New Phytologist Supporting Information

Carbohydrate depletion in roots impedes phosphorus nutrition in young forest trees

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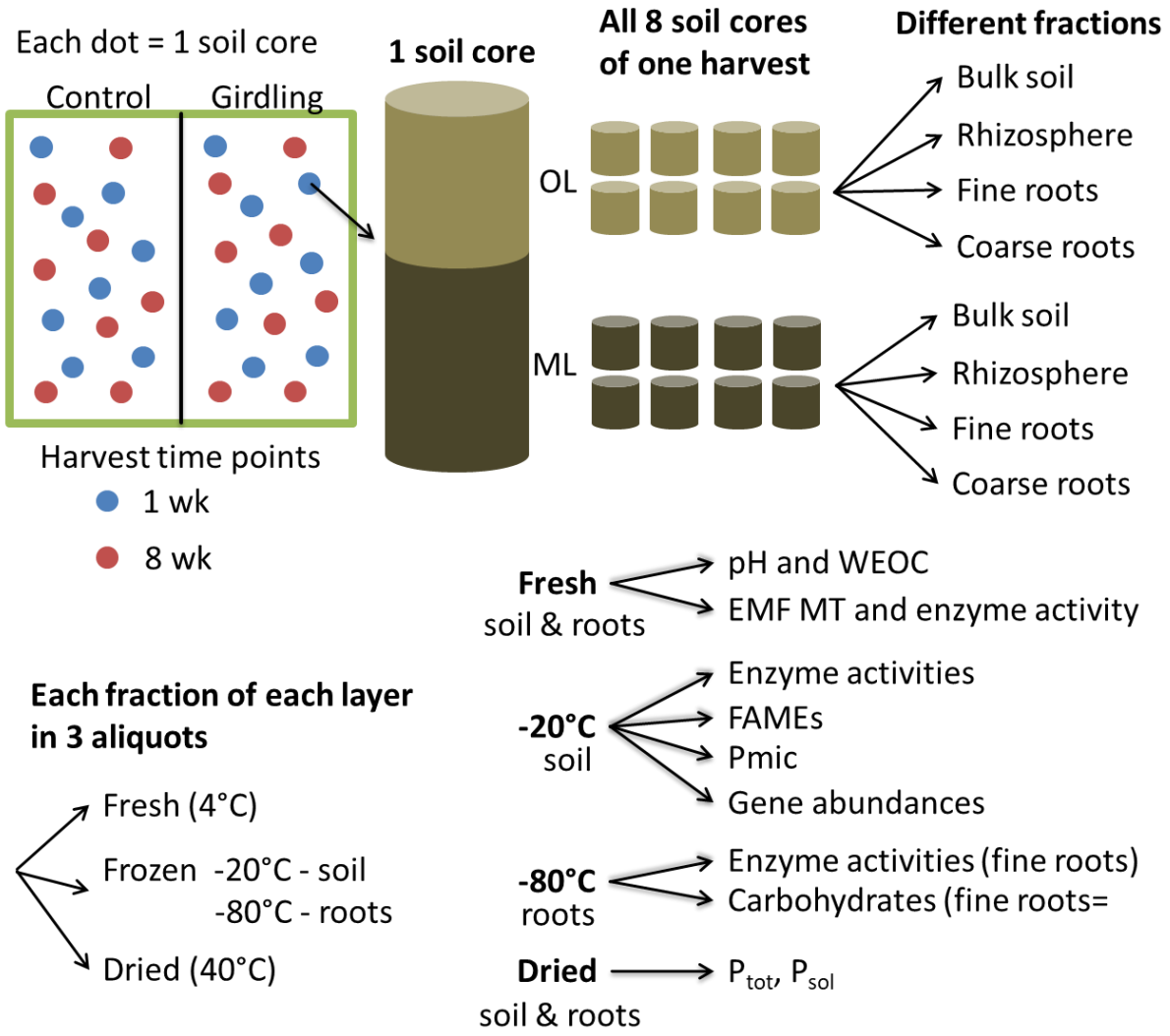
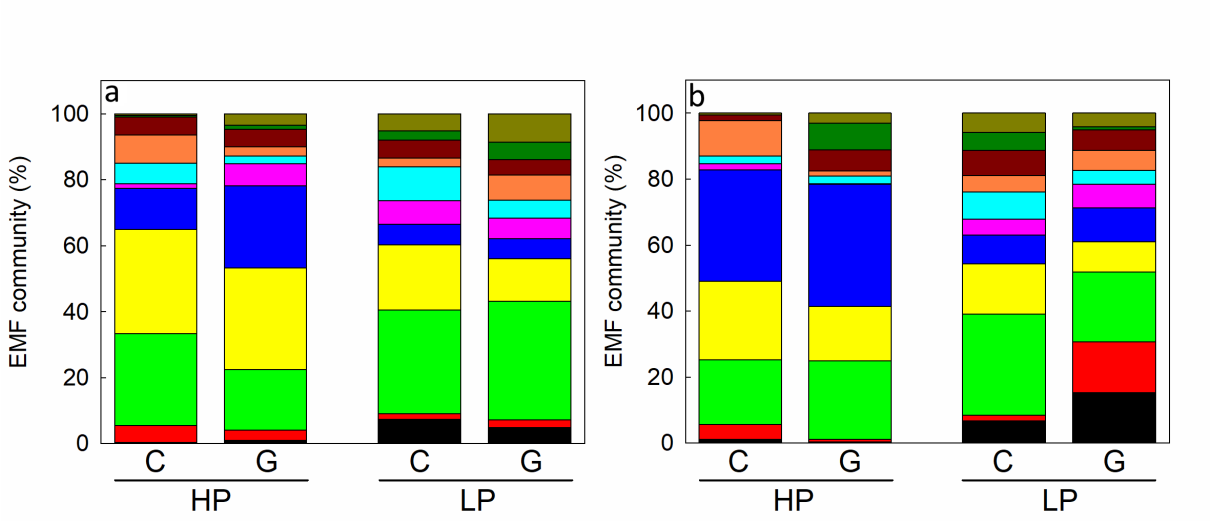
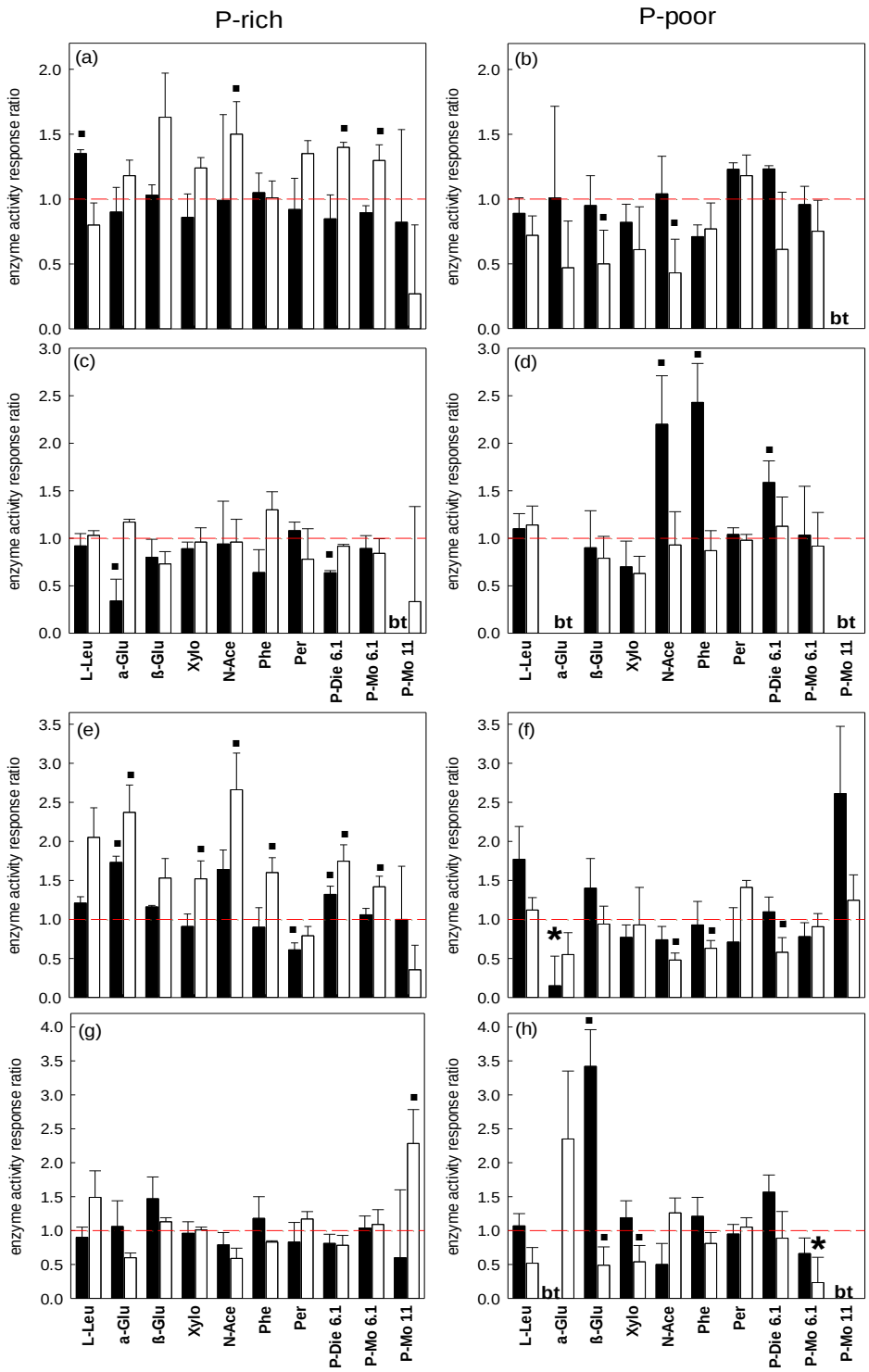


Supplement Figure S1: **Schematic overview on the harvest and sampling scheme**. The plot (green border) was divided (black line) in a control and girdling subplot. In each subplot eight soil cores were taken one week (blue) and eight weeks (red) after the girdling. Each soil core was separated into the organic layer (OL) and mineral topsoil (ML). The eight different soil cores were mixed and fractionated into bulk soil, rhizosphere, fine roots and coarse roots. Each fraction of each layer was divided into three subsamples, of which one was kept fresh, the other frozen and third dry.

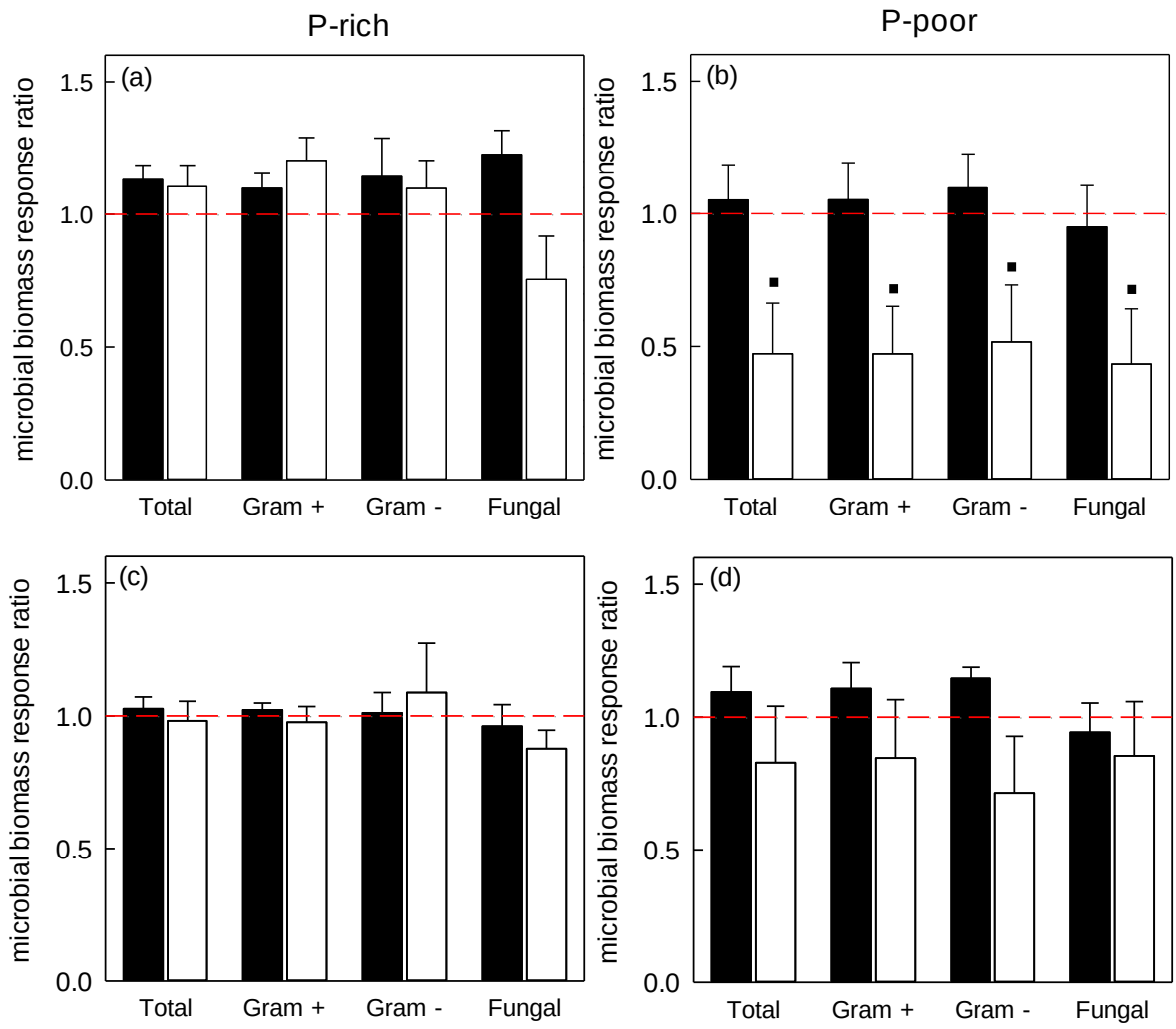


Ectomycorrhizal fungal species	Colour	Reference accession number UNITE	Length and % similarity of nucleotide sequence	NCBI accession number
Unknown EM (Ascomycota)	Black	HG937135	567/567 (100%)	MN970520
Craterellus tubaeformis	Red	UDB015581	422/425 (99%)	MN970524
Imleria badia	Dark green	KT334754	627/627 (100%)	MN970521
Laccaria amethystina	Green	HM189774	712/713 (99%)	MN970515
Lactarius subdulcis	Yellow	KT165260	745/746 (99%)	MN970516
Paxillus involutus	Blue	KT334656	777/778 (99%)	MN970523
Russula fellea	Pink	KT933989	731/731 (100%)	MN970519
Sebacinales sp.	Cyan	AF465191	636/636 (100%)	MN970517
Thelephoraceae sp.	Orange	FN610964	681/682 (99%)	MN970525
Tomentella sp.	Brown	FJ816765	676/679 (99%)	MN970522
Xerocomellus pruinatus	Sand	FN610975	778/780 (99%)	MN970518

Supplement Figure S2: Ectomycorrhizal fungal community on beech (*Fagus sylvatica* L.) roots after girdling (G) and of untreated control plants (C) analyzed in summer (a) and early fall (b). Roots were analyzed one week (a) and eight weeks (b) after girdling. Trees were investigated in P-rich (HP) and P-poor (LP) forests. Roots from the organic layer and the mineral topsoil were analysed separately. Since there were no differences, data for the soil layers were pooled. Data indicate means (HP: n = 6, LP: n = 8). The sample code is shown in the table together with reference accession number (best match in the UNITE Genbank), the sequence length/length in the data base and % similarity of nucleotide sequence, and accession number under which the sequences of the present fungi have been deposited in NCBI Genbank.



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-h). Bars indicate the response ratio of girdled/control. The response ratios were determined one week (black) and eight weeks after girdling (white bars) in the organic layer (a, b, e, f) and the mineral topsoil (c, d, g, h) in a P-rich (left side, a, c, e, g) and a P-poor forest (right side, b, d, f, h). Bars show L-leucine peptidase (L-Leu), α-glucosidase (α-Glu), β-glucosidase (ß-Glu), xylosidase (Xylo), N-Acetyl-glucosaminidase (N-Ace) and phosphodiesterase with MES buffer at pH 6.1 (P-Die 6.1), phosphomonoesterase with MUB buffer at 6.1 (P-Mo 6.1), phosphomonoesterase with MUB buffer at pH 11 (P-Mo 11) and phenoloxidase (Phe), peroxidase (Per) with MUB buffer at pH 3. 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. * indicated $p \leq 0.05$, black squares above bars indicates a marginal difference (trend with $p \leq 0.10$). Controls are marked with the dashed line, bt = below threshold.



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.** Bars indicate means for the response ratio of PFLAs girdled/control determined one week (black) and eight weeks after girdling (white bars). PFLAs were determined in the organic layer of the P-rich (a) and a P-poor (b) forest and in the mineral topsoil of a P-rich (c) and a P-poor forest (d). Bars show: Total microbes (Total), gram positive bacteria (Gram +), gram negative bacteria (Gram -), and fungi (Fungal). 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. Black squares above bars indicates a marginal difference ($p \leq 0.10$). Controls are marked with the dashed line.

Supplement Table S1: Carbon (C) and nitrogen (N) concentrations (mg g^{-1} dry mass) in soil, roots and leaves of beech trees in a P-rich (HP) and P-poor (LP) forests. Harvests of material were conducted in early fall (LP: 17.09.2018, HP: 25.09.2018). Aliquots of 0.7-1 mg dry milled material were weighed into 4mm x 6 mm tin capsules (IVA Analysentechnik, Meerbusch, Germany) using a microbalance (Model: Cubis MSA 2.7S-000-DM, Sartorious, Goettingen, Germany). Samples were analyzed in an Elemental Analyzer (Model SHNC-O EA1108, Carlo Erba Instruments, Milan, Italy). Acetanilide (71.09% C, 10.36% N) was used as the standard for quantification of N and C. For leaves, P concentrations (mg g^{-1}) are also indicated (taken from Lang et al. 2017). Data show means for trees and soil close to the experimental plots used in this study (n = 3 ± SE). To determine the effects of forest site, layer and their interaction (SxL), linear mixed effect models (´lmer´) were used with plot as random factor. Bold letters indicate significance at $p \leq 0.05$.

Site	**HP**		**LP**		**Forest type**		**Layer**		**Si x L**	
Layer	**OL**	**ML**	**OL**	**ML**	**F**	***p***	**F**	***p***	**F**	***p***
Bulk soil										
C	223 ± 18.8	93.9 ± 6.45	243 ± 31.9	28.7 ± 1.2	6.1	**0.043**	67.7	**<0.001**	6.0	**0.044**
N	12.9 ± 0.93	6.97 ± 0.55	11.8 ± 1.36	1.07 ± 0.01	0.0	0.953	61.9	**<0.001**	4.0	0.086
Fine roots										
C	448 ± 3.57	438 ± 12.7	508 ± 0.9	490 ± 2.96	78.9	**<0.001**	23.1	**0.002**	1.6	0.253
N	20.6 ± 0.92	18 ± 0.75	15 ± 0.21	10.7 ± 0.17	60.5	**<0.001**	3.4	0.11	0.2	0.654
Leaves										
C	819 ± 29.2		780 ± 17.9		2.0	0.217				
N	38.4 ± 2.76		34.1 ± 1.28		1.2	0.318				
P	1.41 ± 0.21		1.21 ± 0.08		2.1	0.218				

Supplement Table S2: Functions, primers, standards and thermal profiles for the targeted microbial genes in soil. Gene abundances were determined by real-time PCR assays. Each thermal profile was performed with 35 cycles or 50 cycles respectively.

Target gene	Function	Purpose	Primer	Source of Standard	Thermal profile	No. of cycles
16S rRNA			FP 16S, RP 16S	*E. coli* DSM 30083 (DSMZ)	95°C-45s 55°C-45s 72°C-45s	35
pitA	Pi transporter	Constitutively expressed, low affinity	*pitA*-FW, *pitA*-RW	*Pseudomonas fluorescens*		
pstS	Pi transporter	P starvation response (Pho regulon)	*pstS*-FW, *pstS*-RW	*Bradyrhizobium japonicum*		
phoD	Mineralization	Orthophosphate to microorganisms under P starvation	*phoD*-FW, *phoD*-RW	*Bradyrhizobium japonicum*		
phoN	Mineralization	nonspecific acid phosphatase, acts on ester bonds, carbon, phosphate, nitrogen, or sulfur limitation	*phoN*-FW, *phoN*-RW	*Salmonella enterica* DSM 10062	95°C-15s 60°C-60s 72°C-30s 81°C-60s	50
phnX	Mineralization	Hydrolase specifically acts on carbon-phosphorus bonds	*phnX*-FW, *phnX*-RW	*Salmonella enterica* DSM 17058 (DSMZ)		
appA	Mineralization	Periplasmic phosphoanhydride phosphohydrolase	*appA*-FW, *appA*-RW	*E. coli* DSM 30083 (DSMZ)		
Gcd	Solubilization of Pi	By direct oxidation pathway of glucose and other aldose sugars	*gcd*-FW, *gcd*-RW	*Salmonella enterica* DSM 17058 (DSMZ)		

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. Measurements were conducted in summer (1wk) and early fall (8wk). Data show means for control plots (HP: n = 3, LP: n = 4 ± SE). To determine the effects of forest type, sampling date and their interaction linear mixed effect models (´lmer´) were used with plot as random factor. Bold letters indicate significance at $p \leq 0.05$. When only one sample was available, SE is missing. nd = not determined, EMF = ectomycorrhizal fungi, PFLA = marker phospholipid fatty acids for bacteria and fungi

	Site	**HP**				**LP**				**Site**		**Harvest**		**Site * Harvest**	
	Harvest	**1wk**		**8wk**		**1wk**		**8wk**		**F**	***p***	**F**	***p***	**F**	***p***
	Organic layer														
Acid phosphatase activity	Roots [µmol g^{-1} h^{-1}]	4.83	± 0.64	18.97	± 1.54	20.41	± 0.97	18.90	± 1.52	31.1	**0.002**	30.5	**0.003**	44.8	**0.001**
	EMF [pmol cm^{-2} min^{-1}]	nd		79.57	± 6.96	nd		54.12	± 2.88	9.9	**0.034**				
	Rhizosphere [nmol g^{-1} h^{-1}]	1.5E+04	± 1.5E+03	1.5E+04	± 1.9E+03	9.0E+03	± 9.2E+02	1.3E+04	± 2.3E+03	1.6	0.257	3.8	0.126	1.5	0.295
	Bulk soil [nmol g^{-1} h^{-1}]	1.4E+04	± 2.5E+03	1.3E+04	± 2.1E+03	4.2E+03	± 6.3E+02	9.8E+03	± 2.1E+03	4.8	0.073	10.8	**0.030**	19.7	**0.011**
Gene abundances	*pitA* [copies g^{-1} dw]	3.2E+08	± 6.7E+07	4.2E+08	± 6.2E+07	8.5E+07	± 1.4E+07	2.8E+08	± 3.4E+07	14.4	**<0.001**	8.7	**0.003**	0.9	0.339
	pstS [copies g^{-1} dw]	3.4E+06	± 1.6E+06	1.8E+06	± 5.4E+05	5.8E+05	± 7.4E+04	8.5E+05	± 3.2E+05	3.8	0.106	1.1	0.299	3.1	0.079
	phoD [copies g^{-1} dw]	7.8E+07	± 2.1E+07	9.5E+07	± 2.3E+07	6.1E+06	± 2.5E+05	1.8E+07	± 2.3E+06	16.5	**0.009**	3.0	0.082	0.2	0.673
	phoN [copies g^{-1} dw]	1.5E+06	± 2.1E+05	1.7E+06	± 2.4E+05	1.9E+05	± 2.5E+04	5.8E+05	± 8.8E+04	37.1	**0.002**	34.2	**<0.001**	5.4	**0.020**
	phnX [copies g^{-1} dw]	8.3E+07	± 2.1E+07	8.9E+07	± 1.4E+07	4.1E+06	± 1.2E+06	4.1E+07	± 9.5E+06	15.9	**0.010**	6.0	**0.015**	2.9	0.086
	appA [copies g^{-1} dw]	2.0E+06	± 1.2E+06	1.2E+06	± 5.7E+05	0.0E+00	± 0.0E+00	2.3E+05	± 5.0E+04	3.9	0.104	0.5	0.490	1.9	0.170
	gcd [copies g^{-1} dw]	7.8E+07	± 3.2E+07	9.2E+07	± 1.3E+07	1.7E+07	± 8.7E+05	4.1E+07	± 1.0E+07	9.8	**0.002**	1.1	0.286	0.1	0.776
Microbial biomass	$PLFA_{total}$ [nmol g^{-1} dw]	181.58	± 13.46	299.10	± 1.69	46.29	± 2.75	141.78	± 32.41	47.8	**0.001**	30.9	**0.003**	0.4	0.563
	Gram + [nmol g^{-1} dw]	109.24	± 8.30	142.94	± 3.18	27.11	± 1.67	78.48	± 18.52	39.9	**<0.001**	13.4	**0.004**	0.6	0.464
	Gram - [nmol g^{-1} dw]	24.79	± 3.16	37.00	± 3.79	7.08	± 0.89	22.93	± 4.60	15.6	**0.007**	27.7	**0.003**	0.4	0.557
	Fungal [nmol g^{-1} dw]	14.88	± 1.93	57.32	± 6.65	4.27	± 0.56	15.40	± 4.24	34.6	**0.002**	70.1	**<0.001**	26.8	**0.006**
	Mineral topsoil														
Acid phosphatase activity	Roots [µmol g^{-1} h^{-1}]	4.87	± 0.73	13.86	± 1.88	10.74	± 1.73	8.09	± 0.90	0.1	0.819	17.8	**0.021**	9.3	0.051
	EMF [pmol cm^{-2} min^{-1}]	nd		61.23	± 2.93	nd		50.42	± 1.60	12.2	**0.018**				
	Rhizosphere [nmol g^{-1} h^{-1}]	6.8E+03	± 8.0E+02	9.8E+03	± 2.0E+02	1.4E+03	± 2.3E+02	4.9E+03	± 1.0E+03	53.5	**<0.001**	21.0	**0.001**	0.1	0.737
	Bulk soil [nmol g^{-1} h^{-1}]	6.1E+03	± 5.6E+02	7.7E+03	± 3.1E+02	5.8E+02	± 1.1E+02	1.5E+03	± 2.2E+02	265.3	**<0.001**	38.3	**0.002**	1.9	0.230
Gene abundances	*pitA* [copies g^{-1} dw]	1.0E+08	± 2.0E+07	2.6E+08	± 8.9E+07	4.7E+07	± 8.4E+06	1.4E+08	± 3.7E+07	2.5	0.169	8.3	**0.004**	0.7	0.392
	pstS [copies g^{-1} dw]	1.1E+07	± 2.1E+06	6.5E+06	± 1.8E+06	4.4E+05	± 7.8E+04	5.3E+05	± 1.0E+05	36.0	**<0.001**	2.8	0.092	3.1	0.081
	phoD [copies g^{-1} dw]	6.1E+07	± 2.6E+07	6.1E+07	± 2.8E+07	1.6E+06	± 8.4E+04	4.6E+06	± 7.0E+05	6.8	**0.048**	0.1	0.707	0.1	0.795
	phoN [copies g^{-1} dw]	9.0E+05	± 1.4E+05	1.0E+06	± 3.7E+05	5.2E+04	± 1.8E+03	4.3E+05	± 8.3E+04	13.1	**<0.001**	1.7	0.199	0.3	0.570
	phnX [copies g^{-1} dw]	3.2E+07	± 3.4E+06	5.1E+07	± 5.2E+06	4.7E+05	± 8.9E+04	7.9E+06	± 5.4E+05	143.5	**<0.001**	18.0	**<0.001**	3.5	0.063
	appA [copies g^{-1} dw]	5.8E+05	± 1.4E+05	1.3E+06	± 6.5E+05	3.7E+04		1.3E+05	± 2.8E+04	5.6	0.060	1.8	0.178	1.0	0.329
	gcd [copies g^{-1} dw]	5.2E+07	± 6.6E+06	7.5E+07	± 1.5E+07	8.5E+06	± 2.8E+06	2.6E+07	± 1.9E+06	30.2	**<0.001**	5.7	**0.017**	0.1	0.778
Microbial biomass	$PLFA_{total}$ [nmol g^{-1} dw]	79.47	± 2.97	145.02	± 11.19	11.35	± 1.85	26.24	± 4.25	212.6	**<0.001**	89.8	**<0.001**	34.2	**0.002**
	Gram + [nmol g^{-1} dw]	52.99	± 1.86	88.27	± 7.17	6.85	± 1.05	14.06	± 2.29	231.7	**<0.001**	65.7	**<0.001**	27.8	**0.003**
	Gram - [nmol g^{-1} dw]	10.34	± 0.63	22.39	± 0.70	1.25	± 0.14	5.75	± 1.29	222.6	**<0.001**	92.2	**<0.001**	19.2	**0.001**
	Fungal [nmol g^{-1} dw]	4.15	± 0.78	9.12	± 1.31	1.06	± 0.25	2.14	± 0.45	53.6	**<0.001**	132.0	**<0.001**	53.2	**0.001**

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). Roots from the organic layer and the mineral topsoil were analyzed separately in summer (1wk) and early fall (8wk) after girdling. Data indicate means (HP: n = 3, LP: n = 4) ± SE. To determine the effects of forest type, sampling date, treatment and their interaction linear mixed effect models (´lmer´) were used with plot as random factor. A posthoc Tukey HSD test was performed to compare means. Different letters in rows indicate significant differences at $p \leq 0.05$.

Site	**HP**												**LP**											
Harvest	**1wk**						**8wk**						**1wk**						**8wk**					
Treatment	**C**			**G**			**C**			**G**			**C**			**G**			**C**			**G**		
Organic layer																								
fine roots (g m^{-2})	820.1	± 177.6	a	902.6	± 173.5	a	717.4	± 198.7	a	554.6	± 94.5	a	1942.2	± 298.2	b	983.2	± 175.3	a	1355.1	± 203.8	ab	588.5	± 166.9	a
vital root tips (%)	91.68	± 2.69	b	90.21	± 0.82	b	89.57	± 1.24	b	79.41	± 0.53	a	90.25	± 2.34	ab	89.04	± 1.87	ab	93.17	± 1.48	b	86.31	± 1.26	a
EMF root tips (%)	97.84	± 0.32	d	91.69	± 1.20	a	93.15	± 1.25	ab	94.04	± 0.65	ac	97.15	± 0.38	cd	97.39	± 0.42	cd	95.74	± 0.73	bcd	96.18	± 0.37	bcd
Species richness	7.3	± 1.2	a	7.7	± 1.3	ab	14.0	± 1.7	bc	15.3	± 1.2	c	9.5	± 1.2	ac	9.3	± 1.0	ab	12.8	± 1.4	c	11.3	± 1.2	ac
Shannon diversity	1.72	± 0.21	ab	1.65	± 0.09	a	1.85	± 0.17	ac	2.02	± 0.16	ac	1.89	± 0.14	ab	1.99	± 0.12	ac	2.29	± 0.11	c	2.03	± 0.07	bc
Evenness	0.78	± 0.03	c	0.72	± 0.11	bc	0.46	± 0.03	a	0.50	± 0.07	ab	0.72	± 0.05	bc	0.81	± 0.04	c	0.79	± 0.02	c	0.69	± 0.08	c
Mineral topsoil																								
fine roots (g m^{-2})	529.8	± 64.4	ab	383.8	± 21.5	a	733.8	± 41.6	b	563.6	± 55.2	ab	460.8	± 33.2	ab	460.0	± 53.7	ab	520.4	± 114.1	ab	295.1	± 69.6	a
vital root tips (%)	85.79	± 2.82	ab	85.00	± 2.55	ab	90.17	± 1.04	b	80.04	± 5.23	a	85.91	± 2.59	ab	81.83	± 1.69	a	90.14	± 1.62	b	81.85	± 1.77	a
EMF root tips (%)	95.89	± 0.37	a	93.21	± 1.05	a	95.91	± 1.56	a	94.42	± 2.42	a	96.44	± 0.89	a	96.68	± 0.25	a	93.57	± 0.73	a	93.02	± 0.18	a
Species richness	11.3	± 1.2	ac	10.3	± 0.7	ab	15.0	± 0.6	bc	15.7	± 0.7	c	12.3	± 1.3	ac	10.5	± 1.0	ab	15.3	± 0.6	c	10.0	± 1.1	a
Shannon diversity	2.03	± 0.07	a	2.00	± 0.09	a	2.11	± 0.13	a	2.09	± 0.09	a	2.22	± 0.13	a	2.09	± 0.10	a	2.52	± 0.04	a	2.06	± 0.20	a
Evenness	0.69	± 0.08	ab	0.74	± 0.10	ab	0.56	± 0.05	ab	0.52	± 0.05	a	0.76	± 0.03	ab	0.78	± 0.03	ab	0.82	± 0.03	b	0.82	± 0.09	b

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. Measurements were conducted in summer (1wk) and early fall (8wk). Data show means for control plots (HP: n = 3, LP: n = 4 ± SE). To determine the effects of forest type, sampling date and their interaction linear mixed effect models (´lmer´) were used with plot as random factor. Bold letters indicate significance at p ≤ 0.05. # indicate log transformation prior to statistical analyses to match normal distribution. bt = below threshold.

Site	**HP**				**LP**				**Site**		**Harvest**		**Site x Harvest**	
Harvest	**1wk**		**8wk**		**1wk**		**8wk**		**F**	***p***	**F**	***p***	**F**	***p***
Organic layer - bulk soil														
L-Leucine peptidase MES pH 6.1	623 ± 113		1960 ± 779		164 ± 33.6		339.2 ± 65.0		9.2	**0.029**	5.5	0.065	3.3	0.130
α-Glucosidase MES pH 6.1	77.6 ± 24.5		114 ± 30.2		4.75 ± 1.41		48.2 ± 4.99		18.0	**0.002**	3.7	0.083	0.0	0.909
β-Glucosidase MES pH 6.1	1400 ± 142		2166 ± 368		153 ± 41.5		510 ± 176		5.0	**0.049**	1.0	0.330	1.0	0.350
Xylosidase MES pH 6.1	796 ± 170		729 ± 155		87.1 ± 16.5		252 ± 69.7		35.0	**0.002**	18.7	**0.008**	2.5	0.177
N-Acetyl-glucosaminidase MES pH 6.1	635 ± 91.4		1434 ± 331		228 ± 16.2		689 ± 168		8.2	**0.035**	19.1	**0.007**	1.4	0.293
Phenoloxidase MUB pH 3	30.6 ± 9.40		31.5 ± 7.05		10.9 ± 3.18		21.4 ± 2.08		4.2	0.097	6.2	0.055	4.4	0.090
Peroxidase MUB pH 3	57.8 ± 6.00		37.7 ± 10.4		16.9 ± 1.78		17.7 ± 2.49		25.2	**0.004**	4.6	0.085	5.4	0.068
Phosphomonoesterase MES pH 6.1	13638 ± 2534		13011 ± 2095		4161 ± 632		9801 ± 2144		7.4	**0.042**	3.4	0.123	5.4	0.068
Phosphodiesterase MES pH 6.1	390 ± 30.1		271 ± 19.1		45.9 ± 16.7		245 ± 91.1		10.2	**0.024**	0.5	0.506	8.2	**0.035**
Phosphomonoesterase MUB pH 6.1#	4632 ± 967		3451 ± 696		433 ± 98.8		1298 ± 327		19.0	**0.007**	0.5	0.506	21.4	**0.006**
Phosphomonoesterase MUB pH 11#	52.6 ± 27.7		785 ± 741		bt		2.07 ± 1.31		1.7	0.247	1.5	0.278	1.5	0.280
Organic layer - rhizosphere														
L-Leucine peptidase	799 ± 38.6		1418 ± 231		212 ± 21.4		358 ± 66.4		58.3	**<0.001**	13.3	**0.015**	5.1	0.073
α-Glucosidase #	53.7 ± 23.7		163 ± 18.9		98.7 ± 73.1		60.5 ± 12.7		0.4	0.546	0.6	0.456	2.6	0.139
β-Glucosidase	1670 ± 403		3291 ± 254		321 ± 67.9		619 ± 196		42.4	**0.001**	64.2	**<0.001**	30.5	**0.003**
Xylosidase	1061 ± 385		953 ± 93.9		236 ± 25.8		452 ± 109		10.5	**0.023**	0.1	0.730	1.2	0.327
N-Acetyl-glucosaminidase	945 ± 205		2114 ± 381		868 ± 140		1836 ± 706		0.1	0.710	5.3	**0.044**	0.0	0.832
Phenoloxidase #	42.7 ± 11.7		42.7 ± 10.2		22.9 ± 4.92		46.5 ± 19.2		0.3	0.625	1.1	0.342	1.1	0.342
Peroxidase	134 ± 15.9		82.2 ± 26.3		15.3 ± 1.90		9.06 ± 3.41		40.7	**0.001**	7.2	**0.044**	4.4	0.090
Phosphomonoesterase	14685 ± 1457		15247 ± 1914		8991 ± 918		13095 ± 2300		3.8	0.110	2.4	0.179	1.4	0.289
Phosphodiesterase #	371 ± 28.7		364 ± 52.3		208 ± 4.96		537 ± 139		0.0	0.961	4.5	0.089	4.8	0.079
Phosphomonoesterase	4604 ± 1021		4340 ± 568		1118 ± 232		1605 ± 407		18.6	**0.008**	0.1	0.750	1.3	0.310
Phosphomonoesterase	118 ± 50.8		828 ± 579		9.47 ± 26.8		20.1 ± 8.05		3.5	0.089	2.2	0.170	2.1	0.181
Mineral topsoil - bulk soil														
L-Leucine peptidase#	336 ± 81.0		443 ± 89.0		31.0 ± 11.9		56.8 ± 10.1		23.0	**0.005**	80.7	**<0.001**	30.1	**0.003**
α-Glucosidase	20.8 ± 7.62		21.4 ± 2.63		bt		bt		39.2	**<0.001**	0.0	0.931	0.0	0.931
β-Glucosidase #	443 ± 92.3		811 ± 98.4		29.8 ± 5.38		44.6 ± 10.2		107.5	**<0.001**	11.3	**0.007**	9.6	**0.011**
Xylosidase #	401 ± 67.7		475 ± 43.3		11.8 ± 3.81		40.3 ± 11.3		80.3	**<0.001**	10.7	**0.022**	2.1	0.204
N-Acetyl-glucosaminidase #	321 ± 20.2		536 ± 86.8		42.8 ± 6.46		72.2 ± 21.3		84.3	**<0.001**	9.9	**0.025**	5.7	0.062
Phenoloxidase #	23.7 ± 6.07		22.7 ± 6.97		1.20 ± 0.22		2.26 ± 0.31		15.5	**0.011**	0.0	0.973	4.3	0.092
Peroxidase	43.4 ± 3.91		53.5 ± 7.17		28.0 ± 2.19		30.2 ± 2.16		19.7	**0.007**	3.4	0.124	1.4	0.288
Phosphomonoesterase	6121 ± 556		7732 ± 311		580 ± 93.2		1495 ± 221		291.9	**<0.001**	25.0	**0.004**	1.9	0.226
Phosphodiesterase	249 ± 43.4		284 ± 33.9		11.2 ± 3.61		18.9 ± 4.82		70.9	**<0.001**	2.4	0.179	1.0	0.365
Phosphomonoesterase #	2764 ± 336		3065 ± 266		21.4 ± 13.0		77.9 ± 35.4		132.6	**<0.001**	9.6	**0.027**	4.5	0.088
Phosphomonoesterase	0.02 ± 0.02		11.8 ± 11.8		9.4 ± 9.4		bt		0.0	0.879	0.0	0.881	2.0	0.214
Mineral topsoil –rhizosphere														
L-Leucine peptidase	373 ± 78.8		683 ± 73.8		71.1 ± 15.5		184 ± 48.9		51.0	**<0.001**	16.0	**0.010**	3.5	0.120
α-Glucosidase	20.2 ± 1.87		79.3 ± 37.0		0.00 ± 0.00		0.34 ± 0.34		10.2	**0.010**	3.7	0.084	3.6	0.087
β-Glucosidase	584 ± 134		998 ± 49.3		53.6 ± 5.23		178 ± 36.7		95.6	**<0.001**	21.5	**0.006**	6.2	0.055
Xylosidase	578 ± 72.8		606 ± 93.1		37.9 ± 11.6		163 ± 46.3		74.7	**<0.001**	1.8	0.209	0.7	0.416
N-Acetyl-glucosaminidase	671 ± 98.3		1906 ± 591		595 ± 329		323 ± 78.9		6.7	**0.027**	2.3	0.164	5.5	**0.041**
Phenoloxidase	31.3 ± 8.87		43.2 ± 6.08		5.91 ± 1.39		10.4 ± 1.29		23.2	**0.005**	10.5	**0.023**	2.1	0.203
Peroxidase	114 ± 4.93		106 ± 37.1		46.6 ± 9.48		29.6 ± 3.51		14.0	**0.013**	0.8	0.407	0.1	0.768
Phosphomonoesterase	6787 ± 800		9770 ± 201		1397 ± 202		4865 ± 1016		53.5	**<0.001**	21.0	**0.001**	0.1	0.737
Phosphodiesterase #	306 ± 45.9		565 ± 147		42.4 ± 10.3		92.4 ± 15.2		28.7	**0.003**	6.3	0.054	2.9	0.152
Phosphomonoesterase	3621 ± 212		4078 ± 474		285 ± 238		331 ± 69.4		115.1	**<0.001**	2.2	0.196	1.5	0.276
Phosphomonoesterase	38.0 ± 24.8		98.3 ± 67.5		bt		1.29 ± 1.29		5.0	**0.049**	1.0	0.330	1.0	0.350

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. Bulk soil from the organic layer and the mineral topsoil were analyzed separately in summer (one week, 1wk) and early fall (eight weeks, 8wk) after girdling. Data indicate means (n = 3 (HP), 4 (LP)) ± SE. To determine the effects of forest type, sampling date, treatment and their interaction linear mixed effect models (´lmer´) were used with plot as random factor. Bold letters indicate significance at $p \leq 0.05$.

Site	**HP**				**LP**											
Harvest	**1wk**		**8wk**		**1wk**		**8wk**		**Site**		**Harvest**		**Treatment**		**S*H*T**	
Treatment	**C**	**G**	**C**	**G**	**C**	**G**	**C**	**G**	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Organic layer																
P_{tot}	7.1 ± 0.7	9.2 ± 1.0	8.3 ± 1.7	10.4 ± 3.5	5.5 ± 0.4	7.1 ± 0.7	5.4 ± 1.0	9.9 ± 3.4	4.4	**0.049**	0.2	0.699	3.5	0.075	0.3	0.612
P_{sol}	2.2 ± 0.1	3.4 ± 0.5	2.0 ± 0.9	2.1 ± 0.8	1.2 ± 0.1	1.5 ± 0.1	1.3 ± 0.2	2.2 ± 0.8	7.5	**0.012**	0.2	0.506	4.1	0.060	1.5	0.232
P_{mic}	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.0	0.4 ± 0.03	0.8 ± 0.1	1.5 ± 0.7	4.5	**0.047**	6.3	**0.021**	1.5	0.239	0.5	0.491
Mineral topsoil																
P_{tot}	27.6 ± 2.4	26.0 ± 3.0	33.3 ± 7.0	41.0 ± 7.0	4.3 ± 0.2	4.7 ± 0.4	5.0 ± 0.7	5.9 ± 2.0	55.2	**<0.001**	11.6	**0.004**	1.2	0.285	1.7	0.206
P_{sol}	5.1 ± 0.7	6.6 ± 2.2	8.3 ± 2.6	7.5 ± 1.1	2.0 ± 0.1	2.0 ± 0.2	1.6 ± 0.3	1.8 ± 0.5	12.8	**0.016**	4.8	**0.044**	1.2	0.288	3.2	0.093
P_{mic}	0.4 ± 0.2	0.4 ± 0.2	1.1 ± 0.4	1.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	16.7	**0.010**	19.2	**<0.001**	0.0	0.970	0.1	0.812

Supplement Methods S1

Ectomycorrhizal (EMF) species identification

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 total number of vital root tips is the sum of the vital EMF and vital nonmycorrhizal root tips. EMF colonization and root vitality were calculated as:

$$\text{EMF colonization } (\%) = \frac{\text{number of mycorrhizal root tips}}{\text{number of vital root tips}} \times 100$$

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

To determine EMF species identities, frozen morphotypes were ground in a mortar with a handheld pestle (VWR, Leicestershire, UK) under liquid nitrogen. DNA was extracted using the innuPREP Plant DNA Kit (Analytik Jena AG, Jena, Germany) according to the instructions of the manufacturer. The ITS region of the fungal rDNA was amplified using the PCR primers ITS1F (5′CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). The PCR product was purified using innuPREP PCRpure Kit (Analytik Jena AG, Jena, Germany). Ligation of the PCR product was performed using the pGEM-T Vector Systems (Promega, Madison, WI, USA) following the manual of the manufacturer. The plasmids were transformed into electrocompetent E. coli TOP 10 (Invitrogen, Carlsbad, CA, USA). Positive bacterial clones with target DNA were used as template for further DNA amplification as described above (PCR with primers ITS1F and ITS4 respectively). The amplicons were sequenced by a company (Seqlab, Goettingen, Germany). The sequences were analyzed with Staden package (http://staden.sourceforge.net) and blasted against NCBI GenBank (www.ncbi.nlm.nih.gov) and UNITE (unite.ut.ee) databases. Further information of the fungal identities is found in Supplemental Figure S1.

Ectomycorrhizal phosphatase activities

Individual root tips, assigned to each morphotype, were collected and their enzyme activity were analyzed using a high-throughput microplate fluorometric assay as described in Pritsch et al. (2011). Acid phosphatase (EC 3.1.3.2) activity was measured with fluorescent 4-methylumbelliferone (4-MUF) phosphate (pH 4.5). By hydrolysis, the released MU-fluorescence indicates the substrate turnover rate by phosphatase activity (Jones et al., 2015). Fluorescence measurements were carried out immediately after root tip collection using a fluorescence microplate reader (Victor3, Wallac Perkin–Elmer Life Sciences, Villebon-Sur-Yvette, France), slit width of 5 nm, with an excitation wavelength of 355 nm and the emission wavelength of 460 nm. Phosphatase activity was expressed per unit time and root area (Pritsch et al., 2011).

Projected area of the EMF root tip was measured using the images of the 96-well plates, which were previously used in the fluorescence measurement assay. The plate images were acquired with a high-resolution trans-illumination scanner (ScanMaker i800pluS, Microtek., China). The image analysis was conducted with Adobe Photoshop software (Adobe, California, USA) as described by Buée et al. (2005). In each sample, the enzyme activity was measured on three and, when the number was enough, six root tips of each morphotype. However, after DNA sequencing, some morphotypes resulted in the same fungal species, and for those taxa, the number of enzyme-measured root tips increased.

In each sample, EMF fungal community-weighted mean (CWM) of phosphatase activity was calculated after Garnier et al. (2004).

$$CWM = \sum_{i=1}^{S} Pi * EAi$$

The relative abundance of individual EMF fungal taxa was calculated as number of ectomycorrhizal root tips of each taxa divided by the total number of vital EMF root tips. where *S* is species richness, *Pi* is the relative abundance of species *i*, and *EAi* is the phosphatase activity of the root tips colonized by fungal species *i*.
Phosphatase activity was calculated as the mean value of phosphatase activity of the measured root tips assigned to an individual EMF fungal taxon.

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 quality and quantity of the DNA was checked by using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Schwerte, Germany). Extracted DNA was then stored at − 20 °C until further processing. The extracts were used to determine the abundances of seven P cycle related genes (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, solubilizing P by the oxidation of glucose and aldose sugars (Supplement Table S2) using the primers described by Bergkemper et al. (2016). The 16S rRNA served as proxy for overall bacterial biomass (primers: Bach et al., 2002). Quantitative real-time PCR was conducted on a 7300 Real-Time PCR System (Applied Biosystems, Schwerte, Germany) using SybrGreen as fluorescent dye. The reaction mixture was composed of 12.5 µl PowerSybr Green Master Mix, 2 µl DNA, 0.5 µl bovine serum albumin, 1 µl per primer (*phoN*: 0.2 µl each primer) and was filled up to 25 µl with DEPC water. The Power SybrGreen Master Mix was obtained from Applied Biosystems; primers were synthesized by Metabion (Planegg, Germany), and bovine serum albumin (BSA) was purchased from Sigma (Taufkirchen, Germany). As standards, serial plasmid dilutions ranging from 10^1 to 10^6 (10^2 to 10^7 for 16S rRNA gene) gene copies per µl were used. In order to avoid inhibition of the PCR reaction, an experiment with dilution series of all samples was performed in advance, resulting in an optimal dilution of the samples of 1:64. Quantitative real-time PCR was performed in 96-well plates (Applied Biosystems) for all seven target genes. Each plate contained samples, serial dilutions of the standards in triplicate and at least three no template controls. Each PCR run started with a hot start for 10 min at 95°C. The thermal profile of the PCR, the source of the standard and the primers used are summarized in Supplement Table S1.
To confirm the specificity of the amplicons after each PCR run, a melting curve and a 2% agarose gel were used.

The amplification efficiencies were calculated with the equation $E = [10^{(-\frac{1}{slope})}\text{-}1]$ and resulted in the following values: 16S rRNA gene 75%, *appA* 82%, *gcd* 87%, *phoD* 87%, *phoN* 95%, *phnX* 78%, *pstS* 117% and *pitA* 81%. The R^2 of the standard curve was always above 0.99. The detection limit of the method is 10 gene copies per µl according to the manufacturer's instructions, which corresponds to 10^4 copies g^{-1} dry weight of soil. Samples below 10 copies per µl were treated as 0. This was only necessary for *appA*.

Enzyme activities

All enzyme activities measured in this study are potential activities since they were measured under optimized conditions (in terms of substrate concentration, pH of the buffer, and time).

a) Enzyme activities in fine roots

Frozen roots were milled in a ball mill (MN200, Retsch, Haan, Germany) to a fine powder. For the determination of phosphatase activities, the extraction buffer was prepared one day before use and consisted of 100 mg polyvinylpolypyrrolidone (PVP) (AppliChem, Darmstadt, Germany) in 1.5 ml buffer (0.1 M 2-(N-morpholino)ethansulfonic acid (MES), 0.5% Triton, pH 5.6). Frozen root powder (100 mg) was mixed with the prepared extraction buffer, incubated for 15 min on ice, and centrifuged at 4°C for 30 min at 23940 *g*. The supernatant (0.5 ml) was gel filtered (NAP-5 Columns, No. 17-0853-02, GE-Healthcare illustra, Thermo Fisher Scientific, Schwerte, Germany) and eluted with 1 ml of elution buffer (0.1 M MES, pH 5.6).
Acid phosphatase (EC 3.1.3.2) activity was measured using a modified protocol (Bergmeyer, 2014). 50 µl eluate were mixed with 250 µl reaction solution (5.5 mM pNPP (4-nitrophenyl phosphate disodium salt), J 61401, Alfa Aesar, Thermo Fisher Scientific, Kandel, Germany) in 0.1 M MES, pH 5.6) and incubated at room temperature for 30 min. The reaction was stopped with 500 µl 0.1 M NaOH. The absorption of the solution was measured spectrophotometrically at 405 nm at 25°C. To determine substrate turnover, a calibration curve was produced under the same conditions as above using 10 mM 4-nitrophenol (Fluka #1048) and used to calculate phosphatase activity.
To determine phosphoenolpyruvate carboxylase (PEPC) (EC 4.1.1.31) activity, the extraction buffer was prepared one day before use and consisted of 100 mg polyvinylpolypyrrolidone (PVP) (AppliChem, Darmstadt, Germany) in 1.5 ml buffer (0.1 M Tris-HCl, 5 mM ß-mercaptoethanol, 10% (v/v) glycerol, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM $MgCl_2$, pH 8.0). Frozen root powder (100 mg) was mixed with 1.5 ml of extraction buffer and centrifuged at 4°C for 20 min at 42560 *g*. Then 200 µl supernatant were mixed with 800 µl reaction solution (25 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM D-dithiothreitol, 2 mM $KHCO_3$, 0.1 mM NADPH, 3 units of malate dehydrogenase, 2.5 mM phosphoenolpyruvate, pH 8.0). The reaction solution was prewarmed to 37°C. The decrease in absorbance of the mixture was directly monitored spectrophotometrically at 340 nm at 37°C for 10 min. PEPC activity was determined by the oxidation of NADPH to $NADP^+$ and calculated with the extinction coefficient of 6.22 mM^{-1} cm^{-1}.

b) Enzyme activities in bulk and rhizosphere soil

500 mg frozen soil was mixed with 50 ml deionized H_2O and subsequently dispersed with an ultrasonic disaggregator at 50 J s^{-1} for 120 s. 50 µl of the soil suspension were mixed with 50 µl buffer (0.1 M MES-buffer 2-[N-morpholino] ethanesulfonic acid, pH 6.1, or MUB buffer (stock solution: 1.21 g Tris(THAM) buffer, 1.16 g maleic acid, 1.4 g citric acid monohydrate, 0.63 g boric acid ≥ 99.8% solubilized in $H_2O_{deion.}$, 50 ml 1M NaOH, brought to 100 ml with H_2O_{deion}, working solution: 1:5 dilution of stock solution with H_2O_{deion}. and adjust to pH 6.1 with 1M HCl or to pH 11 with 1 M NaOH) in microplate wells (PP microplate, black 96 wells, Greiner Bio-one GmbH, Frickenhausen, Germany) and 100 µl of fluorescent substrate. The substrates were 7-amino-4-methylcoumarin (AMC) substrate for L-leucine peptidase and 4-methylumbelliferone (4-MUF) substrate for all other enzymes at a final concentration of 10 µM). The following enzyme activities (IUBMB Enzyme nomenclature) were determined in MES buffer: L-leucine peptidase (E.C. 3.4.11.1), α-D-glucosidase (E.C. 3.2.1.20), β-D-glucosidase (E.C. 3.2.1.21), xylosidase (E.C. 3.2.1.37), N-acetyl-glucosaminidase (E.C.

3.2.1.), acid phosphomonoesterase (E.C. 3.1.3.2) and acid phosphodiesterase (E.C. 3.1.4.1) and in MUB buffer: phosphomonoesterase pH 6.1 and pH 11
After an incubation period of 60 min at 30 °C (to stabilize the linearity of the slope), the fluorescence was measured at 0, 30, 60, 120 and 180 min with a fluorescence microplate reader (ELx808, BioTek Instruments Inc., Winooski, VT, USA) with excitation at 360 nm and emission at 460 nm. Between measurements, the microplates were kept in an incubator at 30 °C.
Measurements of potential peroxidase (E.C. 1.11.1.-) and phenoloxidase (E.C. 1.10.3.1) activities were carried out photometrically using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) as the substrate as described by Floch et al. (2007). Peroxidase activity was calculated by subtracting phenoloxidase activity from total oxidase activity (Bach et al., 2013). For total oxidase activity, 50 µl of the soil suspension (as above) was mixed with 140 µl modified universal buffer (MUB; 2.42 g Tris(THAM) buffer, 2.32 g maleic acid, 2.80 g citric acid monohydrate, 1.26 g boric acid ≥ 99.8% solubilized in $H_2O_{deion.}$, adjusted to pH 3, brought to 1 L volume), 50 µl 2mM ABTS substrate solution and 10 µl H_2O_2. in microplate wells (PP microplate, clear 96 wells, Greiner Bio-one GmbH, Frickenhausen, Germany). For phenoloxidase activity, 100 µl soil solution and 100 µl MUB were mixed in each microplate well and 50 µl 2mM ABTS substrate solution was added. A reference sample without substrate was prepared for each sample. Additionally, negative controls without soil were prepared for total enzyme and phenoloxidase activity, respectively. Microplates were pre-incubated at 30 °C for at least 5 min (Floch et al., 2007). Measurement of the resulting stable radical cation ABTS*+ was carried out with a microplate reader (SYNERGY HTX, BioTek Instruments Inc., Winooski, VT, USA) at 30°C at a wavelength 650 nm for 30 min every 3 min.

Determination of microbial phosphorus

Easily soluble, inorganic P (P_{aqua}) and microbially bound P (P_{mic}) were extracted according to Kouno et al. (1995). Corresponding to 2 g dry soil of the mineral layer (1 g dry soil of the organic layer), field fresh soil was extracted with 30 ml deionized H_2O in the presence of an anion exchange membrane (20 mm x 50 mm, RCT®-High-Mechanical-Strength-AMX, Reichelt Chemietechnik, Heidelberg, Germany). To extract the P in microbial cells, a second subsample was prepared as described above and fumigated with 1 ml hexanol. Furthermore, a third subsample was applied for each soil sample with the addition of a defined P concentration (5 µg P g^{-1} dry soil (LP), up to 50 µg P g^{-1} dry soil (HP)) for later correction.

All subsamples were shaken on a horizontal shaker at room temperature for 18 hours and subsequently the membranes were washed with deionized H_2O and transferred to new vessels. To dissolve the P bound on the membranes, 20 ml 0.5 M HCl were added to each vessel and shaken for 1 h. Then, the membrane was removed and 1 ml (HP) to 2 ml (LP) of the acid solution was mixed with 1 ml of Murphy reagent (Murphy & Riley 1962) for color reaction and further measured photometrically at 712 nm with a microplate reader (Biotek ELx808, BioTek Instruments, Winooski, VT, USA). The percent recovery (R) of spiked P was expressed as

$$R\ (\%) = \frac{P_{spike} - P_{aqua}}{Spike} \times 100$$

with P_{spike} and P_{aqua} as the P amounts in the respective subsample, and *Spike* as the added amount of P in the P_{spike} subsample.

P_{mic} was calculated with the following equation:

$$P_{mic}\ (\mu g\ P\ g\ soil^{-1}) = \left(P_{hexanol} - P_{aqua}\right) \times \frac{100}{R}$$

With $P_{hexanol}$ and P_{aqua} as the P concentration values in the respective subsample and R the recovery rate. For each samples, two analytical replicates were performed.

Carbohydrate concentrations in roots

Frozen fine root powder (75 mg) was extracted in 1.5 ml extraction solution (80% dimethyl sulfoxide, 20% of 25 % HCl). The mixture was kept for 30 min in a thermo block (S133-6101, Liebisch, Bielefeld, Germany) at 60°C, cooled on ice, and centrifuged for 5 min at 4°C at 2660 *g* (5417 R, Eppendorf, Hamburg, Germany). The supernatant (200 µl) was mixed with 1200 µl of 0.2 M citrate buffer pH 10.6, centrifuged for 5 min at 4°C at 2660 *g* and further used for carbohydrate analyses (Bergmeyer, 2014). Glucose, fructose and sucrose were determined by the enzymatic activity of glucose-6-phosphate dehydrogenase (EC: 1.1.1.49), phosphoglucose-isomerase (EC: 5.3.1.9) and fructosidase (EC:3.2.1.26) in 50 µl of the supernatant mixed with 50 µl 50 mM citrate, pH 4.6, 250 µl of 4 mM $NADP^+$, 10 mM ATP, 9 mM $MgSO_4$, 0.75 M triethanolamine, pH 7.6 and 400 µl distilled water. The production of NADPH was determined spectrophotometrically (Spectrophotometer DU-640, Beckmann Coulter, Brea, CA, USA) at 340 nm at 25°C. The conversion of starch to glucose was determined three times in 50 µl of the supernatant mixed with 50 µl amyloglucosidase and incubated at 57°C for 20 min using a thermoblock and cooled on ice. Subsequently NADPH production was determined photometrically at 340 nm at 25°C using glucose-6-phosphate dehydrogenase (EC: 1.1.1.49) activity.

Determination of microbial biomass by phospholipid fatty acid determination

2 g of frozen soil were extracted in a solvent of chloroform, methanol, and citrate buffer (pH 4) of 1:2:0.8 (v/v/v) (Bligh & Dyer, 1959). The extract was further separated into glycolipids, neutral lipid fatty acids (NLFA) and phospholipid fatty acids (PLFA) using silica acid SPE cartridges (Bond Elut SI, 500 mg, 3 ml, Agilent Technologies Inc, Santa Clara, CA, USA). The fatty acids were methanolysed with 0.2 M methanolic KOH as described by Ruess et al. (2007). Further, the FAMEs were dissolved in iso-octane and measured on an AutoSystem XL gas chromatograph (Perkin-Elmer Corporation, Norwalk, CT, USA) equipped with a HP-5 capillary column (cross-linked 5% phenyl methyl siloxane; 50 m x 0.2 mm, film thickness of 0.33 µm) and a flame ionization detector. The following temperature set up was used: Initial temperature at 70 °C for 2 min, increased of temperature by 30 °C min^{-1} up to 160 °C, then by 3 °C min^{-1} to 280 °C and held for 15 min. The injection temperature was 260 °C and helium was used as the carrier gas.

Determination of ^{33}P uptake

Before the start of the experiments, roots of extra beech trees were inspected under a stereomicroscope (Leica DFC 420 C, Wetzlar, Germany) and showed that the root tips were 100 % colonized by EMF. For determination of P uptake, a tree was clamped into a tripod and a single fine root (still attached to the tree) was inserted into a reaction vessel with the uptake solution. The remaining root system was covered with wet paper tissues. To prevent dehydration of roots, the tissue coverage was wetted by spraying with artificial soil solution consisting of 100 µM KNO_3, 90 µM $CaCl_2$, 70 µM $MgCl_2$, 50 µM KCl, 24 µM $MnCl_2$, 20 µM NaCl, 10 µM $AlCl_3$, 7 µM $FeSO_4$, 6 µM K_2HPO_4 and 1 µM NH_4Cl (modified by Geßler et al., 2005). The uptake solution consisted of 2 ml artificial soil solution with 1 KBq $^{33}PO_4$ (Hartmann Analytic GmbH, Braunschweig, Germany) per sample. The root was incubated in the uptake solution for 3 h. Pre-tests of incubation times ranging from 2 to 6 h, showed that 3 h were in the linear range and yielded measurable uptake rates. Thereafter, the submersed

part of the root (exposure part) and the subsequent root segment of about 10 mm (transport part), which was not in contact with the uptake solution, were separately cut off and washed three times with artificial soil solution. Afterwards, the root segments were dried for 24 h at 60 °C and used for the determination of dry weight. The dry tissues (complete) were then combusted at 500 °C for 6 h. The ash was mixed with 10 ml of scintillation cocktail (Rotiszint eco plus, Roth, Karlsruhe, Germany), and used for detection of ^{33}P signals (disintegrations per minute (DPM)) with a PerkinElmer scintillation counter (Tri-Carb TR/SL 3180, Waltham, MA, USA). The signal was corrected for the ^{33}P half-life of 25.34 days using QuantSmart (version 4.00, PerkinElmer) to take into account the decay time between harvest and measurements. A background correction was conducted by subtracting blanks (DPM of the scintillation cocktail) from the sample. DPM were converted into Bq (DMP/60) and related to sample dry mass.

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