Supporting Information to Colloid-bound and dissolved phosphorus species in topsoil water extracts along a grassland transect from Cambisol to Stagnosol

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Table S1 The concentration of organic carbon (OC), Al, P, Fe, and Si in asymmetric flow field-flow fractionation (AF4) fractograms of soil particles (d <450 nm) from Cambisol (S1), stagnic Cambisol (S2), and Stagnosol (S3). Data of S1 and S3 are the mean values of three replicate fields with standard deviations. The lowercase indicate significant differences among soil sites (significant difference of soil sites 1 and 3 was tested by t-test, One Way RM ANOVA for AF4 fractions with Fisher LSD, P <0.05).

	First peak (mg kg^{-1}, d=0-20 nm)					Second peak (mg kg^{-1}, d=20-450 nm)				
	OC	Al	P	Fe	Si	OC	Al	P	Fe	Si
S1	2.94±0.89a	0.05±0.03a	0.01±0.01a	0.04±0.01a	0.07±0.02	52.32±10.82	7.65±1.74	0.47±0.17a	7.60±2.11	8.86±1.63
S2	3.15	0.04	0.004	0.05	0.10	60.95	10.6	0.76	13.22	12.51
S3	19.05±3.46b	0.20±0.04b	0.05±0.01b	0.18±0.02b	0.16±0.09	84.00±19.67	8.36±1.99	1.26±0.21b	7.34±0.53	10.32±1.95

All the data of S1 and S3 showed significant difference between AF4 first and second peaks (OC of S1 was log transformed before One Way RM ANOVA analyses because of unequal variances).

Table S2 Detailed phosphorus species[a] determined by ^{31}P-NMR for the studied S1 (Cambisol), S2 (stagnic Cambisol), and S3 (Stagnosol) samples (% of total extracted P)

Sample	AEP	Mono1	Mono2	Mono3	myoIHP	4.7 ppm	α-glyc	β-glyc	Nucl	Pchol	scyllo-IHP
S1 bulk	0.2	3.5	7	2.1	17.4	-	1.4	2.8	4.2	1.4	6.3
S2 bulk	0.7	1.4	7.6	2.1	16.6	-	0.7	2.1	2.1	0.7	7.6
S3 bulk	0.7	2.1	1.4	2.1	12.5	-	-	1.4	2.8	-	4.8
S1 300 kDa-450 nm	-	5	18.9	1.7	13.4	-	1.1	2.8	3.3	1.1	1.7
S2 300 kDa-450 nm	-	3	8.4	1.2	7.2	-	-	0.6	2.4	-	4.8
S3 300 kDa-450 nm	-	1.2	4.4	0.6	7.5	-	0.6	1.2	2.5	0.6	2.5
S1 < 3 kDa	-	-	26.9	-	-	-	-	-	-	-	-
S2 < 3 kDa	-	-	7.3	3.8	2.3	2.5	1	1.5	3.5	-	0.5
S3 < 3 kDa	-	6.8	10.5	1.7	1.7	0.9	-	0.3	1.7	-	0.6
	3.4 ppm	4.2 ppm	5.0 ppm	5.3 ppm	5.9 ppm	neo-IHP	chiro1	chiro2	DNA	OthDi1	OthDi2
S1 bulk	-	-	2.8	-	-	-	4.2	-	1.1	0.8	0.3
S2 bulk	-	-	3.5	-	-	-	4.2	-	0.8	0.4	0.2
S3 bulk	-	-	2.1	-	-	-	2.1	-	0.6	0.7	0.2
S1 300 kDa-450 nm	-	-	-	1.7	-	-	3.3	-	2.8	7.4	0.9
S2 300 kDa-450 nm	-	-	-	0.6	-	-	1.8	-	1.2	2.3	1.7
S3 300 kDa-450 nm	-	-	-	0.6	-	0.6	1.9	-	0.8	1.6	0.4
S1 < 3 kDa	-	-	-	-	-	-	-	-	-	1.9	-
S2 < 3 kDa	-	1	-	-	1	-	-	0.8	4.4	24.2	-
S3 < 3 kDa	0.6	-	-	-	0.6	0.3	1.7	0.9	1.5	6.7	-

[a]2-Aminoethyl phosphonic acid (AEP), orthophosphate monoesters, regions 1, 2, and3 (Mono1, Mono2, and Mono3, respectively), hexakisphosphate (myoIHP), α glycerophosphate (α-glyc), β glycerophosphate (β-glyc), mononucleotides (nucl), choline phosphate (Pchol), scyllo-inositol hexakisphosphate (scyllo-IHP), neo-inositol hexakisphosphate (neo-IHP), chiro-inositol hexakisphosphate 4e/2a (chiro1), chiro-inositol hexakisphosphate 2e/4a (chiro 2), deoxyribonucleic acid (DNA), and orthophosphate diesters, regions 1 and 2 (OthDi1and OthDi2, respectively). No these phosphorus forms were detected in 3-300 kDa of S1, S2, and S3.

Table S3 Chemical shift of peaks detected in ^{31}P-NMR spectra of NaOH-Na_2EDTA samples.

Category[a]	P Form or Compound Class	Chemical Shift (ppm)
Inorganic P		
	Orthophosphate	6.00±0.0
	Pyrophosphate	-4.31±0.26
Organic P		
	Phosphonates	45.81±0.44, 37.73±0.91, 23.95±0.16, 20.40±0.01, 18.96±0.03
Orthophosphate Monoesters		
	myo-IHP	5.61±0.10, 4.69±0.09, 4.30±0.12, 4.17±0.12
	scyllo-IHP	3.85±0.09
	neo-IHP	6.62±0.09, 4.47±0.08
	chiro1	6.47±0.11, 5.01±0.13, 4.52±0.09
	chiro 2	6.76±0.07,5.46±0.05,4.15±0.14
	Unknown	5.01±0.01,5.34±0.07,5.83±0.12
	α-glyc	5.00 ±0.13
	β-glyc	4.60±0.07
	nucl	4.47±0.15
	Pchol	3.98±0.16
	F6P	4.74±0.03
Orthophosphate diesters-- DNA		-0.60±0.19

[a]myo-inositol hexakisphosphate (myo-IHP), scyllo-inositol hexakisphosphate (scyllo-IHP), neo-inositol hexakisphosphate (neo-IHP), D-*chiro*-inositol hexakisphosphate 4e/2a (chiro1), D-*chiro*-inositol hexakisphosphate 2e/4a (chiro 2), α-glycerophosphate (α-glyc), β-glycerophosphate (β-glyc), mononucleotides (nucl), choline phosphate (Pchol), D-Fructose 6-phosphate (F6P), and deoxyribonucleic acid (DNA).

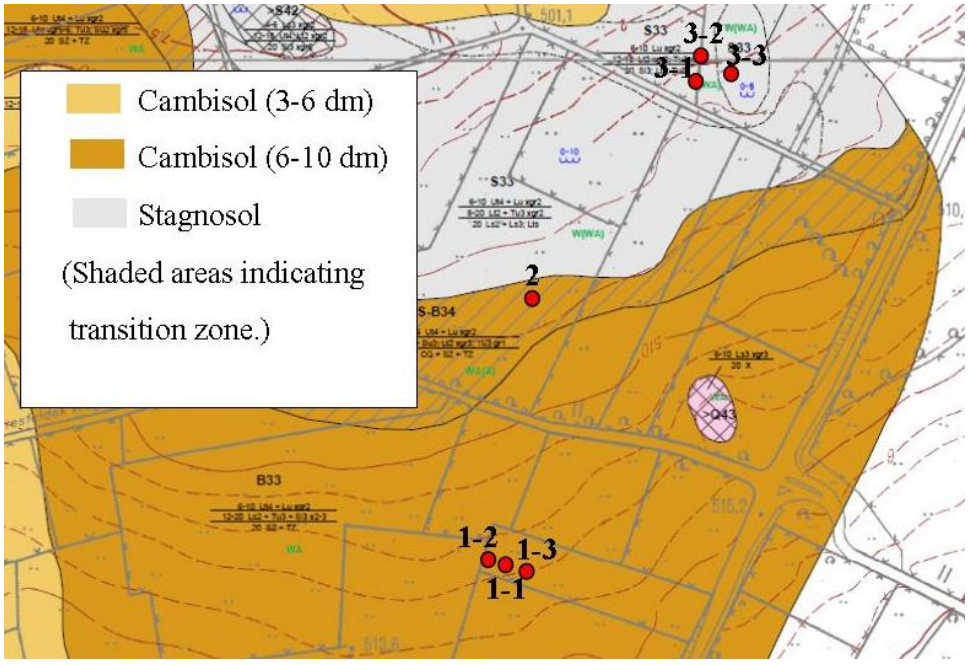
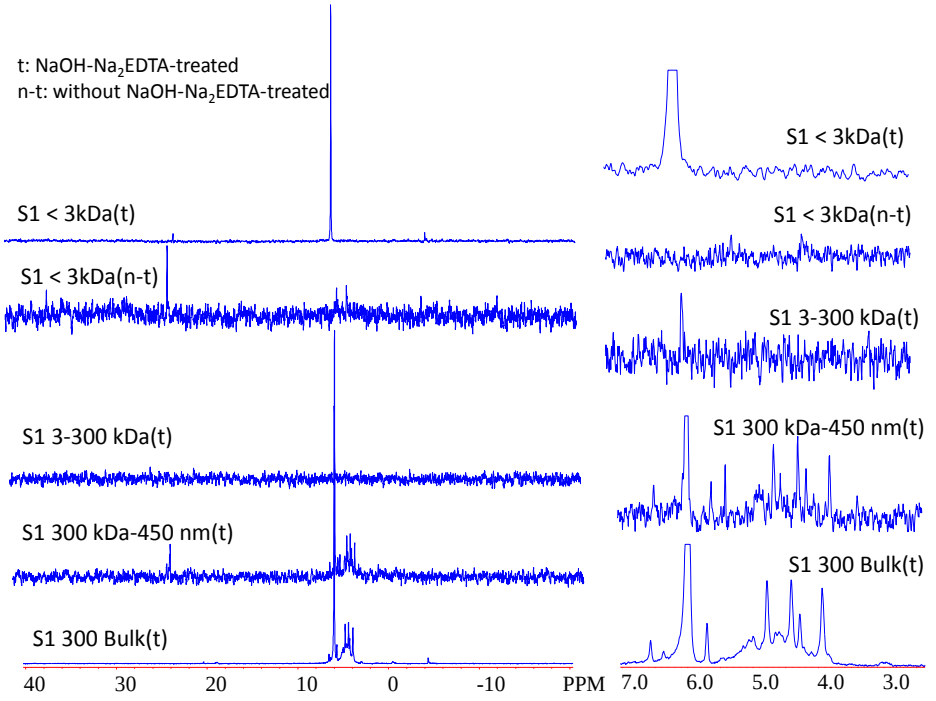


Fig. S1 Excerpt from the soil map of the test site at Rollesbroich (*modified from Geologischer Dienst Nordrhein-Westfalen, 2008*). Numbered red dots indicate location of plots.



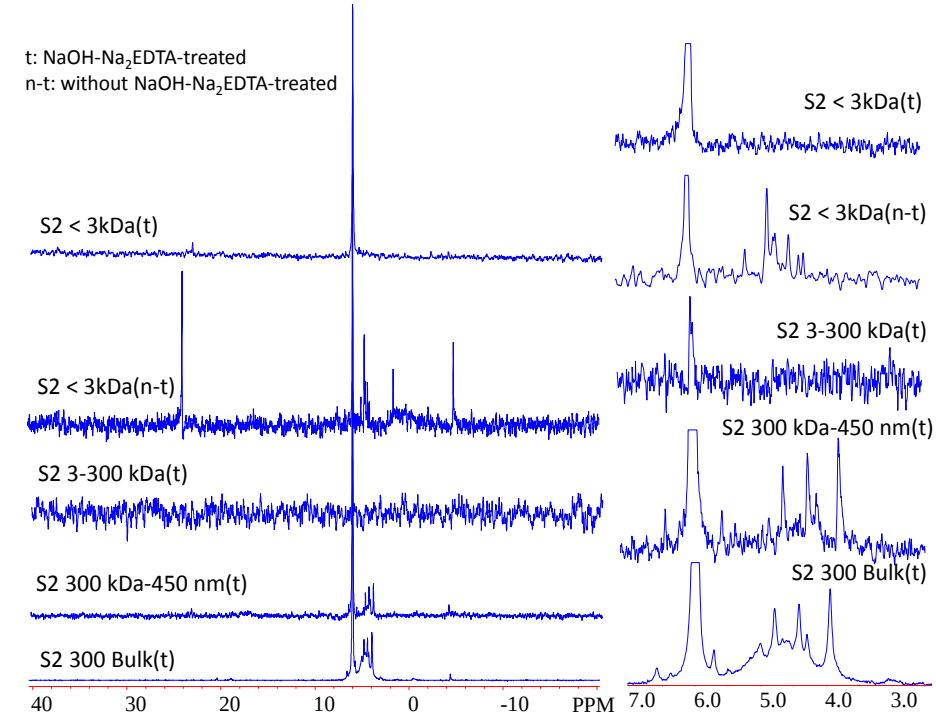


Fig. S2 Solution phosphorus-31 nuclear magnetic resonance spectra of NaOH–Na_2EDTA extracts of bulk, 300 kDa-450 nm, 3-300 kDa, and < 3 kDa fractions in soil water extracts < 450 nm of S1 (Cambisol) and S2 (Stagnic Cambisol). [1] soil fraction < 3 kDa with NaOH-EDTA treatment; [2] soil fraction < 3 kDa without NaOH-EDTA treatment.