Composition of phosphorus in wetland soils determined by SMT and solution ^{31}P -NMR analyses

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Abstract In Eastern China, wetlands are common in the lower reaches of catchments or in coastal zones. Wetlands are at risk from eutrophication because of the large quantities of phosphorus (P) they receive from rivers. They are also decreasing in size. In this contribution, we present information about the composition of P in wetland soils, obtained using the Standards, Measurements, and Testing (SMT) protocol and ^{31}P -nuclear magnetic resonance (NMR) spectroscopy. Average P concentrations varied in the different wetland soils and, in four of the five wetlands sampled, exceeded 500 mg kg^{-1} . HCl-inorganic P (Pi) was the main Pi fraction in wetland soils. The percentage contribution of Pi (89.7 %) to total P was the highest in the Yangtze River estuary wetland. Six P components were detected by ^{31}P -NMR analysis. Mono-P was the main organic P (Po) in wetland soils. Orthophosphate (Ortho-P) was positively and negatively related to NaOH-Pi ($R^2=0.957$, $p<0.001$) and HCl-Pi ($R^2=-0.689$, $p<0.001$), respectively. Orthophosphate monoesters (Mono-P) were positively related to Po ($R^2=0.617$, $p<0.001$) and ortho-P ($R^2=0.624$, $p<0.001$), respectively. The main Po component was Mono-P, and it may be mineralized to ortho-P under the frequently changing redox conditions in wetland soils. The information from this study will

support the development of robust scientific and effective policy for P management in wetlands.

Keywords Wetland · P-NMR · SMT · Phosphorus · Organic phosphorus · Soil

Introduction

Phosphorus (P) is a key element in freshwater ecosystems (Cade-Menun et al. 2006). P from agricultural, industrial, and municipal sources can lead to water quality deterioration and can exacerbate eutrophication of freshwater (Carpenter 2008). Wetlands, as the “kidneys” of the earth, play a critical role in ecological and economic services, by providing habitats for fish and birds, and increasing water quality and retaining nutrients (Hamdan et al. 2012; Pelley 2008; Deegan et al. 2012). Coastal wetlands, which are at the interface between terrestrial and marine ecosystems, play a vital role in P retention and biogeochemistry (Liu 2005). Wetland soils adsorb and immobilize P, and also provide a suitable environment for P uptake by plants and microbes. Nutrient enrichment may promote wetland loss and carbon sequestration, thereby ultimately affecting global warming (Deegan et al. 2012). Studies of the speciation and biogeochemistry of P in wetlands are therefore significant for environmental protection and wetland restoration.

Phosphorus in wetland soils can be divided into inorganic P (Pi) and organic P (Po). Pi and Po can also be broken down into various components. However, only some forms of P in sediment will either be released or support algal growth (Carpenter 2008). Depending on the chemical state, P can be divided into three parts—namely exchangeable P, P associated with Al, Fe, and Mn oxides and hydroxides—and Ca-bound compounds. Further, P can be divided by its bonding

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characteristics into orthophosphate, phosphonates, pyrophosphate, orthophosphate monoesters, DNA-P, and phospholipids (Ruban et al. 2001a; Turner et al. 2005). Although several analytical methods have been developed to determine P fractions, the lack of uniformity between these analytical procedures means that the results from different studies cannot be compared. The Standards, Measurements, and Testing (SMT) protocol, which was launched by the European Commission through the Standards, Measurements, and Testing Program (Ruban et al. 2001b), is the most commonly used method. This method has various advantages; for example, the analysis procedure is straightforward, the reagents are easy to obtain, and there is low interference between the various P forms. However, the SMT method only provides results for total Po and cannot provide a comprehensive description of Po. ^{31}P nuclear magnetic resonance (^{31}P -NMR) spectroscopy is a non-destructive, noninvasive technique that identifies chemical forms and provides comprehensive information about Po components (Hupfer and Rube 2004). It is therefore an ideal technique to use to obtain information about Po species in marine or freshwater sediments (Cade-Menun 2005; Turner et al. 2005; Cade-Menun and Liu 2013). Several Po compounds, such as phosphonates, orthophosphate monoesters, and orthophosphate diesters, have been detected by ^{31}P -NMR (Turner et al. 2005). The combination of SMT and solution ^{31}P -NMR will provide thorough information about P in sediments.

Because of the spread of economic development and urbanization, wetlands have decreased worldwide; in China, 30 % of all remaining natural wetlands and 50 % of coastal wetlands have been lost because of draining, filling, and pollution (Chen et al. 2015). Robust scientific and effective policies to protect and restore wetlands are urgently needed. In the light of this challenge, the aims of this study were to investigate (1) the P characteristics in surface sediments from different wetlands by SMT and solution ^{31}P -NMR fractionation, and (2) the relationships between P fractions and environmental factors. The information will provide a basis for appropriate P management strategies that could be included in wetland protection policies in developing countries.

Materials and methods

Study areas and sediment sampling

Sediments were collected from five typical wetlands, including the Sanjiang Plain wetland (SPW), Liaohe estuary wetland (LEW), Haihe estuary wetland (HEW), Yellow River estuary wetland (YREW_a), and the Yangtze River estuary wetland (YREW_b). The LEW, HEW, YREW_a, and YREW_b are in coastal areas where economic development and urbanization are rapidly increasing. The area of the wetlands is declining, and the environmental and ecological quality is deteriorating.

The SPW, the control wetland in the northeast of China, is in good ecological and environmental condition. The SPW covers an area of $1.09 \times 10^5 \text{ km}^2$ and receives inputs from the Songhua, Heilong, and Wusuli Rivers. The LEW, which is the biggest reed estuarine wetland, covers an area of $0.6 \times 10^4 \text{ km}^2$. The HEW is located in the estuary of the Haihe watershed and includes the Qilihai, Beidagang, and Nandagang wetlands. The YREW_a is located at the estuary of the Yellow River and covers an area of $0.15 \times 10^4 \text{ km}^2$, while the YREW_b is located at the estuary of the Yangtze River and covers an area of $0.3 \times 10^4 \text{ km}^2$. All of the wetlands are in densely populated regions with intensive industrial and agricultural development, and previous studies have shown that their ecological and environmental quality has deteriorated in recent years (Lin et al. 2010; Wang et al. 2013).

Soil samples were collected from the wetlands in September 2013 (Fig. 1). Depending on the area of the wetland, between three and five samples of surface sediment (about 5 cm) were collected using a Peterson grab sampler. Each sampling site comprised three to five subsampling sites, and these subsampling sites were at a distance of more than 500 m from the main sampling site. Samples from the same site were pooled and homogenized to obtain a representative sample. They were stored in a sealed plastic bag and kept at 0 °C during transport to the laboratory. On arrival at the laboratory, samples were freeze-dried at −50 °C (FD-1 freeze-dryer). Dried samples were ground and sieved through a 100-mesh sieve. Samples were quartered and then were stored in sealed plastic bags at room temperature until analysis.

Sediment sample analysis

The SMT protocol was used to determine the P fractions in the sediment (Ruban et al. 2001a and 2001b). Phosphorus was classified into total P (TP), inorganic P (Pi), organic P (Po), P associated with Ca (Ca-P), and P bound to Fe, Al, and Mn oxides and hydroxides (Fe-P) (Fig. 2). The methods for each of these fractions follow. (1) For TP, a sieved sample (0.2 g) was placed in a crucible and calcined at 450 °C in a muffle furnace for 3 h. The ash was cooled and transferred to a centrifuge tube to which 20 mL 3.5 mol L^{-1} HCl was added. To make it easier to transfer the ash, HCl can be added directly to the crucible. The tube was covered and stirred overnight (16 h), and then centrifuged at 2000 g for 15 min. After centrifugation, the extract was collected in a test tube to allow determination of concentrated TP in the sediment. (2) For Pi and Po, a sieved sample (0.2 g) was placed in a centrifuge tube, and 20 mL 1 mol L^{-1} HCl was added with a pipette. The tube was covered and stirred overnight (16 h), and then centrifuged at 2000 g for 15 min. After centrifugation, the extract was collected in a test tube for determination of Pi. The Po content was calculated as the difference between TP and Pi. (3) For HCl-Pi and NaOH-Pi, a sieved sample (0.2 g) was accurately weighed into a centrifuge tube, and 20 mL 1 mol L^{-1} NaOH was

Fig. 1 Location of five sampling areas in Eastern China, including Sanjiang Plain wetland, Liaohe estuarine wetland, Haihe estuarine wetland, Yellow River estuary wetland, and Yangtze River estuary wetland. Each sample area included three to five sample sites, and three to five sub-samples were collected at each sample site

added. The tube was covered and stirred overnight (16 h), and then centrifuged at 2000 g for 15 min. Complete mixing was necessary, as the sediment must be kept in suspension for this extract. After centrifugation, NaOH-Pi was extracted from the supernatant, while HCl-Pi was extracted from the residue. The extract was collected, and 10-mL sample was measured by pipette and set aside. After adding 4 mL 3.5 mol L⁻¹ HCl, the tube was covered. Next, the tube was stirred vigorously for 20 s and then left to stand overnight (16 h). A brown precipitate appeared and, once settled, was centrifuged at 200 g for 15 min. NaOH-Pi was determined in the supernatant. The residue of the previous centrifugation was washed with 12 mL 1 mol L⁻¹ NaCl and stirred for 5 min. This suspension was centrifuged at 2000 g for 15 min, and the supernatant was discarded. The washing step was repeated once, and then 20 mL 1 mol L⁻¹ HCl was added to the tube and stirred overnight (16 h). After centrifugation at 2000 g for 15 min, HCl-Pi was determined in the extract. The

P in all of the extracts was measured by molybdate colorimetry (Aspila et al. 1976).

The pH was determined by pH electrode at a sediment:water ratio of 1:2.5 (Lu 1999). Loss-on-ignition (LOI) analysis followed the standard methodology (Heiri et al. 2001). Total carbon (TC) and total nitrogen (TN) were analyzed by elemental analyzer (Vario EL III, Elementar, Germany).

NaOH–EDTA extraction and solution ³¹P-NMR

Po analysis included two parts, namely, NaOH–EDTA extraction and ³¹P-NMR analysis. For the ³¹P-NMR analysis, sediment samples were ground into powder and sieved through a 100-μm mesh. They were then pre-extracted with a solution of 0.05 mol L⁻¹ EDTA and 0.25 mol L⁻¹ NaOH for 16 h at room temperature at a sediment:extract ratio of 1:10 (Turner et al. 2003a). Pi (Pi in NaOH–EDTA) and TP (TP in NaOH–EDTA)

Fig. 2 The chart of SMT fraction system

Table 1 Physicochemical properties of wetland soils

Sites	pH	TP ^a /(mg · kg ⁻¹)	TN/%	TC/%	LOI/(g · kg ⁻¹)	TC/TN
Sanjiang Plain wetland	6.97	706.1	0.14	1.50	4.52	10.67
Liaohe estuarine wetland	8.35	482.4	0.15	1.82	5.68	12.32
Haihe estuarine wetland	8.09	627.6	0.24	3.19	6.22	15.11
Yellow River estuary wetland	8.16	654.1	0.20	3.05	5.47	27.81
Yangtze River estuary wetland	8.24	576.1	0.05	1.37	2.95	30.62

^a Average value

TP total phosphorus, TN total nitrogen, TC total carbon, LOI loss-on-ignition

were determined in an aliquot of the extract using the molybdate colorimetry method before and after digestion by K₂S₂O₈, respectively (Shinohara et al. 2012). The difference between TP and Pi was Po in the NaOH–EDTA extract. The remaining solution was frozen and lyophilized for ³¹P-NMR analysis.

Although Po may be lost by incomplete dissolution during the freeze-drying and re-dissolution process, lyophilized samples in this study were completely re-dissolved. Lyophilized NaOH–EDTA extracts (300 mg) were re-dissolved in 0.6 mL D₂O and 0.1 mL 10 mol L⁻¹ NaOH; they were then ultrasonicated for 30 min and equilibrated for 5 min. To reduce interference from paramagnetic ions (Fe³⁺ and Mn²⁺), 2 % (v/v) of BD (0.11 M NaHCO₃ + 0.11 M Na₂S₂O₄) was added to the extracts. The supernatants were centrifuged for 15 min at 14,000 rpm and transferred to 5-mm NMR tubes. All of the preparation procedures were completed within 1 h to minimize loss of Po. Solution ³¹P-NMR spectra were obtained using a Bruker 400-MHz spectrometer (Bruker, Billerica, MA, USA) operated at 129.53 MHz at a temperature of 25 °C. We used a 90° observation pulse, a relaxation delay of 3 s, and an acquisition time of 0.6 s. Depending on the concentration of Po, spectra were collected with around 3000–20,000 scans (Beijing NMR center). Although T₁ relaxation times were not explicitly determined in this study, for most samples (70 %) analyzed for Mn and Fe, a T₁ relaxation time of 3 s should be adequate to obtain quantitative spectra for a variety of P forms (McDowell et al. 2006). Chemical shifts were recorded relative to an 85 % H₃PO₄ standard (δ = 0 ppm). Signals were assigned to P compounds based on data in the literature (Turner et al. 2003b; Cade-Menun 2005). Peak assignments were made using ³¹P-NMR chemical shifts of phosphonate (phon-P: 12–23 ppm), orthophosphate (ortho-P: 6–7 ppm), orthophosphate monoesters (mono-P: 4–6 ppm), phospholipids (lipid-P: 1–3 ppm), deoxyribonucleic acid phosphorus (DNA-P: 0 ppm), and pyrophosphate (pyro-P: -3.5–4.5 ppm). The spectral processing was carried out using NMR Utility Transform Software (NUTS) for Windows (2010 edition; Acorn NMR, Livermore, CA, USA). The contribution of each P compound group obtained from the different areas of each P species was calculated relative to TP in the NaOH–EDTA extract determined by the molybdenum blue method (Turner et al. 2003b; Shinohara et al. 2012).

Statistical analysis

The experimental data were analyzed using SPSS 19.0 for Windows. The relationships between the P and sediment properties were assessed using Spearman's correlations.

Results and discussion

Phosphorus in the surface sediment from different wetlands

Concentrations of P in soil varied in different wetlands (Table 1). The average TP concentration (706.1 mg kg⁻¹) was the highest in SPW, followed by YREWa (654.1 mg kg⁻¹), HEW (627.6 mg kg⁻¹), and YREWb (576.1 mg kg⁻¹); all of these concentrations were higher than the TP concentrations in the Poyang Lake wetland (360.7 mg kg⁻¹ in surface soil) (Nie et al. 2014). The P concentrations in SPW were high because of the good ecological condition and the high coverage and variety of plants. Plant residue decomposition can introduce large amounts of P to wetland soils (Cheesman et al. 2010). Unlike SPW, HEW, YREWa, and YREWb are in the lower reaches of their catchments where socioeconomic development and

Fig. 3 Concentration of TP and percentage components of TP (Po, HCl-Pi, and NaOH-Pi) in different wetland soils. A indicates Sanjiang Plain wetland, B Liaohe estuarine wetland, C Haihe estuarine wetland, D Yellow River estuary wetland, and E Yangtze River estuary wetland

urbanization are more intensive, meaning that the wetlands received large amounts of pollution, resulting in P accumulation in the wetland soils (Pernet-Coudrier et al. 2012). Wetland organisms immobilize P from the P-enriched runoff in their tissue, which is then incorporated into the wetland soils when the organism decays. This process includes physical, chemical, and biological processes (Turner et al. 2006).

Most variation in the TP concentrations in the different wetlands was caused by variations in Pi. The percentage contribution of Pi to TP ranged from 73.4 to 89.7 %. The Pi (89.7 %)

proportion was the highest in YREW_b. The pH in SPW was higher than that in the other four wetlands. TN and TC contents were higher in HEW and YREW_a than in the other two wetlands. The LOI varied between the wetlands, but was the lowest in YREW_b.

Results from the SMT fractionation are shown in Fig. 3. NaOH-Pi represents the P bound to Al, Fe, and Mn oxides or hydroxides, and Fe-P was the dominant P fraction in the sediment (Ruban et al. 2001a). Fe-P and Al-P are also easily influenced by the pH and oxidation-reduction potential

Fig. 4 ^{31}P -NMR spectra of NaOH-EDTA extracts of wetland soils

(Ruban et al. 1999) and can be released into pore water and surface water under reducing conditions. The average concentration of NaOH-Pi ranged from 47.2 to 310.7 mg kg⁻¹. NaOH-Pi was not the main component in the wetland soils, but accounted for between 7 and 44 % of TP in sediment. The concentration of NaOH-Pi was the highest in SPW (310.7 mg kg⁻¹, or 44 % of TP). HCl-Pi represents Ca-bound P in sediment and is often referred to as apatite-P (Ruban et al. 2001b). The concentration of HCl-Pi ranged from 207.8 to 462.6 mg kg⁻¹ and represented, on average, 60.9 % of TP. Most of the Pi in wetland soil was apatite-P (Ahlgren et al. 2005).

Phosphorus forms identified by ³¹P-NMR

The NMR spectra for the wetland soils are shown in Fig. 4. Six ³¹P NMR peaks were detected in the NaOH-EDTA extracts of the wetland soils. All NMR spectra showed peaks in the areas of Po and Pi, which included ortho-P, pyro-P, phon-P, mono-P, lipid-P, and DNA-P. Poly-P was not detected in any of the wetland soils.

The concentrations and relative distribution of the various P compound groups in the different wetland soils are presented in Fig. 5. Po in the NaOH-EDTA fraction ranged from 5.7 to 70.5 mg kg⁻¹. Ortho-P was the dominant form of P in the

NaOH-EDTA extracts of the wetland soils; concentrations of ortho-P ranged from 74.9 to 427.2 mg kg⁻¹ and accounted for 68–94 % of TP in the NaOH-EDTA extracts. The variation in the Po concentration may be attributable to the wetland plants. Previous research has shown that the contribution of Po to TP was greater in a cattail marsh than in a submerged aquatic vegetation wetland (Turner et al. 2006). Biogenic-P compounds were identified as molybdenum-nonreactive P compounds in soil (Rydin 2000; Shinohara et al. 2012). Concentrations of biogenic-P ranged from 5.9 to 76.0 mg kg⁻¹ and included pyro-P, mono-P, DNA-P, lipid-P, and phon-P. Concentrations of mono-P ranged from 4.0 to 66.9 mg kg⁻¹ and accounted for 4–15 % of TP in the NaOH-EDTA extracts. Mono-P was the main component in biogenic-P in all of the wetland soils. The second group of biogenic-P in the wetland soil was diester-P, which included DNA-P and lipid-P. Contributions of phon-P and pyro-P to biogenic-P were minimal in the wetland soils.

Mono-P is considered as stable Po in the wetland soil. The majority of mono-P comes from manures, plants, and algae, and is considered to be biologically unavailable and immobile in sediment (Turner et al. 2002; McKelvie 2007). However, some studies have demonstrated that mono-P may be a source of phosphate under anaerobic and aerobic conditions (Golterman et al. 1998). The redox conditions of wetlands

Fig. 5 Concentrations of P compounds in NaOH-EDTA extracts of wetland soils by ³¹P-NMR (mg kg⁻¹)

change frequently, so mono-P may be mineralized under hypoxic conditions in the wetland soils. Diester-P represents labile P in wetland soils. Previous studies in Lake Erken in Sweden have shown that it has a half-life of 21 years (Ahlgren et al. 2005; Cade-Menun 2005). The concentrations of DNA-P and lipid-P, the main components of diester-P, ranged from 0.1 to 22.7 mg kg⁻¹, and 0.1 to 3.5 mg kg⁻¹, respectively. Although the diester-P contents were lower than the concentrations of mono-P in Po, DNA-P and lipid-P may be the main source of P for wetland soils under frequent changes in redox condition. Pyro-P represents active Pi in the soil and can be directly used by aquatic organisms. Phon-P, which was considered as stable Po with C-P binding ability, was also detected in the wetland soils (Turner et al. 2005). These results reflect those from studies of eutrophic lakes, such as Taihu Lake (Bai et al. 2009)

Correlations between various P fractions and physicochemical properties

Correlation coefficients between various P forms and physicochemical properties are shown in Table 2. While NaOH-Pi and HCl-Pi were the main fractions of Pi, they were negatively correlated in the wetland soils ($R^2 = -0.683$, $p < 0.001$). Ortho-P detected by the ³¹P-NMR analysis was positively and negatively correlated with NaOH-Pi ($R^2 = 0.957$, $p < 0.001$) and HCl-Pi ($R^2 = -0.689$, $p < 0.001$), respectively. This was because the NaOH-EDTA acted as a chelating agent and chelated with Fe and Al, forming a clathrate during the extraction process. The HCl-Pi, which contained Ca-bound compounds, was not easily chelated by NaOH-EDTA (Cade-Menun and Preston 1996; Cade-Menun and Lavkulich 1997; Ruban et al. 2001a). This was also confirmed by the negative relationship of NaOH-Pi with pH ($R^2 = -0.869$, $p < 0.001$). The Po concentration was

positively correlated with LOI ($R^2 = 0.555$, $p < 0.01$), indicating that the organic matter in the different wetlands had an influence on the Po content. Some studies have indicated that the organic matter associated with Po was the stable, humified soil organic matter (Hamdan et al. 2012). Mono-P was positively related with Po ($R^2 = 0.617$, $p < 0.001$) and ortho-P ($R^2 = 0.624$, $p < 0.001$), which indicates that Po was the main component and that mono-P might be mineralized to ortho-P under the frequently changing oxidation–reduction conditions in wetland soil (Golterman et al. 1998). This was also preliminarily confirmed in a large treatment wetland in Florida and rewetted soil in England and Wales (Turner and Haygarth 2001; Turner et al. 2006). The activity of diester-P was higher than that of mono-P and so could be easily mineralized.

Conclusions

In this study, we used the SMT protocol and ³¹P-NMR technology to determine the composition of P in typical wetland soils. The P content varied between the different wetlands, and the concentration was the highest in SPW (706.1 mg kg⁻¹). Variation in TP concentrations in the different wetlands was mostly attributable to changes in Pi, the percentage of which ranged from 73.4 to 89.7 %. The percentage of Pi (89.7 %) was the highest in YREW_b. With the exception of SPW, HCl-Pi, derived by the SMT fractionation process, was the main component of Pi in most wetlands (310.7 mg kg⁻¹ Pi, 44 % of TP). Six P components were detected in the ³¹P-NMR analysis, including ortho-P, pyro-P, phon-P, mono-P, lipid-P, and DNA-P. Mono-P, which ranged from 4.0 to 66.9 mg kg⁻¹, was the main component of Po. Relationships between the P components and physicochemical properties in wetland soils were variable. NaOH-Pi and HCl-Pi were negatively

Table 2 Pearson correlation coefficients between various phosphorus fractions and physicochemical properties in the wetland soils

	TTP	NaOH-Pi	HCl-Pi	Po	Ortho-P	Mono-P	Diesters-P	CNRatio	pH	LOI
TP	1	0.425	0.263	0.634 ^b	0.427	0.514 ^a	0.465 ^a	-0.020	-0.370	0.331
NaOH-Pi		1	-0.683 ^b	0.547 ^a	0.957 ^b	0.655 ^b	0.958 ^b	-0.433	-0.869 ^b	0.008
HCl-Pi			1	-0.387	-0.689 ^b	-0.369	-0.673 ^b	0.555 ^a	0.556 ^a	-0.004
Po				1	0.622 ^b	0.617 ^b	0.655 ^b	-0.420	-0.411	0.555 ^a
Ortho-P					1	0.624 ^b	0.991 ^b	-0.441	-0.711 ^b	0.032
Mono-P						1	0.721 ^b	-0.617 ^b	-0.611 ^b	0.503 ^a
Diesters-P							1	-0.494 ^a	-0.733 ^b	0.112
CNRatio								1	0.381	-0.554 ^a
pH									1	-0.094
LOI										1

NaOH-Pi NaOH-Pi in SMT, HCl-Pi HCl-Pi in SMT, Po Po in wetland soil, Ortho-P orthophosphate, Mono-P orthophosphate monoesters, Diesters-P orthophosphate diesters, CNRatio the ratio of total carbon to total nitrogen, LOI loss-on-ignition

^a Correlation is significant at the 0.05 level (two-tailed)

^b Correlation is significant at the 0.01 level (two-tailed)

correlated in the wetland soil ($R^2 = -0.683$, $p < 0.001$). Ortho-P from the ^{31}P -NMR analysis was positively and negatively correlated with NaOH-Pi ($R^2 = 0.957$, $p < 0.001$) and HCl-Pi ($R^2 = -0.689$, $p < 0.001$), respectively. Mono-P was positively correlated with Po ($R^2 = 0.617$, $p < 0.001$) and ortho-P ($R^2 = 0.624$, $s < 0.001$). Diester-P, which includes DNA-P and lipid-P, had a higher activity than mono-P, and so may be easily mineralized.

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