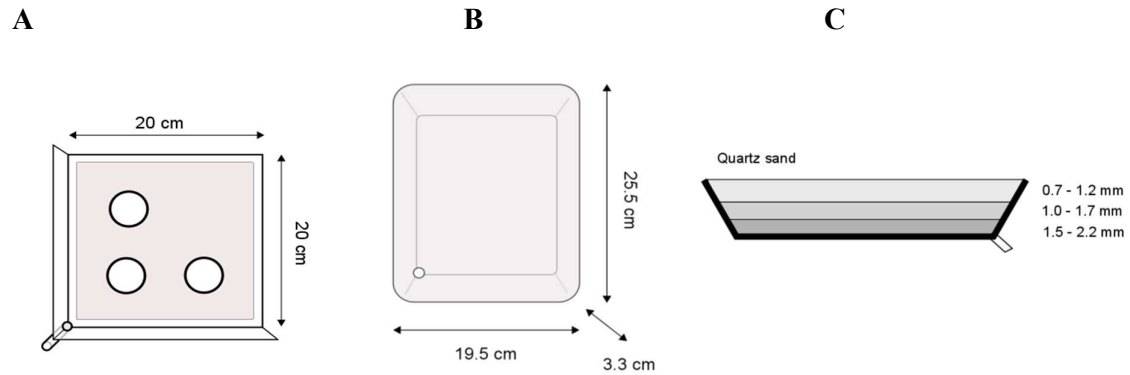


Supplementary Material

1 Lysimeter design

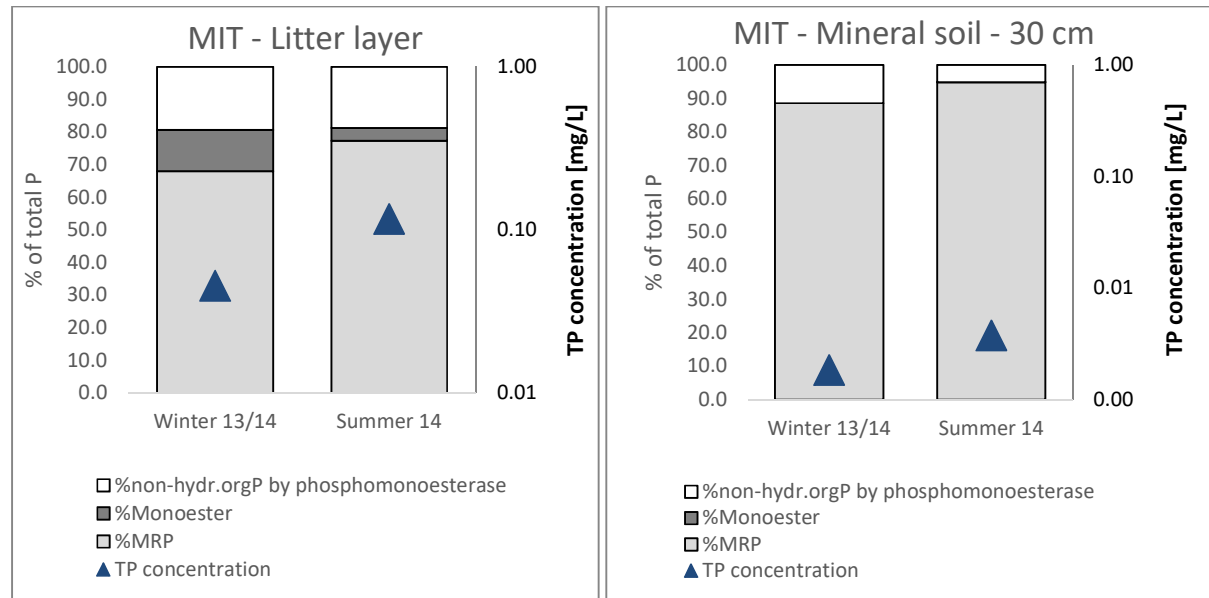


Supplementary Figure 1. Zero-tension lysimeter design. (A) lysimeter for the litter and Oe/Oa horizon, (B) lysimeter for the mineral topsoil, (C) lysimeter for the mineral topsoil: filling with different grained quartz sand.

2 Data from Mitterfels (MIT)

Supplementary Table 1: Characteristics of the the study site Mitterfels (MIT), for more detailed information please see Lang et al. (2017).

Mitterfels	
Location	N: 48.976008° E: 12.879879°
Elevation (m a.s.l.)	1023
Mean annual temperature (°C)	4.5
Mean annual precipitation (mm)	1299
Forest stand	Beech
Parent material	Paragneis
Soil type	Dystric Cambisol
pH (CaCl₂) at 0-5 cm	2.9
Humus layer	Moder
Horizon depth (litter - Oe/Oa -A(e)h)	4/8/6



Supplementary Figure 2. Phosphorus (P) forms in soil solutions from the litter and the A horizon at Mitterfels (MIT) in Winter 2013/2014 and Summer 2014, as revealed by enzyme addition assays with phosphomonoesterase addition; DIP: dissolved inorganic P, DOP = dissolved organic P.

Supplementary Table 2: Results of enzyme addition assays conducted with phosphomonoesterase addition in 2013/2014 on soil solutions from the site Mitterfels (MIT), and results from enzyme addition assays from literature with soil solutions and soil extracts.

Study	Phosphomonoesterase-labile DOP [% of DOP]*			non-hydrolyzable DOP [% of DOP]**			Medium	Ecosystem
	min	max	mean	min	max	mean		
	This study MIT-Litter layer	17	40	29	60	83		
This study MIT-Mineral topsoil	0	1	0	99	100	100	leachate	forest soil

* hydrolyzable by phosphomonoesterase, or classified as simple or labile monoesters/monoester-like

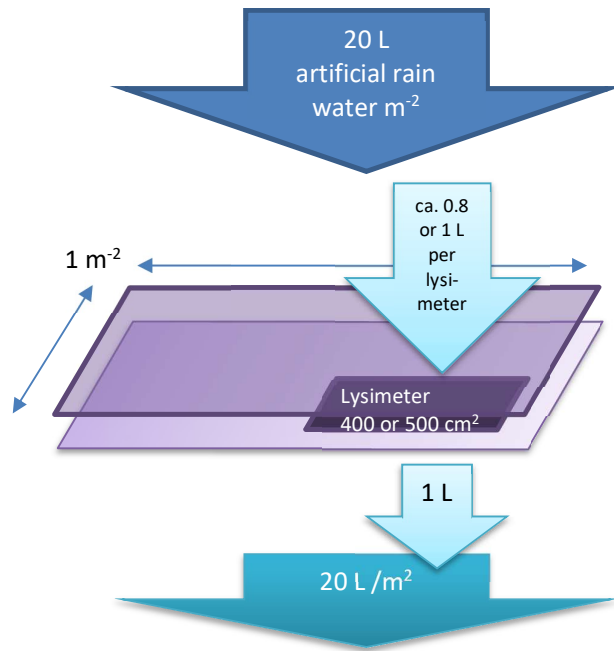
** = enzyme-stable, depends on how many different enzymes were added/were naturally in the solution

DOP: dissolved organic phosphorus

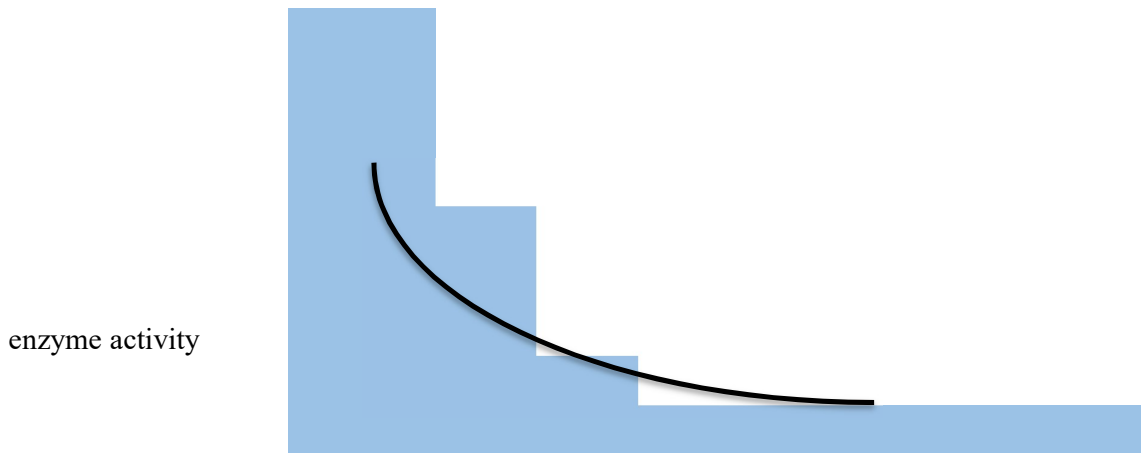
Supplementary Table 3: Back-estimated initial distribution of phosphorus (P) forms in soil solutions based on the organic P hydrolysis model and the P forms measured 22 hours after sampling for the site Mitterfels (MIT) beneath the organic layers (litter and Oe/Oa horizon and the mineral soil (30 cm depth). total P = total phosphorus. *: non-hydrolyzable by phosphomonoesterase, **: no back-estimation possible because of lack of presence of monoester-labile organic P at time of measurement.

Site	Soil depth	Season	Hours	Total P	Inorganic P	Monoester-labile DOP	Non-hydr. org. P at 22 hours*	Monoester-labile DOP
	cm		after sampling	μmol L ⁻¹	% of total P	% of total P	% of total P	% of DOP
MIT	0	Winter 13/14	0 (estimated)	3.76	58	23	19	56
MIT	0	Winter 13/14	22 (measured)	1.46	68	13	19	40
MIT	0	Summer 14	0 (estimated)	3.76	58	23	19	56
MIT	0	Summer 14	22 (measured)	3.76	77	4.0	19	17
MIT	30	Winter 13/14	0 (estimated)	0.06	89	0.0**	11**	0**
MIT	30	Winter 13/14	22 (measured)	0.06	89	0.0	11	0
MIT	30	Summer 14	0 (estimated)	0.12	95	0.3	5.2	4.7
MIT	30	Summer 14	22 (measured)	0.12	95	0.0	5.2	0.8

3 Estimations of phosphomonoesterase activity fluxes in soil solutions and soil extracts



Supplementary Figure 3. Sampling design for leachate samples: 1-hour irrigation with 20 L of artificial rain water. Samples were taken in several leachate volume steps of 100 mL (first two steps), and 250 mL (all following steps). For this study the sample of step 3 was used. Analysis of other parameters done on all samples show an exponential decrease with progressing irrigation and wash out towards a steady state. We assume that this is similar for enzyme activity. Therefore, the rate of step 3 represents a reasonable value for the enzyme activity rate of 1 Liter of leachate.



	1	2	3	4	5
Sampling steps [mL]	100	100	250	250	250
Cumulative sampling volume [mL]	100	200	450	700	950

- Assumption 1: There is an exponential decrease of enzymatic activity during sampling (similar to measured P concentrations)
- Assumption 2: Sampling step 3 is a good approximation of an average value for a liter of leachate
- Assumption 3: At a certain time point there will be a low, but constant value (production = export)

4 Derivation of DOP hydrolysis model

Solution by Michaelis-Menten (MM) model:

The equation of MM model governing the reaction kinetics is a differential equation

$$v = V_{max} \frac{[S]}{K_m + [S]}$$

where 'v' is the instantaneous speed of reaction, [S] is the instantaneous concentration of substrate, while V_{max} and K_M are constants. At any instant of time, speed 'v' of the reaction is the rate of generation of product P, i.e.

$$v = \frac{dP(t)}{dt}$$

So,

$$\frac{dP(t)}{dt} = V_{max} \frac{[S]}{K_m + [S]}$$

At any instant of time 't', concentration of substrate [S] is equal to its concentration at $t = 0$ (i.e. beginning) minus concentration of product. At time ' $t = 0$ ', the concentration of substrate is S_0 .

$$[S] = S_{@t=0} - P(t) = S_0 - P(t)$$

Leading to:

$$\frac{dP(t)}{dt} = V_{max} \frac{S_0 - P(t)}{K_m + S_0 - P(t)}$$

Here, P(t) is product concentration at time 't', while S_0 , V_{max} and K_M are constants. This equation can be solved with any computation program (here done with MATLAB and R), with desired time spans and time steps. Here, it was applied as following:

$$DIP_t = \int_{t'=0}^{t'=t} V_{max} * \frac{labileDOP_0 - DIP_{t'}}{K_M + labileDOP_0 - DIP_{t'}} dt'$$

The equation yields the product concentration P(t) alias DIP_t as a function of time, and can be used to calculate the relevant substrate concentrations (labileDOP_t).

$$labileDOP_t = labileDOP_0 - DIP_t$$

[3]

5 Michaelis-Menten parameters V_{\max} and K_M : Data and statistics

Supplementary Table 4: Michaelis-Menten parameters V_{\max} and K_M for phosphomonoesterase in soil solutions. SE = standard error, n = 12.

	Treatment	Horizon	V_{\max} leachate		K_M leachate		V_{\max} soil		K_M soil	
				SE		SE		SE		SE
			[$\mu\text{mol/L/h}$]		[$\mu\text{mol/L}$]		[$\mu\text{mol/g/h}$]		[$\mu\text{mol/L}$]	
BBR	Control	Litter	4.10 ± 0.6		17.3 ± 3.9		61.7 ± 11		142 ± 32	
BBR	Control	Oe/Oa	2.80 ± 0.2		9.75 ± 1.4		15.3 ± 1.4		37.8 ± 7.1	
BBR	Control	A	2.87 ± 0.1		4.14 ± 0.7		16.4 ± 5.2		33.4 ± 7.6	
BBR	+N	Litter	2.35 ± 0.3		11.5 ± 4.3		41.5 ± 10		130 ± 14	
BBR	+N	Oe/Oa	3.71 ± 0.7		20.0 ± 5.2		19.6 ± 3.3		66.1 ± 22	
BBR	+N	A	2.56 ± 0.3		3.07 ± 0.4		12.3 ± 1.3		17.2 ± 3.5	
BBR	+P	Litter	4.95 ± 1.6		24.9 ± 14		52.1 ± 2.3		137 ± 7.4	
BBR	+P	Oe/Oa	2.94 ± 0.5		10.8 ± 1.8		22.6 ± 3.6		67.6 ± 3.6	
BBR	+P	A	2.47 ± 0.2		3.15 ± 1.5		12.4 ± 2.5		16.4 ± 1.6	
BBR	+NxP	Litter	4.97 ± 1.3		16.1 ± 4.0		41.6 ± 1.0		125 ± 3.5	
BBR	+NxP	Oe/Oa	2.28 ± 0.4		8.22 ± 1.4		14.6 ± 1.6		71.0 ± 15	
BBR	+NxP	A	3.07 ± 0.3		4.86 ± 0.8		7.90 ± 0.9		22.0 ± 8.2	
LUE	Control	Litter	3.74 ± 0.3		97.9 ± 44		128 ± 14		106 ± 21	
LUE	Control	Oe/Oa	5.84 ± 0.0		63.9 ± 0.0		23.1 ± 3.8		61.5 ± 5.0	
LUE	Control	A	2.56 ± 0.7		38.9 ± 14		13.0 ± 6.4		67.3 ± 22	
LUE	+N	Litter	4.57 ± 1.0		34.2 ± 7.1		108 ± 28		90.0 ± 8.2	
LUE	+N	Oe/Oa	1.89 ± 0.3		37.6 ± 18		37.7 ± 14		110 ± 50	
LUE	+N	A	5.17 ± 1.9		68.1 ± 25		14.6 ± 4.2		58.7 ± 15	
LUE	+P	Litter	2.45 ± 0.4		43.1 ± 11		101 ± 6.3		90.9 ± 16	
LUE	+P	Oe/Oa	2.18 ± 0.3		84.6 ± 30		27.8 ± 5.5		98.7 ± 25	
LUE	+P	A	4.57 ± 1.3		204 ± 123		5.74 ± 1.0		20.7 ± 4.9	
LUE	+NxP	Litter	8.09 ± 1.3		161 ± 86		86.4 ± 8.6		68.5 ± 3.4	
LUE	+NxP	Oe/Oa	2.38 ± 0.3		197 ± 7.9		21.9 ± 1.7		60.9 ± 5.6	
LUE	+NxP	A	6.18 ± 1.3		132 ± 28		9.90 ± 1.6		53.9 ± 5.4	

Supplementary Table 5: Statistical results from linear mixed model, where the log transformed independent kinetic parameters K_M and V_{max} in soil solutions and soil extracts were modelled. In the model, site, nitrogen addition (+N), and phosphorus (+P) addition are fitted as fixed effects with an interaction between site, +N, and +P for the N×P treatment; Blocks are fitted as random effects. Presented are the F value (ratio of variance within and between the groups), and the p value with significance, where * indicates $p \leq 0.1$, ** $p \leq 0.05$, and *** $p \leq 0.01$. Data was analysis as subsetted to horizons; previous model without subsets showed horizons significant with $p < 0.01$.

	log K_M soil			log V_{max} soil			log K_M leachate			log V_{max} leachate		
	F value	p value	Sign.	F value	p value	Sign.	F value	p value	Sign.	F value	p value	Sign.
Litter												
Site	12.5	<0.01	***	16.90	0.01	**	14.20	<0.01	***	0.40	0.55	
N	0.9	0.35		4.80	0.05	*	0.00	0.83		1.00	0.33	
P	0.7	0.42		0.50	0.48		0.50	0.49		1.30	0.28	
Site:N	0.2	0.67		0.10	0.71		0.30	0.58		6.10	0.03	**
Site:P	0.7	0.41		0.30	0.59		0.00	0.85		0.40	0.53	
N:P	0.1	0.73		0.40	0.53		2.00	0.18		4.30	0.06	*
Site:N:P	0	0.88		0.00	0.86		0.80	0.39		0.50	0.49	
Oe/Oa												
Site	1.50	0.24		1.60	0.27		40.50	<0.01	***	0.10	0.83	
N	0.20	0.67		0.00	0.94		0.30	0.62		3.20	0.11	
P	0.90	0.35		0.00	0.83		1.70	0.22		4.10	0.08	*
Site:N	0.40	0.55		0.60	0.46		0.00	0.83		3.00	0.12	
Site:P	0.70	0.40		0.40	0.56		7.70	0.02		0.20	0.66	
N:P	1.80	0.20		6.00	0.03	**	0.70	0.42		1.50	0.25	
Site:N:P	0.00	0.83		0.10	0.80		6.80	0.03		8.20	0.02	**
A horizon												
Site	12.90	<0.01	***	0.50	0.50		16.90	0.04	**	2.70	0.13	
N	0.20	0.63		0.10	0.71		1.30	0.31		1.40	0.26	
P	3.80	0.07		3.70	0.08	*	2.10	0.20		1.10	0.33	
Site:N	3.00	0.10		3.70	0.08	*	0.50	0.50		1.10	0.33	

Site:P	0.50	0.50		0.00	0.88	3.30	0.12	0.90	0.37
N:P	4.50	0.05	*	0.00	0.99	0.80	0.40	0.00	1.00
Site:N:P	0.20	0.66		0.50	0.50	0.20	0.66	0.60	0.47

Supplementary Table 6: Statistical results from linear mixed model, where the phosphorus (P) forms total P (P_{tot}), dissolved inorganic P (PO₄-P), and dissolved organic P (DOP) in soil solutions are modelled. P_{tot} and PO₄-P were log transformed due to non-normal distribution of the residuals. In the model, site, event, nitrogen addition (+N), and phosphorus (+P) addition are fitted as fixed effects with an interaction between site, +N, and +P for the N×P treatment; Blocks are fitted as random effects. Presented are the F value (ratio of variance within and between the groups), and the p value with significance, where * indicates $p \leq 0.1$, ** $p \leq 0.05$, and *** $p \leq 0.01$. Data was analysis as subsetting to horizons; previous model without subsets showed horizons significant with $p < 0.01$.

	log P _{tot}			log PO ₄ -P			DOP		
	F value	p value	Sign.	F value	p value	Sign.	F value	p value	Sign.
Litter									
Site	1.4	0.31		0.5	0.54		13.7	0.02	**
N	0.1	0.82		0.2	0.68		0.5	0.48	
P	4	0.05	*	4.5	0.04	**	0	0.83	
Event	151.7	<0.01	***	125.2	<0.01	***	19.8	<0.01	***
Site:N	7.7	<0.01	***	9.7	<0.01	***	1.1	0.30	
Site:P	0.3	0.59		0	0.83		0.3	0.56	
N:P	2.1	0.16		1	0.33		0.2	0.70	
Site:N:P	16.7	<0.01	***	11.2	<0.01	***	1.6	0.22	
Oe/Oa									
Site	0.9	0.39		0.5	0.53		11.3	<0.01	***
N	2.1	0.15		2	0.17		3.9	0.06	*
P	1	0.32		0.7	0.41		0.6	0.43	
Event	37.3	<0.01	***	38.2	<0.01	***	15	<0.01	***
Site:N	1.4	0.25		2.6	0.12		0.1	0.73	
Site:P	0.1	0.72		0.7	0.42		0.2	0.64	

N:P	5.5	0.03	**	5.7	0.02	**	0.5	0.48	
Site:N:P	1.7	0.21		2.1	0.16		0	0.89	
<hr/>									
A horizon									
Site	1.4	0.30		1.2	0.33		10.3	0.03	**
N	0.1	0.80		0.1	0.73		0.2	0.70	
P	0.5	0.50		2.2	0.15		0.1	0.72	
Event	28.3	<0.01	***	19.2	<0.01	***	14.1	<0.01	***
Site:N	0.6	0.43		0.3	0.59		2.8	0.10	
Site:P	0	0.95		0	0.92		2	0.16	
N:P	0.7	0.42		0	0.94		0.2	0.65	
Site:N:P	0.7	0.42		0.6	0.45		0.1	0.76	

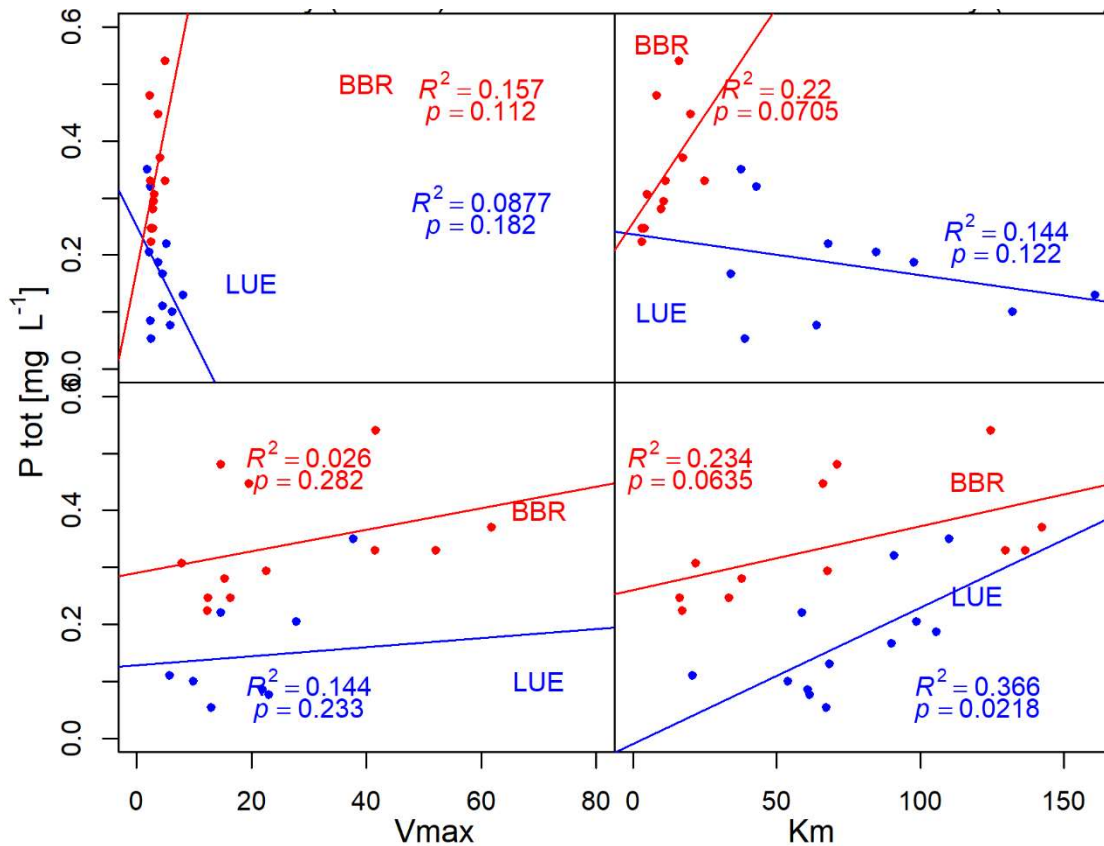
6 Relation total P to Michaelis-Menten parameters and soil parameters

A: V_{\max} in soil solution (top)

B: K_M in soil solution (top)

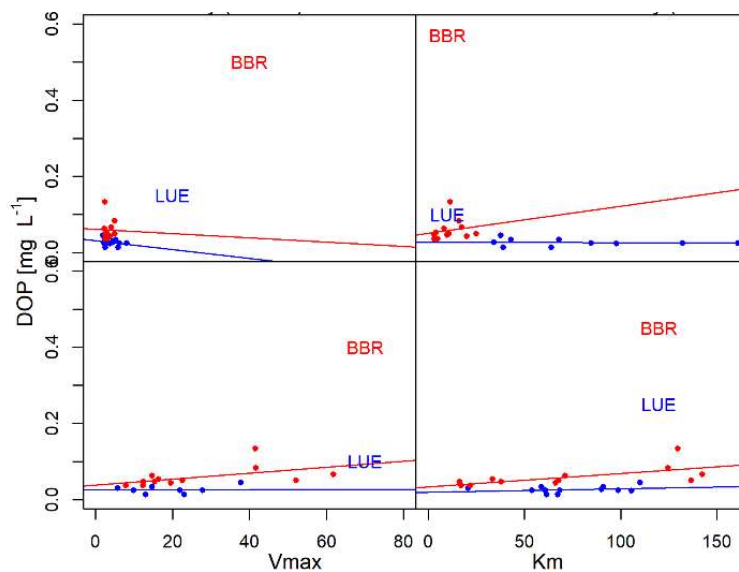
and soil extract (bottom)

and soil extract (bottom)

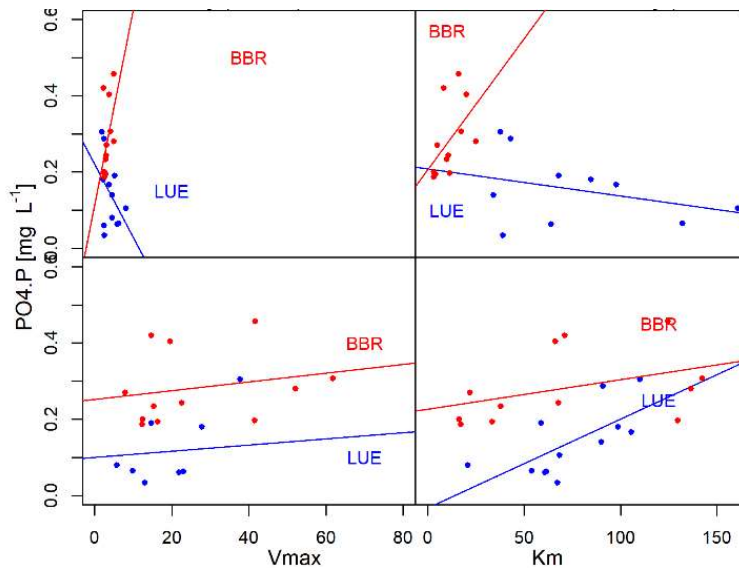


Supplementary Figure 4. V_{\max} and K_M for phosphomonoesterases in soil solutions and soil extracts versus total phosphorus (P) concentration in soil solutions. A: V_{\max} in soil solutions (top) and soil extracts (bottom), B: K_M value in soil solutions (top), and soil extracts (bottom); K_M in $\mu\text{mol/L}$, V_{\max} in $\mu\text{mol/L/h}$ for soil solutions and $\mu\text{mol/g/h}$ for soil extracts.

A: V_{\max} in soil solution (top) and soil extract (bottom) B: K_M in soil solution (top), and soil extract (bottom)

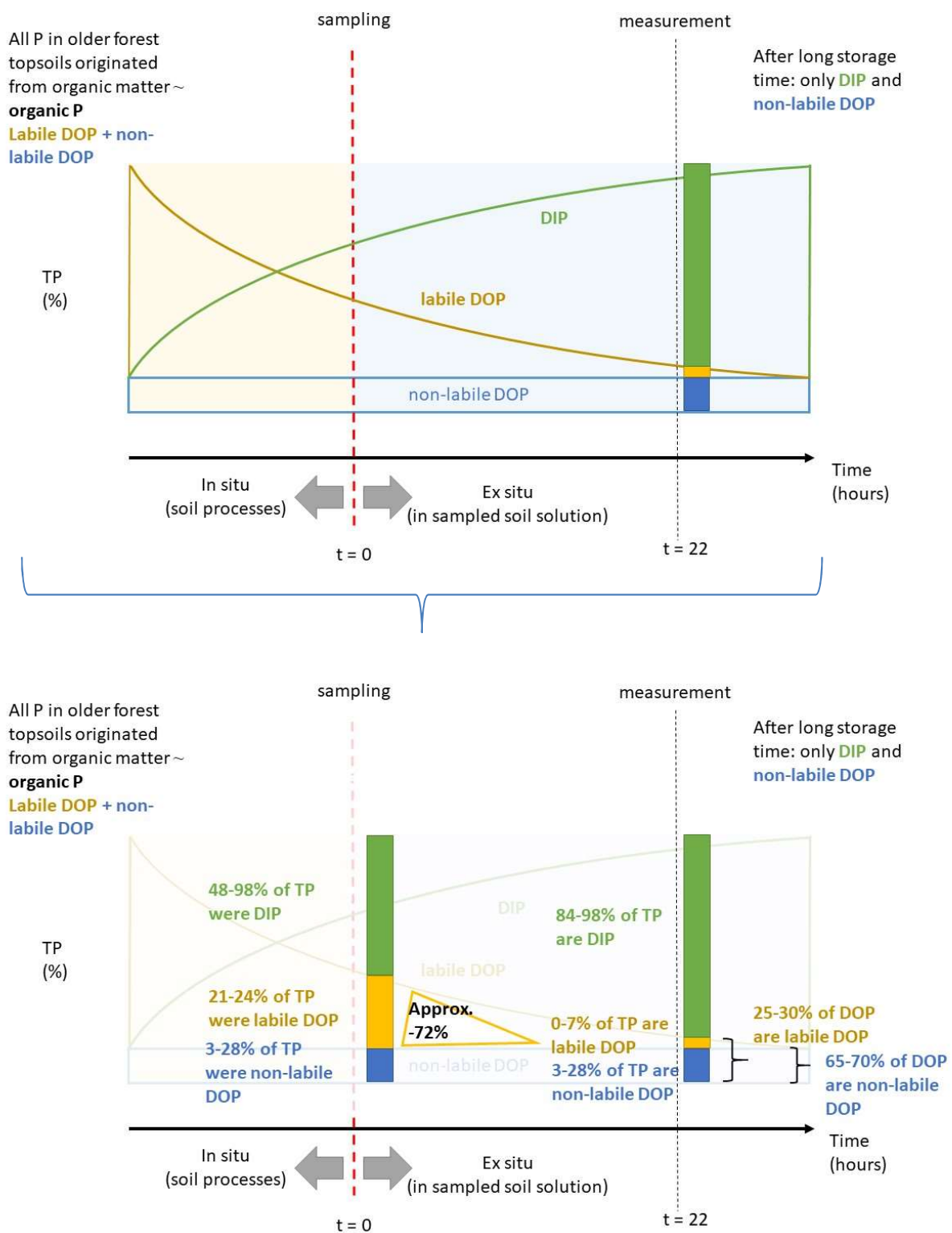


A: V_{\max} in soil solution (top) and soil extract (bottom) B: K_M in soil solution (top) and soil extract (bottom)



Supplementary Figure 5. V_{\max} and K_M for phosphomonoesterases in soil solutions and soil extracts versus dissolved organic phosphorus (DOP) and phosphate ($\text{PO}_4\text{.P}$) concentration in soil solutions. A: V_{\max} in soil solutions (top) and soil extracts (bottom), B: K_M value in soil solutions (top), and soil extracts (bottom); K_M in $\mu\text{mol/L}$, V_{\max} in $\mu\text{mol/L/h}$ for soil solutions and $\mu\text{mol/g/h}$ for soil extracts.

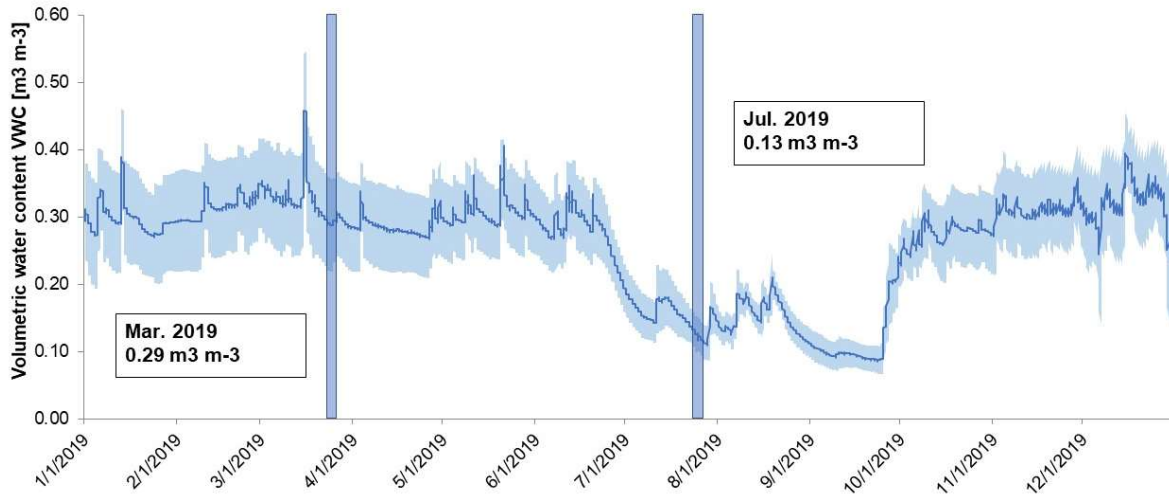
7 Back-estimation of initial labile DOP values – graphical



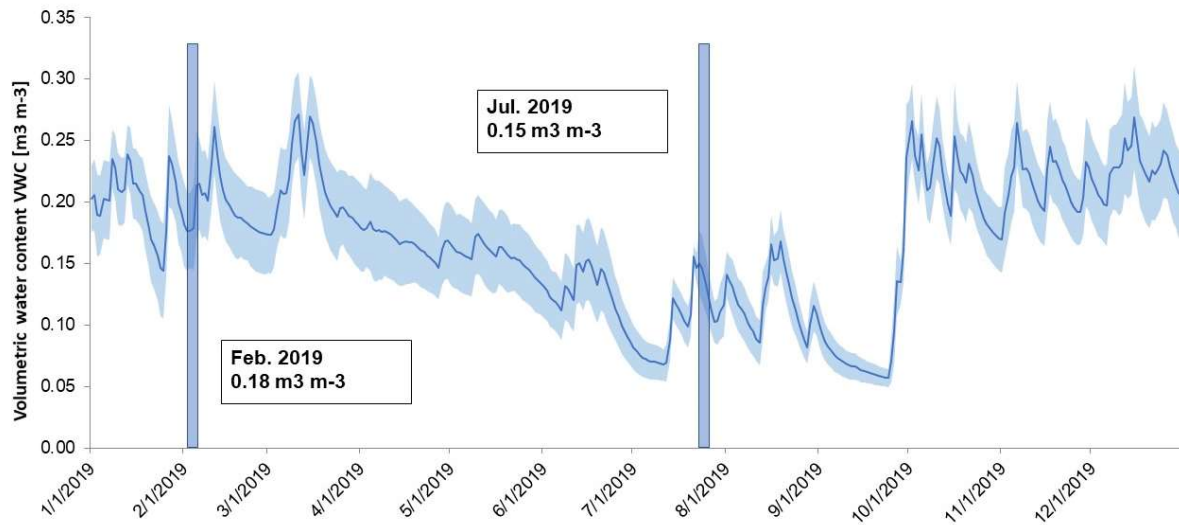
Supplementary Figure 6: Scheme of back-estimation of initial labile DOP values.

8 Volumetric water content at BBR and LUE at 5 cm soil depth

BBR (n=27)



LUE (n=27)



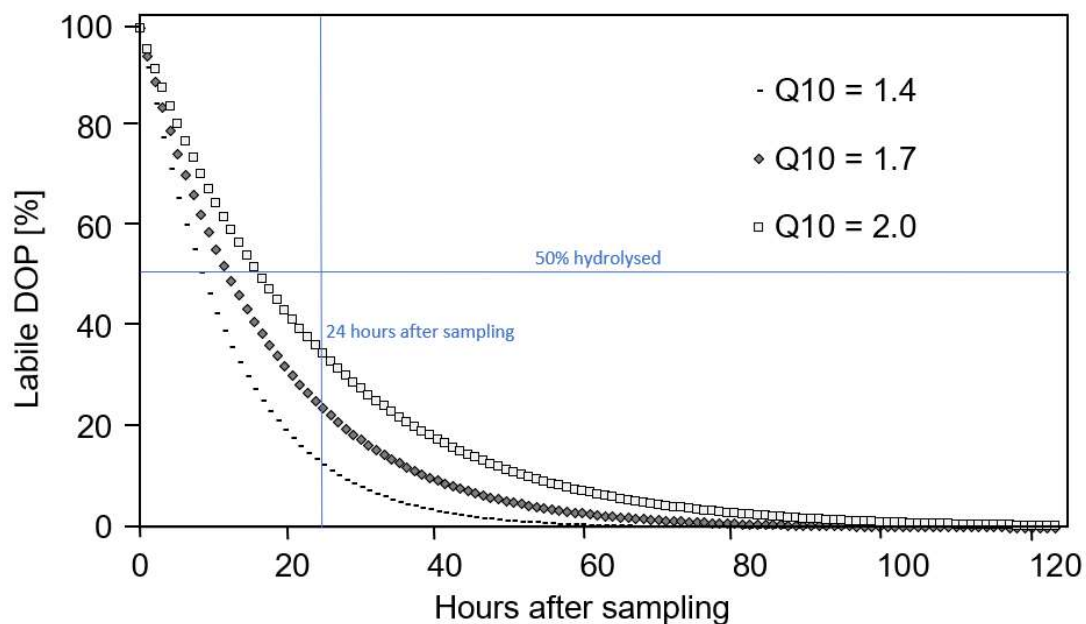
Supplementary Figure 7: Volumetric water content (VWC), measured with Decagon EC5 sensors at 5 cm soil depth.

9 Sensitivity analysis of parameter of the organic P hydrolysis model

9.1 Influence of Q_{10} value

Supplementary Table 7: Literature overview on Q_{10} values for phosphomonoesterases.

Source	Enzyme	Q_{10} mean	Q_{10} min	Q_{10} max	Comment
(Menichetti et al., 2015)	Acid phosphomonoesterase	1.07	0.79	1.32	Tropical soils, 0-20cm depth
(Hui et al., 2013)	Acid phosphatase	1.77	-	-	very different origins
(Min et al., 2019)	Acid phosphatase	2.27	± 0.25	± 0.25	soils along a latitudinal boreal forest transect at 5, 15, and 25°C
(Trasar-Cepeda and Gil-Sotres, 1988)	Acid phosphatase	1.25	1.1	1.38	Ranker and Cambisols, surface horizons
(Kroehler and Linkins, 1988)	Acid phosphatase	2.2	-	-	Brooks Range Alaska, tundra, Eriophorum plant roots
Average		1.7			

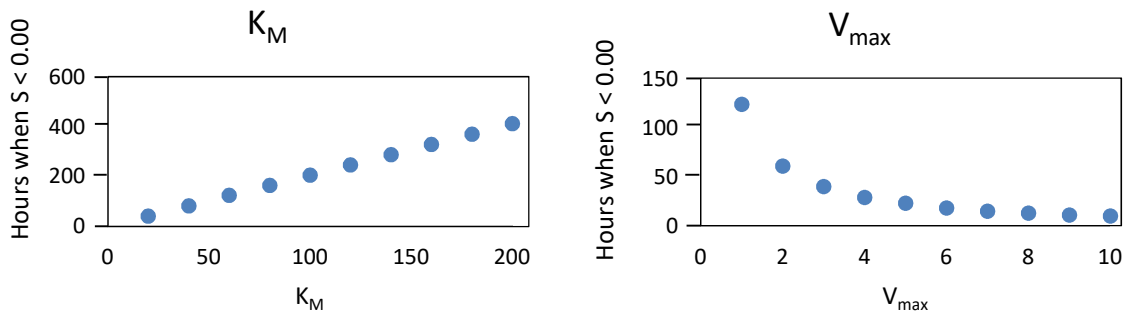


Supplementary Figure 8: Influence of Q_{10} value on hydrolysis rate of labile DOP.

9.2 Influence of Michalis-Menten parameters

Calculated were the hours needed to reach a labile DOP (S : in $\mu\text{mol L}^{-1}$) concentration of $0.00 \mu\text{mol L}^{-1}$ as dependent on a range of K_M and V_{\max} values.

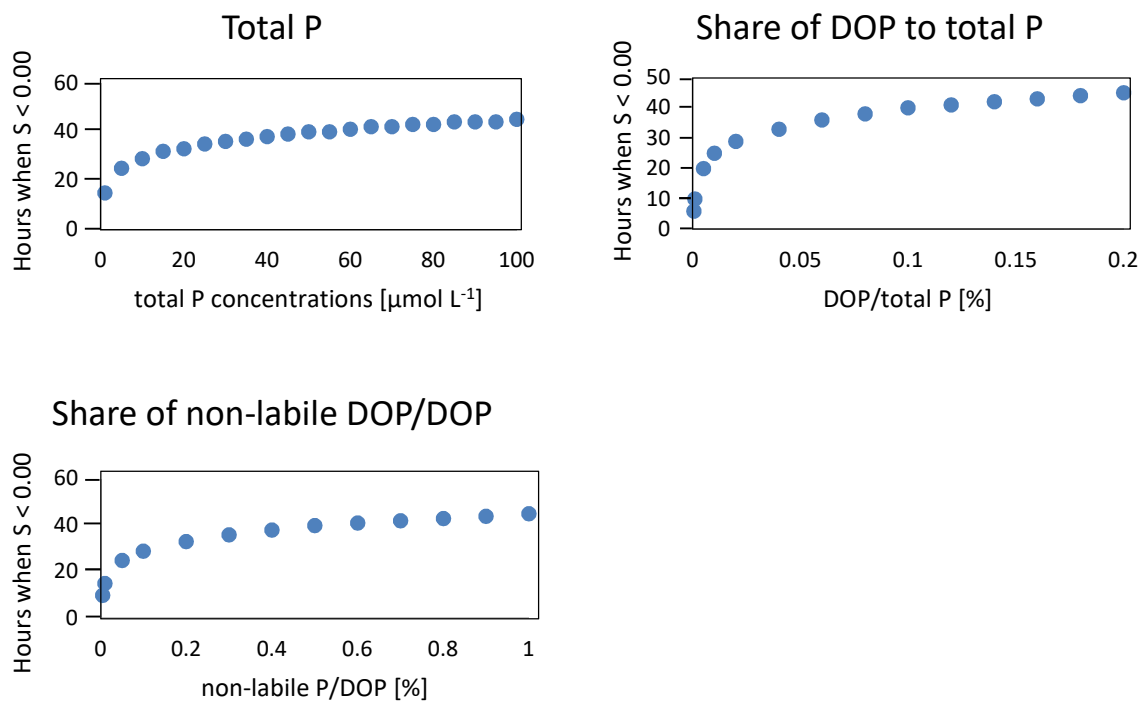
Ecosystem-relevant ranges of values based on literature research and own data				
Variable	Unit	Average	MIN	MAX
V_{\max}	$\mu\text{mol/L/h}$	3	0.5	10
K_M (constant)	$\mu\text{mol/L}$	20	1	200



9.3 Influence of P concentration and fraction of dissolved organic P

Calculated were the hours needed to reach a labile DOP (S : in $\mu\text{mol L}^{-1}$) concentration of $0.00 \mu\text{mol L}^{-1}$ as dependent on a range of P concentrations.

Ecosystem-relevant ranges of values based on literature research and own data				
Variable	Unit	Average	MIN	MAX
total P	$\mu\text{mol L}^{-1}$	50	0	97
Share DOP of total P at $t = 0$	%	0.1	0.00001	0.2
Share non-labile P of DOP	%	0.5	0.05	1



9.4 Influence of P concentration and fraction of dissolved organic P

We calculated the ratios of all consecutive steps for both, time (hours) and variables.

A doubling of P concentration or P fractions did almost not change the hydrolysis rate (same time span until labile DOP is hydrolyzed). In contrast, a doubling of K_M lead to a doubling of the hydrolysis rate, and a doubling of V_{max} lead to a decrease of the hydrolysis rate by 0.5.

