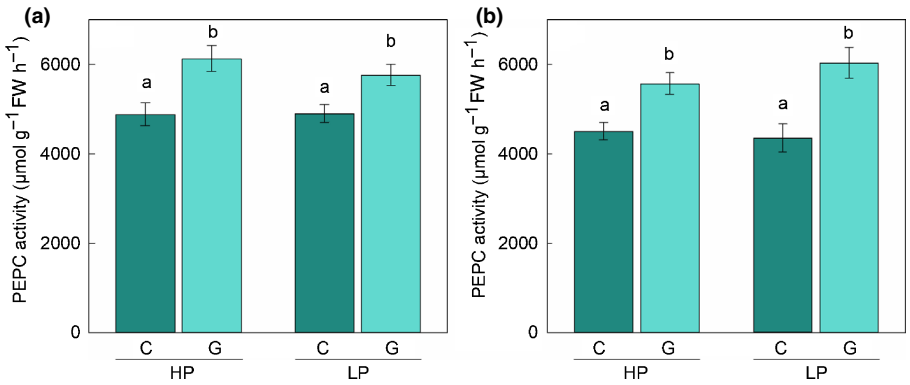

Fig. 2 PEPC activity (µmol g^{-1} FW h^{-1}) of fine roots of beech trees (*Fagus sylvatica*) after girdling (G, light colour) and of untreated controls (C, dark colour) in phosphorus (P)-rich (HP) and P-poor (LP) forests. Roots from the organic layer (a) and the mineral topsoil (b) were analysed separately. 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 post hoc Tukey HSD was performed to detect differences between means. Different letters indicate significant differences of the means at $P \leq 0.05$.

or time point, whereas the HP roots from the organic layer showed a strong enhancement only 1 wk after girdling and the HP roots from the mineral topsoil showed a moderate enhancement 8 wk 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 colonisation of root tips and the community composition of the ectomycorrhizal fungi were unaffected by girdling (ANOSIM: $R^2 = 0.418$, $P > 0.05$, Fig. S2), with the exception of the LP mineral topsoil, where mycorrhizal species richness significantly decreased from an average of 10 to 5 species 8 wk after girdling (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: $R^2 = 0.988$, $P < 0.05$, 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 (Fig. S3). In agreement with these results, no changes in the abundance of genes for microbial P mobilisation 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 with the results of the HP soils, the response ratio of gene copy numbers for P transporters and P mineralisation of soil microbes (*pitA*, *pstS*, *phoD*, *phoN*, *phnX*) showed a transient increase 1 wk 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 (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 (Table S3).

We also measured soil enzyme activities involved in carbon or nitrogen mineralisation (Table S5). We found no significant increases in response to girdling (Fig. S3). However, many of the carbon-related enzymes in the rhizosphere (organic layer) showed trends towards increased activities 8 wk after girdling (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 wk after girdling, a *c.* 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 8 wk.

We found that root biomass decreased in response to girdling, especially in the LP forest (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 8 wk, whereas in the LP forest, a strong decline was already apparent 1 wk 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%) 8 wk after girdling (Table S4). In contrast to roots, the stock of P in soil and the stock of P_{mic} were unaffected by girdling (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. At 1 wk 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 that of the roots of the nongirdled trees (Fig. 6). In both girdled and

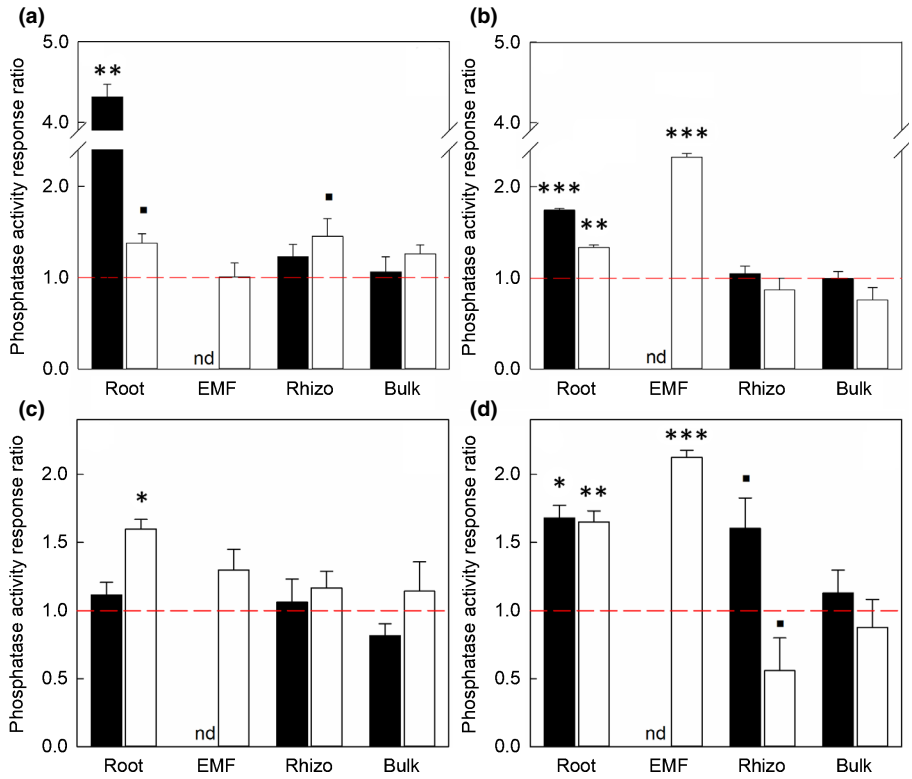

Fig. 3 Response ratio of acid phosphatase activities after girdling in relation to nongirdled controls. Bars indicate the response ratio of phosphatase activities (μmol g^{-1} FW h^{-1}) for girdled/control determined in fine roots (Root) of *Fagus sylvatica*, mycorrhiza (EMF), rhizosphere (Rhizo) and bulk soil (Bulk) 1 wk (black bars) and 8 wk after girdling (white bars). The response ratios were determined for phosphatase activities in the organic layer of a phosphorus (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 nongirdled treatments were tested using Student's paired *t*-test and indicated by asterisks (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$). Black squares above bars indicate a marginal difference (trend with $P \leq 0.10$). Controls are marked with the dashed line. nd, not determined.

nongirdled plants, *c.* 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 mobilisation and the plant P supply

In this study, we investigated the links between root carbohydrate resources, P uptake and P mobilisation 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 1 wk after girdling in the upper soil layer, and were stronger in roots with higher starch contents than in those containing less starch. This finding suggested 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 fertilisation (Zavišić *et al.*, 2018), supporting the idea 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 emphasises 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 supported the idea 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*

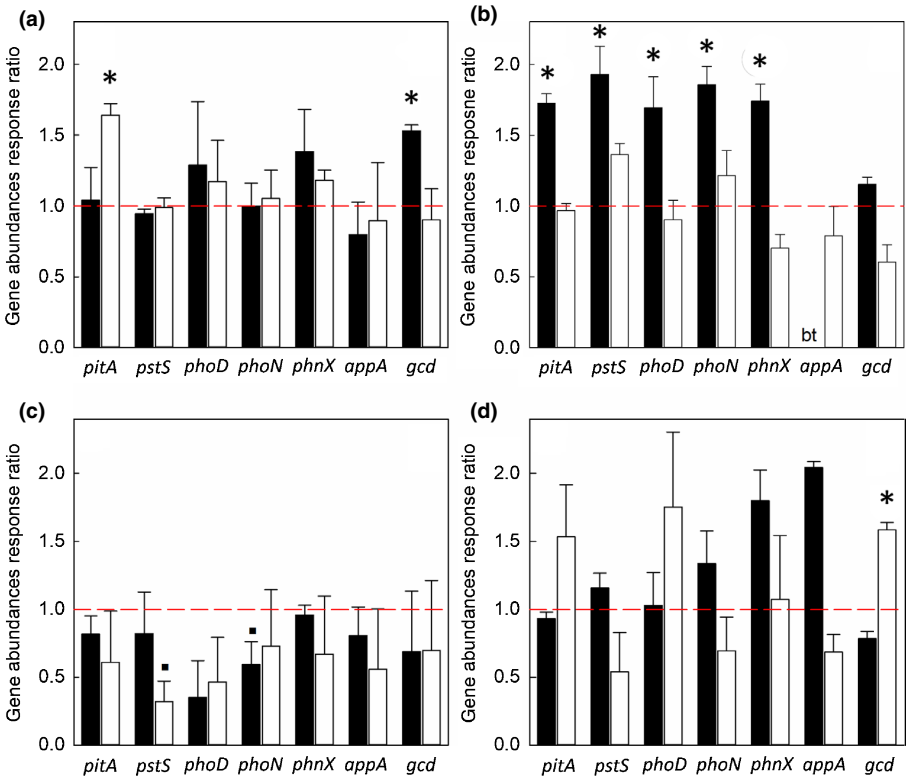

Fig. 4 Response ratios of gene abundances for phosphorus (P) transporters (*pitA*, *pstS*), P mineralisation (*phoD*, *phoN*, *phnX*, *appA*) and P_i solubilisation (*gcd*) of soil microbes after girdling in relation to nongirdled controls. Bars indicate the response ratio for girdled/control of young *Fagus sylvatica* trees determined 1 wk (black bars) and 8 wk 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 nongirdled treatments were tested using Student's paired *t*-test and indicated by asterisks (*, $P \leq 0.05$). Black squares above bars indicate a marginal difference ($P \leq 0.10$). Controls are marked with the dashed line. bt, below threshold.

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 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 & 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 colonised 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 have shown 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 10 wk after girdling compared with nongirdled 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 nongirdled young trees. Therefore, it is unlikely that the stability of the root P concentrations was the result of lower export from belowground to aboveground tissues. Our results suggested that P homeostasis was achieved by a combination of biomass tradeoff and P resorption from declining roots.

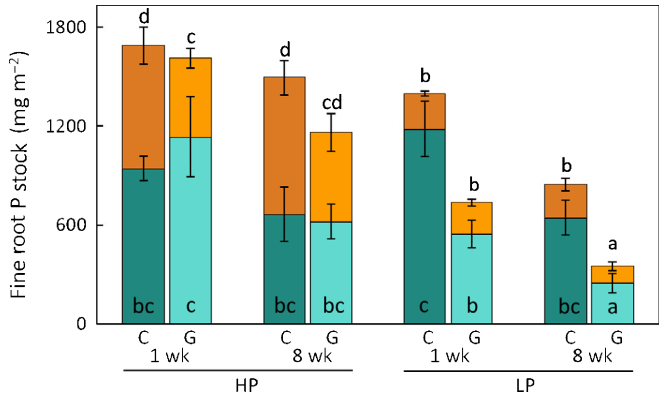

Fig. 5 Phosphorus (P) stocks in fine roots (mg m^{-2}) in the soil beneath girdled (G, light colours) and nongirdled control (C, dark colours) trees (*Fagus sylvatica*). Trees were investigated in P-rich (HP) and P-poor (LP) forests. Fine roots from the organic layer (turquoise bars) and the mineral topsoil (orange bars) were analysed separately 1 wk and 8 wk 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 effects models 'lmer' were used to plot as random factor and a post hoc Tukey honest significant difference (HSD) test was performed to detect differences between means. Different letters above the bars indicate significant differences at $P \leq 0.05$.

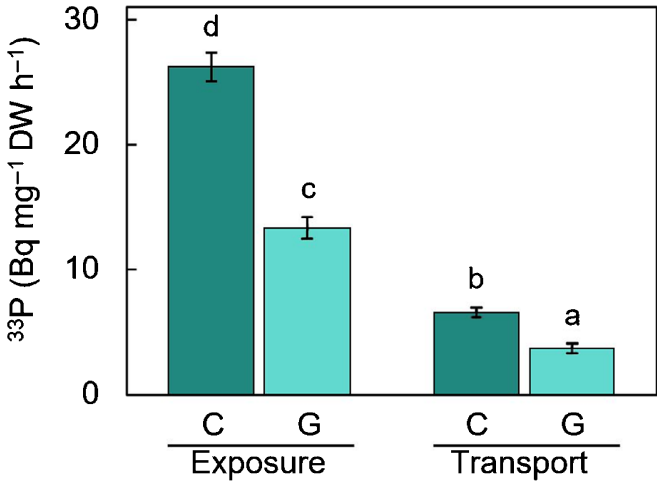

Fig. 6 ^{33}P uptake (Bq mg^{-1} DW h^{-1}) of beech (*Fagus sylvatica*) roots 1 wk after girdling (G, light colour) and of nongirdled control plants (C, dark colour). Uptake was determined for the root part which was exposed to the labelling solution (exposure) and the upstream part of the roots to 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 post hoc Tukey honest significant difference (HSD) test was performed to detect differences between means. Different letters indicate significant differences at $P \leq 0.05$.

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 girdling 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 10 than in the HP forest, girdling transiently affected bacterial P mobilisation, 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). As 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 autumn 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 colonising only a small portion of the root tips were lost. However, very rare species were excluded by our sampling design as we included only mycorrhizal species that colonised 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 that showed little or no change initially but showed significant increases in enzyme activities for the degradation of organic matter with a delay of *c.* 1 yr after girdling (Weintraub *et al.*, 2007; Kaiser *et al.*, 2010). Significant decreases in root carbohydrates were, however, already observed within the first year after girdling 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 emphasises 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 compared with 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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Author contributions

SC and AP designed the study. SC, RP, BS, KM, PM-G, 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.

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Data availability

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

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Harvest and sampling scheme.

Fig. S2 Ectomycorrhizal fungal community on beech (*Fagus sylvatica* L.) roots after girdling (G) and of untreated control plants (C) analysed in summer and early autumn.

Fig. S3 Response ratios of enzyme activities after girdling in relation to nongirdled controls in the bulk soil and the rhizosphere.

Fig. S4 Response ratio of microbial biomass (based on total PLFAs) as well as of group-specific PLFAs after girdling in relation to nongirdled controls.

Methods S1 Detailed description of the laboratory methods.

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.

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

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

Table S4 Fine root biomass, root tip vitality, ectomycorrhizal colonisation 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 nongirdled beech trees (C).

Table S5 Soil enzyme activities (nmol g^{-1} h^{-1}) in nongirdled control plots in the organic layer and mineral topsoil of P-rich (HP) and P-poor (LP) forests.

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

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