

Does Soil Disturbance Affect Soil Phosphorus Fractions?

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ABSTRACT

Increased turnover of organic matter as a result of soil disturbance (e.g. by soil tillage) is described in principle, but the direct influence of soil disturbance on soil P turnover especially for organic farming systems has not been sufficiently proven. The objective of the study was to evaluate the short term effect of soil disturbance on different soil P fractions in a soil shaking experiment. Four soils were incubated for 10 days in the dark with three different disturbance treatments: 1) no disturbance, 2) overhead shaking for 2 h at the beginning of the experiment and 3) continuous overhead shaking at 5 r. p. m. The four investigated soils were: 1) a silty loam soil with long term bio-compost application and 2) the corresponding soil without bio-compost application, 3) a long-term organically managed clay loam soil and 4) a clay loam soil with long time application of pig manure, all not and from Baden-Württemberg, Germany. We determined NaHCO₃-, NaOH- and H₂SO₄-extractable inorganic and organic P fractions (P_i and P_o, resp.) in a sequential extraction. Furthermore, the potentially plant available P as Calcium-acetate-lactate-extractable P (CAL-P) and P extractable by electro-ultra-filtration (EUF-P), and *aqua regia* extractable total P (P_T) were determined. Furthermore, we determined microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP), and acid phosphatase activity in soil. The organically managed soil had the highest P_T contents (1300 mg·kg⁻¹). The soil with pig manure application had the smallest potentially labile P fractions (NaHCO₃-P_i and -P_o and NaOH-P_i). The ecologically managed soil had the biggest organic P fractions (114 mg·kg⁻¹ NaHCO₃-P_o and 463 mg·kg⁻¹ NaOH-P_o), but, this soil was the lowest in CAL-P (5 mg·kg⁻¹). Short term soil disturbance had effects on labile organic P fractions of two of the four analyzed soils, but inorganic P was rather unaffected. In the compost amended COMP(+) soil, there was an incorporation of P from the less available NaOH-P fractions into the more available NaHCO₃-P_o fraction. However, if taking all investigated soils and treatments into account, the effects of soil disturbance were limited and not consistent.

Keywords: Phosphorus Fractions; Soil Disturbance; Microbial Phosphorus; Organic Phosphorus; Microbial Nitrogen; Acid Phosphatase Activity

1. Introduction

Investigating nutrient mobilization and immobilization as a result of soil tillage, it has to be distinguished between long term and short term effects [1]. Long term effects include changes in organic matter and nutrient distribution as well as organic matter and nutrient turnover within the soil profile after conversion from highly intensive to less intensive tillage systems, e.g. from ploughing to no-till, and *vice versa*. Many investigations are published on this subject including the effect of nutrient stratification [2-8]. In contrast to these investigations, this study focused on short term effects, *i.e.* the direct influence of soil disturbance on soil P turnover.

Increased turnover of organic matter as an immediate result of soil disturbance is described in principle [9,10].

Aggregate crushing usually has positive effects on C and N mineralization as summarized in the review presented by [11]. N mobilization by soil disturbance and tillage is well documented in literature. An influence of soil tillage on N availability was postulated by [12] after comparison of measured and model simulated values. An influence of soil tillage intensity on N mineralization was reported by other authors (e.g. [13,14]). A considerable influence of different intensities of simple soil mixing on the adenylyate energy charge during incubation for eight days at 25°C was reported by [9]. The energy level experienced by soils during a typical cultivation operation by repeated dropping of defined weight from a defined height on a soil core was imitated by [15]. The authors found a clear positive effect on soil CO₂ evolution during the first

hours following this treatment which was negatively correlated with the total soil C content. This loss of C by an increment of microbial activity as measured by soil respiration can be due to the fact that tillage/disturbance can open up pores, thus exposing previously protected soil organic matter to the attack by microorganisms [16].

Most investigations focusing on the effect of soil disturbance/tillage on soil P refer to long term field experiments with combined effects of fertilization and tillage. Soil disturbance enhances P allocation in inorganic fractions due to mineralization [17] whereas no tillage promotes an increase of enzymatic activities including acid phosphatase [18] and a nutrient stratification [7,8], showing more P fertilization placement effects rather than tillage effects. Other researches indicate that an adequate tillage can prevent topsoil P accumulation and P losses by erosion, but too heavy tillage enhances leaching of P in particulate P forms [19,20]. In contrast to C and N, however, no detailed knowledge exists about P mobilization by soil disturbance and tillage in the short term, and in particular considering organically and long-term compost managed soils.

In organic farming, compost and manure are important sources as P fertilizers. Compost/manure added to soil can enhance the availability of phosphorus through the direct release of P from the compost or indirectly through the release of humic acids to the soil. Humic substances can promote release of P by complexing Fe and Al derived from Fe- and Al-phosphates [21], and by the formation of Ca-humates when reacting with Ca-phosphates [22], both resulting in a release of the corresponding phosphate anions. Furthermore, organic farming promotes accumulation of organic matter, and this also leads to improved physical properties such as a lower bulk density with lower dry strength and increased friability [23]. Moreover, to organic farming currently higher levels of biological activity were associated [24]. However, a positive effect of soil tillage on soil nutrient availability was postulated especially for organic farming systems but was not sufficiently proven [25].

Chemical sequential extraction procedures are widely used to subdivide extractable soil P into inorganic (P_i) and organic (P_o) fractions differing in extractability [26, 27]. In this procedure, the inorganic pool extracted with 0.5 M sodium bicarbonate is attributed mainly to being labile P_i in the solid soil phase attached to crystalline surfaces [27]; moderately labile P_i extracted with 0.1M NaOH comprises chemisorbed P_i of lower plant-availability associated with amorphous and crystalline Fe and Al hydroxides [26]; and no labile primary Ca-bound P is extractable through 0.5 M HCl or H_2SO_4 [28]. Regarding organic fractions, easily mineralisable $NaHCO_3$ - P_o and moderate labile NaOH- P_o comprise P linked to organic matter [28,29]. The non-extractable recalcitrant P_i and P_o

comprise residual P of very low availability [30]. Microbial P is extracted within or besides sequential fractionation procedure after soil fumigation by $CHCl_3$ fractionation and $NaHCO_3$ extraction [31].

The objective of this study was to evaluate the short term effect of soil disturbance on different soil P fractions in a soil shaking experiment. It was hypothesized that soil disturbance (here soil shaking) mobilizes soil P, eventually leading to a redistribution of soil P towards more labile fractions.

2. Materials and Methods

2.1. Soils and Compost

Four soils were sampled at 0 - 30 cm depth from three different sites in Baden-Württemberg, Southern Germany at November 2009. Five sub-samples of each 20 cm diameter core were taken across the sampling sites (20 × 50 m).

1) COMP(-): Silty loam soil (Loess derived Luvisol) taken from a long term (since 1997) compost fertilization experiment at the experimental farm "Heidfeldhof", belonging to the University of Hohenheim, located in the south-west of Stuttgart, Germany (48°43'00"N; 9°11'40"E), control variant without compost application.

2) COMP(+): The same soil as COMP(-) but with high annual application rates of mature bio-compost corresponding to 400 kg total N $ha^{-1} a^{-1}$ or approx. 40 t DM $ha^{-1} a^{-1}$. The compost derived mainly from kitchen waste and garden plant residues digested in a central compost plant. The compost contained around 4500 mg P kg^{-1} DM, with an average of 1566 mg- kg^{-1} $NaHCO_3$ -P, 342 mg- kg^{-1} NaOH-P and 2472 mg- kg^{-1} H_2SO_4 -P, and 84% P_i of the total P (average of years 2000 and 2008).

3) ECO: Clay loam soil (Regosol) from a site under long term bio-dynamic farming (ECO) according to the regulations of the German "Demeter" association; taken 2 km south of Rosenfeld, Germany (48°16'32"N; 8°43'00"E).

4) MAN: Clay loam soil (Gleyic Vertisol) with long term liquid pig manure application from a site at the experimental farm of the Agricultural Technology Centre (LTZ) in Karlsruhe-Durlach, Germany (49°14'25"N; 9°59'43"E).

All soils were sieved (<2 mm Ø), adjusted to approx. 50% of max water holding capacity and stored at 4°C until the experiment started. Three treatments were carried out in five replicates during 10-days at 15°C in the dark: 1) control soil with no disturbance during incubation (T-0), 2) soil disturbance by shaking during the first two hours of incubation on an overhead multi-axle rotating shaker at 5 r. p. m. (T-1), 3) soil disturbance by continuous shaking of the soil during 10 days on an overhead multi-axle rotating shaker at 5 r. p. m. (T-2).

The shaking treatments energy can be estimated from the size of the bottles (250 mL), and the number of rotations for each treatment. Considering a bottle size of approx. 10 cm height, 600 rotations in the 2 h and 72,000 in the 10 d treatment, the energy applied was according the formula $K = m * g * h$ (K : kinetic energy, m : weight; g : gravity acceleration = $9.8 \text{ m}\cdot\text{s}^{-2}$ and h : height) of $294 \text{ J}\cdot\text{kg}^{-1}$ and $35,280 \text{ J}\cdot\text{kg}^{-1}$, respectively. The 2 h treatment can be compared to the energy of $150 \text{ J}\cdot\text{kg}^{-1}$ of typical soil cultivation [15,32], but the 10 d treatment energy input was several times higher than for average soil cultivation.

For all treatments, 50 g moist soil was incubated in 250 mL polyethylene bottles. After ten days, soils in all bottles were homogenized and further processed as described below. From the years 2000 and 2008 of the long term compost experiment, compost samples were available and subject to P fractionation in the same way as the soil samples (see below).

2.2. Soil and Compost Analyses

Soil pH was determined in 0.01 M CaCl_2 in a soil-solution relation of 1 g: 2.5 mL. Organic C (C_{org}) and total N were measured with an C-N-auto-analyzer (“Vario MAX CN” type, “Elementar” Company). Microbial biomass C and N (MBC and MBN, respectively) were estimated by fumigation-extraction [33]. Briefly, one 25 g portion soil was fumigated for 24 h at 25°C with ethanol-free CHCl_3 . Following fumigant removal, the soil was extracted with 100 mL 0.5 M K_2SO_4 by 30 min horizontal shaking at 200 r. m. p. and filtered yielding a clear filtrate. Another non-fumigated 25 g portion of soil was extracted similarly at the time fumigation commenced. The extracts were frozen until analyses for total C and N concentrations on a TOC/TIC analyzer (Multi N/C 2100, Analytik Jena, Germany). Microbial biomass C (MBC) was calculated as follows: $\text{MBC} = \text{EC}/k_{\text{EC}}$; where EC = (organic C extracted from fumigated soils)-(organic C extracted from non-fumigated soils) and $k_{\text{EC}} = 0.45$ [34, 35]. Microbial biomass N (MBN) was calculated the same way but using $k_{\text{EN}} = 0.54$ [36].

Phosphorus in soils and compost was fractionated according to a modified “Hedley” fractionation method [26, 37]. Briefly, 0.5 g soil were extracted with 0.5 M NaHCO_3 followed by extraction with 0.1 M NaOH . Finally, the remaining soil was extracted with 0.5 M H_2SO_4 . The “inorganic” molybdate reactive phosphorous (P_i) in all extracts was measured by the method of [38] at 700 nm and pH 5.0 spectrophotometrically (Hitachi U-3300), whereas “organic” phosphorous (P_o) in all extracts was calculated as the difference between total P and P_i . Thus, this fractionation procedure yielded six different analytical fractions: $\text{NaHCO}_3\text{-}P_i$, $\text{NaHCO}_3\text{-}P_o$, $\text{NaOH-}P_i$, $\text{NaOH-}P_o$, $\text{H}_2\text{SO}_4\text{-}P_i$ and $\text{H}_2\text{SO}_4\text{-}P_o$. Total P (P_T) in soil

samples were extracted with aqua regia (1:3 HNO_3 and HCl) extraction) and determined with ICP-OES (“Vista Pro” type, “Varian” company) [39].

Microbial biomass P (MBP) was estimated by Chloroform Fumigation Extraction [31]. Fumigation was done as described for microbial C. Following fumigant removal, the soil was extracted with 0.5 M NaHCO_3 by 30 min. horizontal shaking at 200 r. p. m. and filtered. Non-fumigated soil was treated similarly, except that the desiccator contained no CHCl_3 , and was not evacuated. Inorganic P was analyzed in aliquots of the extracts by the ammonium molybdate-ascorbic acid method described by [38]. A spike of KH_2PO_4 equivalent to $25 \mu\text{g P g}^{-1}$ soil was used to correct for P_i fixation for each soil during the NaHCO_3 extraction. Biomass P was calculated $\text{MBP} = \text{EP}/k_{\text{EP}}/\text{recovery}$, where EP is the difference between $\text{NaHCO}_3\text{-}P_i$ extracted from fumigated and non-fumigated soil, and recovery is the recovering factor obtained for correct P_i fixation mentioned above [31,40] and k_{EP} is 0.4 [41].

Plant available P was characterized by Calcium Acetate Lactate (CAL) extraction [42]. 5 g soil was extracted by shaking for 90 min with 100 ml of a 0.05 M Ca acetate-0.05 M Ca lactate-0.3 M CH_3COOH pH 4.1 solution followed by filtration. P in the extract was determined colorimetrically by using ammonium molybdate reduced with ascorbic acid and Zn-chlorhydrate and followed by spectrophotometrical measurement of the absorbance at 480 nm (Hitachi U-3300).

Acid phosphatase (orthophosphoric-monoester phosphomonoesterase) activity (P-ase) was determined by the *p*-nitrophenyl phosphate method with modifications, as described by [43,44]. Briefly, soil samples (1 g) were incubated with 1 mL 50 mM *p*-nitrophenol phosphate and 4 mL 0.1 M tris buffer pH 5.5 for 1 h at 20°C . At the end of the incubation period, 1 mL 0.5 M CaCl_2 was added, and the solution was quickly filtered. Then, the filtrate was treated with 4 mL 0.5 M NaOH . Samples were homogenized and centrifuged at 2500 g for 10 min. *p*-nitrophenol released was determined spectrophotometrically by measuring the absorbance of the supernatant at 400 nm (Hitachi U-3300).

Soil P as determined by electro ultrafiltration (EUF) was extracted according [45,46]. The first P fraction (P-1) was collected the first 30 min. at 20°C with an electric current of max. 200 V and 15 mA; this fraction is assumed to contain available P. The second P fraction (P-2) was collected during minutes 30 and 35 at 80°C with an electric current of max. 400 V and 150 mA; this fraction is assumed to contain labile P [45].

2.3. Statistics

Results of the soil analyses are presented as mean values of five repetitions. The data were subjected to analyses of

variance using the ANOVA procedures of the SAS/STAT, version 6 [47]. Data were arcsine transformed to meet requirements of normality if needed and further compared by the Duncan's multiple range test. Statistical significance was determined at $P \leq 0.05$. Pearson correlations and principle component analysis (PCA) were performed using SAS/STAT, version 6 [47].

3. Results

Several years of compost addition (COMP(+)) increased concentrations of available CAL-P and also P determined

by electro-ultrafiltration (P1(EUF) and P2(EUF)) compared to COMP(-) (**Table 1**). However, the increase of P_t by $130 \text{ mg (kg soil)}^{-1}$ did not affect most of the sequential P-Fractions. Only $\text{H}_2\text{SO}_4\text{-P}_i$ was increased by $180 \text{ mg kg soil}^{-1}$. $\text{H}_2\text{SO}_4\text{-P}_o$ was extremely small and only detectable in the COMP(-) soil (not indicated in **Table 1**). Furthermore, compost addition enhanced microbial biomass (MBC and MBN) almost two-fold (**Table 1**).

The other two soils, ECO and MAN, were not included in the statistical analysis as they have different parental materials and textures and soils differed consid-

Table 1. Soil quality indicators and phosphorus fractions in the original soils.

Soil	unit	COMP (-)	COMP (+)	ECO	MAN
pH (CaCl ₂)		6.46 b	7.22 a	6.22	7.50
C _{org}	mg·g ⁻¹	9.8	17.7	36.2	24.3
N _T	mg·g ⁻¹	1.26	2.12	3.76	2.52
C _{org} /N _T		7.7	8.3	9.6	9.6
P _T	mg·kg ⁻¹	789 b	922 a	1300	1060
CAL-P	mg·kg ⁻¹	67 b	144 a	5	23
P1 (EUF)	mg·kg ⁻¹	32.4 b	48.6 a	11.1	9.8
P2 (EUF)	mg·kg ⁻¹	9.3 b	15.1 a	4.1	3.5
<i>P fractions</i>					
NaHCO ₃ -P _i	mg·kg ⁻¹	185	185	49	40
NaHCO ₃ -P _o	mg·kg ⁻¹	51	68	115	91
NaOH-P _i	mg·kg ⁻¹	197	186	143	50
NaOH-P _o	mg·kg ⁻¹	112	79	464	176
H ₂ SO ₄ -P _i	mg·kg ⁻¹	212 b	392 a	316	370
<i>Microbial biomass</i>					
MBC	mg·kg ⁻¹	148 b	241 a	589	463
MBN	mg·kg ⁻¹	20 b	40 a	63	52
MBP	mg·kg ⁻¹	19	11	5	3
MBC/C _{org}	%	1.5	1.3	1.6	1.9
MBN/N _T	%	11.7	12.4	5.5	5.3
MBP/P _T	%	2.1	1.9	1.7	2.1
Molar C:N:P		20:2:1	57:8:1	304:28:1	399:38:1
Acid P-ase	mg PNF kg ⁻¹ ·h ⁻¹	105 b	441 a	946	376

COMP(-): Silty loam soil without compost application; COMP(+): Silty loam soil with long term compost application; ECO: Clay loam soil under long term bio-dynamic farming; MAN: Clay loam soil with long term liquid pig manure application; C_{org}: organic carbon; N: total N; P_T: total P extractable with aqua regia. CAL-P: extractable with calcium acetate lactate solution; P1(EUF): P extractable by electro-ultra-filtration, 1st. fraction; P2(EUF): P extractable by electro-ultra-filtration, 2nd. fraction; NaHCO₃-P_i: inorganic P extractable with NaHCO₃; NaHCO₃-P_o: organic P extractable with NaHCO₃; NaOH-P_i: inorganic P extractable with NaOH; NaOH-P_o: organic P extractable with NaOH; H₂SO₄-P_i: inorganic P extractable with H₂SO₄; MBC: microbial biomass C; MBN: microbial biomass N; MBP: microbial biomass P; acid P-ase: acid phosphatase activity; PNF: *p*-nitrophenol). Different letters in the same P fraction show significant differences determined by *t* test ($P < 0.05$, MAN and ECO soil were not included).

erably in the determined P fractions. In general, ECO and MAN contain more P_o and less P_i in both NaHCO_3 - and NaOH -extractable fractions compared to $\text{COMP}(-)$ and $\text{COMP}(+)$ soils. The ECO soil had the highest concentration of P_T and of the P_o fractions compared with the other soils. Furthermore, ECO and MAN have in general higher microbial biomasses and P-ase activities than $\text{COMP}(-)$ and $\text{COMP}(+)$ soils (**Table 1**). $\text{MBC}/C_{\text{org}}$, MBN/N_T and MBP/P_T ratios were fairly constant among all soils, except MBN/N_T that was almost twice as high in ECO and MAN compared to $\text{COMP}(-)$ and $\text{COMP}(+)$ soils (**Table 1**).

Significant effects of the soil shaking treatments on the investigated variables were limited and not consistent (**Table 2**). In the $\text{COMP}(+)$ soil, $\text{NaOH}-P_o$ decreased while NaHCO_3-P_o , $\text{H}_2\text{SO}_4-P_i$ (2 h shaking only) and MBN (10 d shaking only) increased. In the ECO soil, $\text{H}_2\text{SO}_4-P_i$ and CAL-P increased after 10 d shaking. Only in the ECO soil, acid P-ase activity increased after 10 d shaking. In the MAN soil, NaHCO_3-P_i decreased (10 d shaking only) while NaHCO_3-P_o increased.

Furthermore, both shaking treatments led to a decrease of soil microbial biomass (significant for MBC and MBN). The $\text{COMP}(-)$ soil was unaffected by soil shaking, except an increase of MBP after 10 d shaking.

In general, NaHCO_3-P_i is correlated with CAL-P and MBP (**Table 3**). NaHCO_3-P_o is positively correlated with $\text{NaOH}-P_o$ and P-ase and MBC and MBN, but negatively with CAL-P and MBP. The $\text{H}_2\text{SO}_4-P_i$ fraction did not correlate with any other parameter. P-ase correlated positively with microbial elements, negatively with available P_i but positively with P_o forms.

PCA analysis (**Figure 1**) shows that organic C, microbial C (MBC) and N (MBN), P-ase, total C and N, and P_o (NaOH and NaHCO_3) were strongly related to PC1, whereas available and labile P_i fractions were negatively related to PC1 and positively to PC2. No labile P_i and pH were dispersed on the plot.

As indicated in **Figure 2**, A correlation could be detected between CAL-P (as an indicator of plant available P) and the NaHCO_3-P_i fraction (considered as labile P_i). However, data were strongly clustered due to the four

Table 2. Phosphorus fractions and microbial biomass variations after the disturbance experiment.

Soil	-----COMP(-)-----			-----COMP(+)------			-----ECO-----			-----MAN-----		
	Treatment	no shaking	2 h	10 d	no shaking	2 h	10 d	no shaking	2 h	10 d	no shaking	2 h
unit	$\text{mg}\cdot\text{kg}^{-1}$	% change		$\text{mg}\cdot\text{kg}^{-1}$	% change		$\text{mg}\cdot\text{kg}^{-1}$	% change		$\text{mg}\cdot\text{kg}^{-1}$	% change	
Total P	840	1	2	1005	-5	-6	1415	4	1	1076	8	1
CAL-P	62	-3	-1	119	0	0	2	70	304*	4	0	36
<i>P fractions</i>												
NaHCO_3-P_i	156	-5	-2	208	2	6	35	15	0	23	9	-31*
NaHCO_3-P_o	8	58	-1	7	149*	235*	49	2	-22	26	38*	45*
$\text{NaOH}-P_i$	136	-8	0	167	9	-1	163	-5	-1	66	4	5
$\text{NaOH}-P_o$	99	-1	18	158	-74*	-69*	241	0	-6	91	-5	8
$\text{H}_2\text{SO}_4-P_i$	232	-10	6	284	20*	-5	222	31	35*	265	12	-12
<i>Microbial biomass</i>												
MBC	148	23	-24	171	57	58	442	43	17	480	-17*	-23*
MBN	23	-26	-19	40	-15	-23*	52	20	26	54	-48*	-37*
MBP	37	3	44*	46	2	3	29	-16	7	23	-3	-15
Molar C:N:P	11:1:1	11:1:1	7:1:1	10:1:1	15:2:1	15:1:1	40:4:1	68:6:1	43:5:1	55:5:1	47:3:1	49:4:1
MBP/P_T (%)	4.4	2	41	4.6	7	10	2.0	-13	6	2.1	-10	-16
Acid P-ase ⁽¹⁾	226	3	17	238	-2	-13	596	7	41*	307	-13	-2

COMP(-): Silty loam soil without compost application; COMP(+): Silty loam soil with long term compost application; ECO: Clay loam soil under long term bio-dynamic farming; MAN: Clay loam soil with long term liquid pig manure application; C_{org} : organic carbon; N: total N; P_T : total P extractable with aqua regia. CAL-P: extractable with calcium acetate lactate solution; P1(EUF): P extractable by electro-ultra-filtration, 1st fraction; P2(EUF): P extractable by electro-ultra-filtration, 2nd fraction; NaHCO_3-P_i : inorganic P extractable with NaHCO_3 ; NaHCO_3-P_o : organic P extractable with NaHCO_3 ; $\text{NaOH}-P_i$: inorganic P extractable with NaOH ; $\text{NaOH}-P_o$: organic P extractable with NaOH ; $\text{H}_2\text{SO}_4-P_i$: inorganic P extractable with H_2SO_4 ; MBC: microbial biomass C; MBN: microbial biomass N; MBP: microbial biomass P; acid P-ase: acid phosphatase activity; PNF: *p*-nitrophenol). Different letters in the same P fraction show significant differences determined by *t* test ($P < 0.05$, MAN and ECO soil were not included).

Table 3. Pearson correlation table for soil phosphorus fractions and microbial biomass after incubation.

		1	2	3	4	5	6	7	8	9	10
1	NaHCO ₃ -P _i										
2	NaHCO ₃ -P _o	-0.71									
3	NaOH-P _i	0.57	-0.12								
4	NaOH-P _o	-0.48	0.56	0.28							
5	H ₂ SO ₄ -P _i	0.11	0.04	0.25	-0.12						
6	Total P	-0.67	0.81	0.10	0.75	0.26					
7	CAL-P	0.97	-0.65	0.64	-0.44	0.18	-0.59				
8	MBC	-0.72	0.86	-0.03	0.64	0.17	0.88	-0.60			
9	MBN	-0.51	0.62	0.22	0.68	0.28	0.85	-0.30	0.80		
10	MBP	0.77	-0.57	0.53	-0.31	0.14	-0.54	0.71	-0.63	-0.36	
11	Acid P-ase ⁽¹⁾	-0.57	0.62	0.26	0.84	0.10	0.84	-0.54	0.72	0.76	-0.36

Abbreviations: see **Table 1**. Bold numbers denotes significant correlations at $p < 0.05$, $n = 60$.

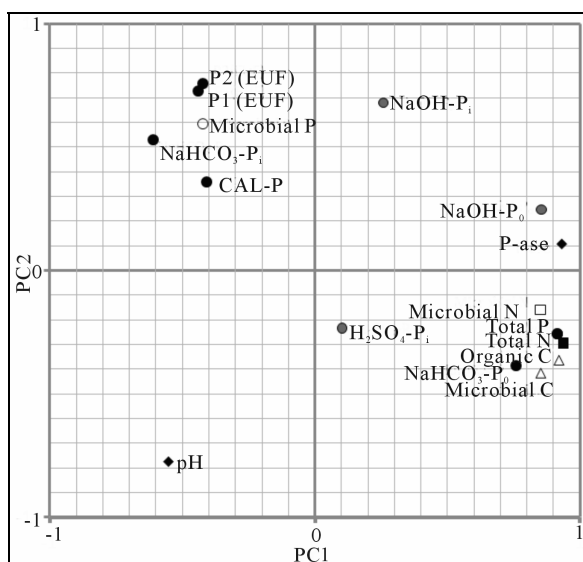


Figure 1. PCA analysis of incubated soils. Only the two principal components were considered. Abbreviations: see Table 1.

soils used in this investigation.

4. Discussion

The different soils had contrasting soil properties concerning P fractions. The high compost amendment resulted in a higher H₂SO₄-P_i and, as a consequence, in a higher P_T content. However, the other fractions were almost unaffected, except available CAL-P. It is reported that P derived from organic fertilizer can be found as P_i after few hours [48], or as labile and recalcitrant P_o after 56 ds in soil [49] and after 25 years [50] or 34 years [51]. However, how compost P is transformed after application

may depends on the nature of the residues, soils, crop P uptake, management, climate and others factors. Under high yields, soil P_o can be depleted [52] as well as available P [53]. High P_i in compost could be responsible that large parts of P could be found in the P_i but not in the P_o fraction. Nevertheless, P-ase was four times higher in the COMP(+) soil compared to COMP(-) soil. Thus, microbial activity might be responsible for high available P (CAL-P) in this soil.

CAL-P was relatively high in COMP(+) and COMP(-) soils considering the P classification of official extension services (optimal and elevated, respectively, [50]). CAL measurements resulted in 5 to more than 10 fold higher P concentrations for COMP soils compared to ECO and MAN. Same differences between the soils were visible for other fractions considered as easily available (EUF and NaHCO₃-P_i), although, not to the same extent. Although CAL-P and NaHCO₃-P_i were closely correlated, ($r = 0.90$, **Table 3**) for all soils, this relationship disappears if focusing on a single soil only. A relationship between the P-fractions considered as labile (EUF, NaHCO₃-P_i, CAL-P and MBP) is also indicated by a common cluster in the PCA (**Figure 1**).

CAL-P measurements separate COMP (+) and COMP (-) more clearly than the NaHCO₃-P_i-Fraction (**Figure 2**). This may be due the higher pH of COMP (+). In soils high in pH, the acidic CAL solution (pH 4.1) may extract certain amounts of less plant available Ca-Phosphate.

Disturbance affected the labile NaHCO₃-P_o fractions mostly in the COMP(+) and MAN soils. As reported earlier, MBP is positively related to NaHCO₃-P_o fractions [41], but this was not the case in our study. In contrast, MBP could rather be related to NaHCO₃-P_i (**Table 3**), and this was also confirmed by the PCA analysis (**Figure**

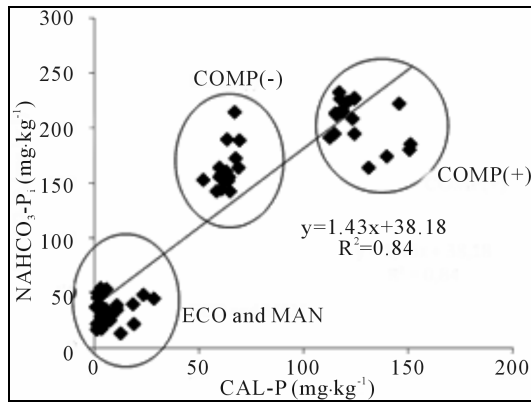


Figure 2. Regression between CAL-P and NaHCO₃-P_i. Abbreviations: see Table 1.

1). This relation was also maintained if respective PCA's were done for each soil separately (not shown). Nevertheless, NaHCO₃-P_o could be closely related to P_{ase} indicating a participation in the microbial turnover (**Figure 1**).

Both COMP(+) and MAN soils have the highest pH, and the highest H₂SO₄-P_i fraction related to Ca bound Phosphates [28]. This can be a consequence of the slightly alkaline pH of both, compost residues and pig manure in COMP and MAN soils, respectively [54], and due to MAN soil natural properties. An apparent translocation from NaOH-P_o to NaHCO₃-P_o is visible for the COMP(+) soil indicating an increase of availability of P_o. However the absolute decrease in NaOH-P_o cannot fully be explained by the increase in NaHCO₃-P_o or changes in any other fraction, indicating that further fractions are existing as a (preliminary) sink of originally organic P. Reference [55] proposed soil microbial residues as an intermediate sink for N which might also be true for P. Higher P mobilization in the COMP(+) compared to the other soils can be due to a higher pH, lower resistance to mechanical disruption or more labile organic matter [56], lower aggregate size and higher friability [57], as a consequence of compost addition.

In MAN soil, on the other hand, labile NaHCO₃-P_i was apparently converted to NaHCO₃-P_o. Microorganisms could have been grown and died during the time elapsed between incubation and determination, stimulated by soil rewetting by incubation, thus absorbing P_i and releasing P as organic components recovered by NaHCO₃-P_o [55, 58]. As microbes died and cell P was reconverted to P_i no change in MBP could be observed, but decrements of MBC and MBN as these elements cycle faster, as they are parts from no structural components of the cell. This conversion of labile P_i to P_o has not been observed for the other soils. This can be due to the nature and structure of the soil or due to the effects of the applied pig manure.

Regarding ECO soil, there was an increase of available CAL-P with 10 d as similarly observed with soil tillage

[59]. But this enhancement was not reflected by changes in the P fractionation, not even in the most available NaHCO₃-P_i fraction. The enhancement of P availability in the ECO soil may be attributed to an enhanced enzymatic activity after 10 d shaking. The COMP(-) soil was the less affected by disturbance. Only MBP was enhanced after 10 d shaking. This P uptake by microbes was not reflected in any change of the other P fractions. None of the other soils showed similar significant MBP changes.

In their meta study, [60] reported that a mean molar C:N:P ratio of 60:7:1 for the soil microbial biomass can be found and that differences from this general ratio are less pronounced than similarities over a wide range of ecological properties. While the original COMP(+) soil is very similar to this ratio, the C:N:P ratio of the original COMP(-) soil indicates a much narrower relationship between P and the two other elements. In the original MAN and ECO soils, however, the ratios are much wider than reported by [60]. After incubation, however, the ratios decreased in all soils indicating a relative enrichment of soil microbial biomass in all treatments.

Our values for MBP and MBP/P_T in COMP(+) and COMP(-) were similar to those reported by [61] for German soils. MBP and MBC were negatively correlated (**Table 3**), and MBP can be related to the other P_i forms, but MBC rather to MBN and P_o, as shown by PCA analysis. This can be explained by different distributions of C and P throughout the cell structures, thus, differing in cycling and microbial acquisition, or by the development of different microbial communities [62,63]. It is reported that environmental changes affect microbial communities more than microbial activity, and that microbial P is more susceptible to changes than N and C, resulting in a faster microbial P recycling [62,64], but in our study microbial P was almost unaffected in spite of changes for microbial C and N in ECO soil and a redistribution of P fractions in COMP(+) soil. Almost all changes were in microbial C and N as well as in P_o fractions, thus confirmed by a closer relationship between these variables as revealed by PCA analysis.

5. Conclusion

Short term soil disturbance had effects on labile organic P fractions of two of the four analyzed soils, but inorganic P was rather unaffected. These changes can be associated to changes in the microbial biomass C and N as shown by PCA analysis, but microbial P was rather unaffected. In the compost amended COMP(+) soil, there was an incorporation of P from the less available NaOH-P_o fractions into the more available NaHCO₃-P_o fraction. However, if taking all investigated soils and treatments into account, the effects of soil disturbance are limited and not consistent. Soils with high organic fertilization do not

necessarily have higher organic P fractions and higher enzymatic activity than those without organic fertilization.

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