The effect of soil organic matter on long-term availability of phosphorus in soil: evaluation in a biological P mining experiment

Supporting Information

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Numbers of Tables: 3

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+ additional information on the endpoint “critical cumulative P uptake”

S1. Set-up biological mining experiment

Table S1: Nutrient application. Composition of the nutrient solution added to the soils at the start of the biological mining experiment

Nutrient	Concentration added at t=0	Chemical form added
Macronutrients		
Nitrogen (N)*	400 mg N kg DS^{-1}	NH_4NO_3
Potassium (K)	100 mg K kg DS^{-1}	KCl
Sulphur (S)	43 mg S kg DS^{-1}	$MgSO_4.7H_2O$, $CuSO_4$, $ZnSO_4$, $CoSO_4.7H_2O$
Magnesium (Mg)	32 mg Mg kg DS^{-1}	$MgSO_4.7H_2O$
Micronutrients		
Chlorine (Cl)	95 mg Cl kg DS^{-1}	KCl, $MnCl_2.4H_2O$
Manganese (Mn)	3 mg Mn kg DS^{-1}	$MnCl_2.4H_2O$
Copper (Cu)	2.3 mg Cu kg DS^{-1}	$CuSO_4$
Zinc (Zn)	2 mg Zn kg DS^{-1}	$ZnSO_4$
Boron (B)	0.7 mg B kg DS^{-1}	H_3BO_3
Molybdenum (Mo)	0.3 mg Mo kg DS^{-1}	$Na_2MoO_4.2H_2O$
Cobalt (Co)	0.1 mg Co kg DS^{-1}	$CoSO_4.7H_2O$

*After 6 harvests (Day 131), it was decided to switch N fertilisation from NH_4NO_3 to $CaNO_3$ due to the observed gradual decline in soil pH.

S2. Point of P deficiency: alternative definitions

The critical cumulative uptake of phosphorus (CCP), used as one of the two endpoints from the biological mining experiment, is the cumulative P uptake up to the point where P becomes deficient. In the main manuscript, this point of deficiency was defined based on the relative yield of the ryegrass. Alternatively, deficiency can be detected based on

(1) **Shoot P concentration**. The onset of P deficiency is defined as the point at which shoot P concentrations decreased below 2.0 mg P g dry matter^{-1}, which is typically associated with a 10% reduction in yield (Smith et al., 1985; Reuters & Robinson, 1997). In this study, such a growth reduction was often only observed at shoot P concentrations < 1.9 mg P g dry matter^{-1}. Therefore, this criterion resulted in slightly lower values of the CCP compared to yield- or uptake-based deficiency (Figure S1). Nevertheless, as Figure S2 shows, correlations with soil properties and indices of P availability remain unaffected.

(2) **Shoot P uptake**. Dry matter yield and shoot P concentration trends did not always match; in some cases, harvests with reduced yields still had an adequate shoot P concentration, while in other cases, decreased shoot P concentrations were found in harvests with increased biomass yield, likely due to growth dilution. To overcome this mismatch, their product, i.e. the shoot P uptake was introduced as an alternative to detect the point of deficiency. This method is based on the idea that P uptake, corrected for growth period, is adequate as long as it is ≥ 90% of the uptake at Harvest 2, if the shoot P concentration in the latter would only be the minimal required 2.0 mg P g dry matter^{-1}. In other words, P uptake was adequate up to the point where the relative uptake rate (RU) decreased below 90%, i.e.

$$RU(\%) = 100 * \frac{P_{shoot}(t_i) \cdot yield(t_i)/(t_i - t_{i-1})}{2\ mg\ P\ g\ DM^{-1} \cdot yield(t_2)/(t_2 - t_1)} < 90\% \quad \text{(Equation S1)}$$

With t_i the day of the harvest, P_{shoot} the shoot P concentration in mg P g dry matter^{-1} and both yields expressed in g dry matter per kg dry soil. The minimal required 2.0 mg P g^{-1} was used as a reference

P_{shoot} at Harvest 2 because, for most soils, the shoot P concentration in the biomass at that harvest was considerably larger. This luxury uptake is not essential and should, therefore, not be used as a reference to define the point of deficiency. The relative uptake rate largely agreed with the relative yield in defining the CCP per soil (Figure S1) and yielded similar correlations with soil properties and indices of P availability (Figure S2).

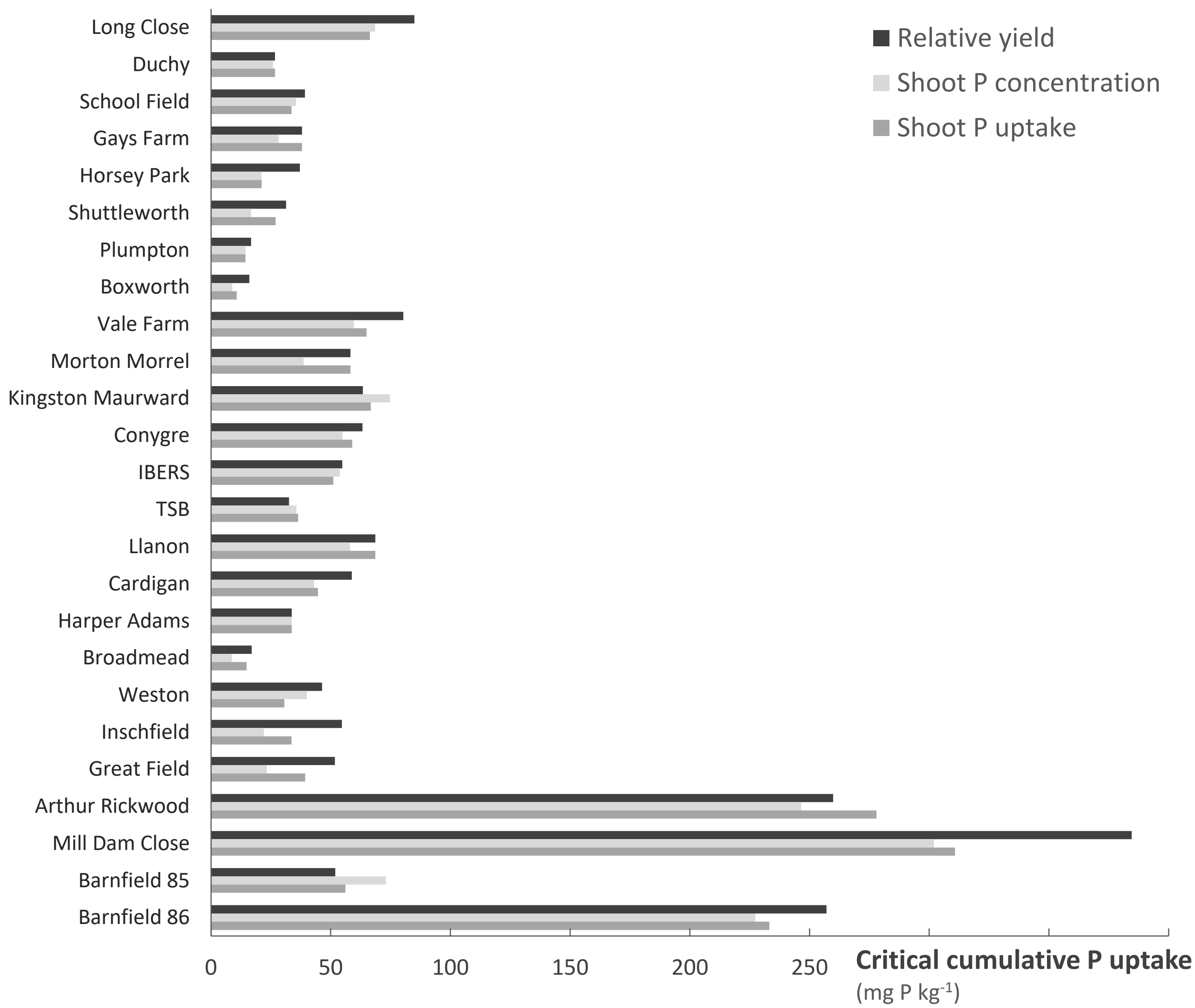

Figure S1: **Critical cumulative P uptake.** The critical cumulative P uptake (CCP), i.e. the cumulative P uptake up to the point where P becomes deficient, per soil. The three methods that were used to detect the point of deficiency, either based on yield, shoot P concentration, or shoot P uptake, largely agreed.

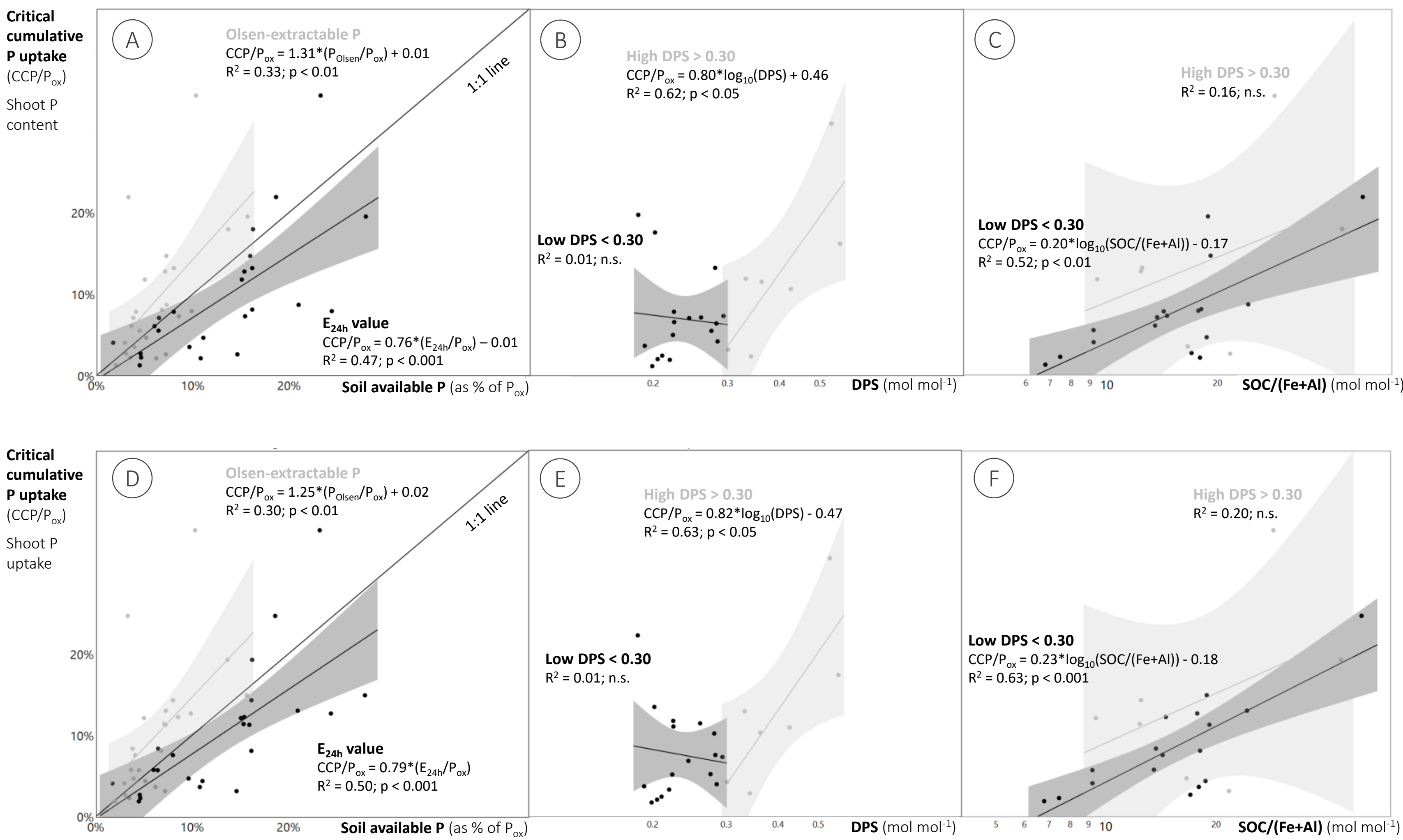

Figure S2: Critical cumulative P uptake vs. typical P availability indices and soil properties. The CCP in the biological P mining experiment (CCP/P_{ox}) matched well with the isotopically exchangeable soil P pool (E_{24h}/P_{ox}) and was significantly correlated with Olsen-extractable P (A, D). Variation between soils was most significantly explained by the ratio of P (B, E) and organic carbon (C, F) to amorphous Fe and Al oxyhydroxides, respectively termed the degree of phosphorus saturation (DPS) and the SOC/(Fe+Al) ratio. Upper panels A-C show the CCP determined based on shoot P concentration, while the CCP in the bottom panels D-F was determined based on relative P uptake. Note that DPS and the SOC/(Fe+Al) ratio are presented on a $\log_{10}$ scale.

S3. P uptake in biological mining and correlation to available P in soil P tests

Table S2: P uptake and availability indices. Results from the biological mining experiment and how they relate to different indices of P availability. Total (TCP) and critical (CCP) cumulative P uptake were calculated as explained in Section 2.3. The labile Q_1 and total desorbable Q_1+Q_2 pool were determined in a P desorption experiment on 20 out of 25 soils (Table 1).

	Ryegrass data			P availability indices									
soil	Total yield (g kg^{-1})	TCP (mg P kg^{-1})	CCP (mg P kg^{-1})	P_{Olsen} (mg P kg^{-1})	CCP/P_{Olsen}	E_{24h} (mg P kg^{-1})	CCP/P_{E24h}	Labile Q_1 (mg P kg^{-1})	CCP/Q_1	Total desorbable Q_1+Q_2 (mg P kg^{-1})	TCP/(Q_1+Q_2)	Desorption at 448d (mg P kg^{-1})	TCP/(Q_1+Q_2)
1	114	175	85	36	2.37	70	1.21	88	0.97	374	0.47	342	0.51
2	116	152	27	41	0.65	54	0.49	62	0.43	375	0.41	255	0.60
3	58	97	39	40	0.99	86	0.46	77	0.51	326	0.30	264	0.37
4	45	76	38	32	1.19	78	0.49	66	0.58	271	0.28	258	0.30
5	56	75	37	24	1.53	36	1.03	66	0.56	179	0.42	169	0.45
6	31	43	31	21	1.49	52	0.60	-	-	-	-	-	-
7	20	32	17	41	0.41	75	0.22	54	0.31	157	0.20	140	0.23
8	34	48	16	25	0.65	50	0.32	33	0.48	191	0.25	119	0.41
9	57	108	80	36	2.21	73	1.09	56	1.43	221	0.49	213	0.50
10	76	118	58	33	1.79	94	0.62	74	0.78	195	0.61	190	0.62
11	56	113	63	42	1.52	90	0.70	63	1.01	286	0.39	268	0.42
12	50	89	63	42	1.51	50	1.27	57	1.12	179	0.49	177	0.50
13	57	99	55	32	1.69	53	1.03	-	-	-	-	-	-
14	22	43	33	26	1.24	15	2.13	51	0.64	266	0.16	220	0.20
15	111	155	69	31	2.19	53	1.29	70	0.98	303	0.51	264	0.59
16	52	94	59	35	1.67	50	1.17	54	1.09	231	0.41	222	0.42
17	34	67	34	28	1.19	67	0.50	55	0.62	189	0.36	183	0.37
18	22	33	17	25	0.67	44	0.38	29	0.58	203	0.16	110	0.30
19	70	98	46	32	1.44	57	0.81	31	1.52	138	0.71	104	0.94
20	57	77	55	36	1.51	80	0.68	79	0.69	434	0.18	285	0.27
21	42	61	52	27	1.89	50	1.05	-	-	-	-	-	-
22	195	375	260	37	6.97	210	1.24	-	-	-	-	-	-
23	184	446	385	128	3.00	387	0.99	-	-	-	-	-	-
24	97	160	52	36	1.44	79	0.65	70	0.74	258	0.62	235	0.68
25	141	328	257	68	3.76	154	1.67	142	1.81	349	0.94	346	0.95

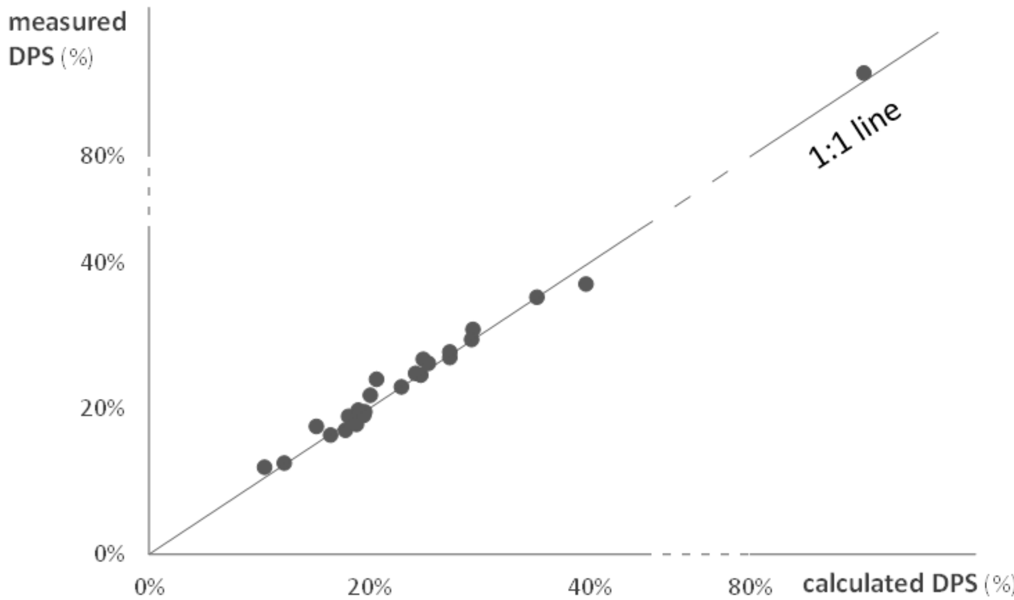

Figure S3: Measured vs. predicted final DPS. The final DPS was measured by oxalate-extraction of the soils after the P depletion experiment and compared with the DPS as calculated from the initial DPS and total P uptake measured in above-ground biomass.

S4. Soil properties affecting long-term P availability

Table S3: Pearson correlation coefficients between P uptake data and soil properties. Correlation analysis was performed on all soils, excluding calcareous Soil 7. Logically, ratios compared to P_{ox} are correlated to oxalate-extractable elements and ratios compared to P_{di} to DCB-extractable elements; for absolute values, correlation coefficients are given for both. For the OC content, correlation coefficients are presented excluding highly organic Soil 23 (23% OC), results including this soil are added between brackets when considerably different. Additionally, the correlation coefficients for the two groups of P saturation, i.e. DPS below (n=18) or above (n=7) 0.30 mol mol^{-1}, are given separately for the DPS and SOC/(Fe+Al) ratio. Significant correlations are indicated by *p<0.05, **p<0.01 and ***p<0.001.

	Critical cumulative P uptake			**Total P uptake**		
	CCP (log_{10}) (-)	**CCP/P_{ox}** (%)	**CCP/P_{di}** (%)	**TCP** (log_{10}) (mg P kg^{-1})	**TCP/P_{ox}** (%)	**TCP/P_{di}** (%)
Normally distributed soil properties						
pH	-0.39	0.09	0.04	-0.42*	0.15	0.11
% sand	-0.09	0.01	0.06	-0.26	-0.07	-0.02
% silt	0.06	-0.09	-0.25	0.19	-0.08	-0.27
% clay	0.06	-0.07	0.18	0.16	0.19	0.32
Org C	0.22 (0.46*)	-0.37 (0.19)	-0.31 (0.25)	0.31 (0.47*)	-0.41 (0.14)	-0.31 (0.19)
Log_{10} transformed soil properties						
$P_{ox\|di}$	0.46*| 0.47*	-0.26	-0.31	0.53*| 0.50*	-0.39	-0.47*
$Fe_{ox\|di}$	0.35 | 0.09	-0.38	-0.37	0.40 | 0.16	-0.45*	-0.39
$Al_{ox\|di}$	-0.02 |-0.00	-0.45*	-0.44*	0.11 | 0.13	-0.43*	-0.43*
$Fe_{ox\|di}$+$Al_{ox\|di}$	0.25 | 0.08	-0.43*	-0.41*	0.33 | 0.16	-0.48*	-0.42*
DPS	0.35 | 0.50*	0.46*	0.09	0.29 | 0.43*	0.33	-0.09
DPS<0.30		-0.17			-0.02	
DPS>0.30		0.78*			0.77*	
SOC/(Fe+Al)	0.19 | 0.33	0.57**	0.41*	0.16 | 0.32	0.59**	0.39
DPS<0.30		0.69**			0.61*	
DPS>0.30		0.41			0.51	