Biogenic Phosphorus Compounds in Sediment and Suspended Particles in a Shallow Eutrophic Lake: A P-31-Nuclear Magnetic Resonance (P-31 NMR) Study

ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · SEPTEMBER 2012

Impact Factor: 5.33 · DOI: 10.1021/es301887z · Source: PubMed

9 AUTHORS, INCLUDING:

Akio Imai
National Institute for Environmental Studies
79 PUBLICATIONS **1,048** CITATIONS
SEE PROFILE

Nobuyuki Kawasaki
UNISEL | Universiti Selangor
18 PUBLICATIONS **202** CITATIONS
SEE PROFILE

Kazuhiro Komatsu
National Institute for Environmental Studies
19 PUBLICATIONS **82** CITATIONS
SEE PROFILE

Shingo Miura
National Institute for Environmental Studies
6 PUBLICATIONS **33** CITATIONS
SEE PROFILE

Environmental Science & Technology

Article

pubs.acs.org/est

Biogenic Phosphorus Compounds in Sediment and Suspended Particles in a Shallow Eutrophic Lake: A ^{31}P-Nuclear Magnetic Resonance (^{31}P NMR) Study

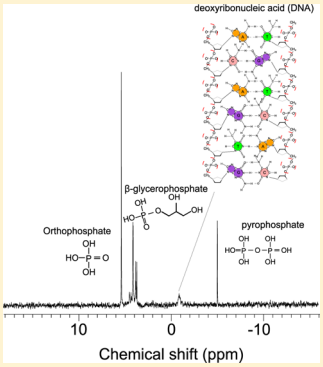
Ryuichiro Shinohara,[†,*] Akio Imai,[†] Nobuyuki Kawasaki,[†,‡] Kazuhiro Komatsu,[†] Ayato Kohzu,[†] Shingo Miura,[†] Tomoharu Sano,[†] Takayuki Satou,[†] and Noriko Tomioka[†]

[†]National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

[‡]Faculty of Science and Biotechnology, University of Selangor, Jalan Timur Tambahan 45600 Bestari Jaya, Salangor, Malaysia

Supporting Information

ABSTRACT: Differences in biogenic phosphorus (P) compounds between sediment and suspended particles in aquatic environments are important for understanding the mechanisms of internal P loading, but these differences are still unknown. We used solution-state ^{31}P-nuclear magnetic resonance spectroscopy (^{31}P NMR) with NaOH-ethylenediaminetetraacetic extraction to detect the multiple P compounds in suspended particles and sediment in the eutrophic Lake Kasumigaura, including orthophosphate monoesters, orthophosphate diesters, pyrophosphate, and polyphosphate. We tested the hypothesis that there is a significant difference between these groups in suspended particles and sediment. Biogenic P other than orthophosphate was found in significantly higher proportions in suspended particles (74.3% of total P) than in sediment (25.6%). Orthophosphate monoesters were comparatively more abundant in suspended particles, as indicated by the ratio of orthophosphate diesters to monoesters (average, 0.31 for suspended particles; 1.05 for sediment). The compounds identified as orthophosphate monoesters by ^{31}P NMR spectroscopy originated mainly from phospholipids (α-glycerophosphate and β-glycerophosphate) and ribonucleic acid (RNA-P), whereas the orthophosphate diesters included mostly DNA (DNA-P). These results suggest that the dynamics of orthophosphate diesters, the production of DNA-P, or the degradation of phospholipids, play an important role in P cycling in Lake Kasumigaura.



INTRODUCTION

Suspended particles in aquatic environments eventually settle and accelerate the accumulation of phosphorus (P) in surface sediment. This process contributes to internal P loading through increased P supply from bottom sediment, thereby delaying recovery from a eutrophic state even after the reduction of external loading.[1] The release of P from suspended particles and sediment is an important process for internal P loading.[2,3] The mechanisms of this release are a function of the P compounds present in the suspended particles and sediment, because P compounds are directly linked to the mechanisms of P degradation and release, through enzymatic hydrolysis and fluctuation of redox potential/pH.[2,4,5] Thus, to understand internal P loading it is necessary to identify the specific P compounds in suspended particles and sediment and any differences in the amounts present.

Studies of sediment P release have focused on the reduction reactions of minerals (e.g., Fe and Mn [6]) that liberate orthophosphate to the sediment pore water.[7] Biogenic P compounds, which are analyzed as the molybdenum-nonreactive P in an NaOH extract, also release P under redox conditions similar to those where Fe(III) is reduced to Fe(II).[8,9] The mechanism could involve the release from polyphosphate-incorporating bacteria,[10] as well as phytate-P mineralization.[11] Conceivably, the biogenic P in sediment could be supplied through the sedimentation of suspended particles including detritus, phytoplankton, and bacteria.[12,13] In fact, surface sediment includes polyphosphates partially derived from the sedimentation of polyphosphate-incorporating phytoplankton and bacteria.[9,14] However, despite their importance, little information is currently available about the specific biogenic P compounds in suspended particles and sediment and any differences between them.

We detected and compared P compounds in suspended particles and surface sediment by applying ^{31}P-nuclear magnetic resonance spectroscopy (^{31}P NMR), a powerful and nondestructive method for analyzing P compounds.[15−17] ^{31}P NMR analysis has been used to detect multiple groups of P compounds, including biogenic P, orthophosphate, orthophosphate monoesters, orthophosphate diesters, pyrophosphate, and polyphosphate.[10,18] This tool is also used to track specific P compounds and their proportions, such as *myo*-inositol hexakisphosphate,[19] an important orthophosphate monoester compound.[20]

Received: May 15, 2012
Revised: August 28, 2012
Accepted: September 6, 2012
Published: September 20, 2012

These analytical characteristics of ^{31}P NMR could supply important information on the P cycle in the water column and in sediment. First, P analysis by ^{31}P NMR distinguishes labile compounds. For example, orthophosphate diesters such as DNA (DNA-P), ribonucleic acid (RNA-P), and phospholipids, are likely degraded more rapidly than orthophosphate monoester compounds.[21] Furthermore, the difference between P compounds in suspended particles and sediment reflects the transformation, degradation, or production of biogenic P, and the orthophosphate diester-to-monoester ratio in settling particles has been used as an index of the degradation or mineralization of these P groups.[22] We have also applied this ratio to suspended particles and sediment while concurrently determining the specific compounds responsible for changes in the ratio. Second, each group of biogenic P exhibits a different attenuation rate with sediment depth,[23,24] which indicates that the biogenic P supplied through sedimentation eventually alters long-term P accumulation and degradation in the sediment. This accumulation of P onto the sediment could affect P release from sediment, an important component of internal P loading.[25]

The objective of the current study was to analyze and compare P compounds in sediment and suspended particles by using ^{31}P NMR. Because solution NMR detects the different ester linkages of monoesters and diesters, we applied an extraction method using NaOH-ethylenediaminetetraacetic acid (EDTA) to efficiently extract organic P.[26−28] We tested the hypothesis that biogenic P compounds in suspended particles are different from those in sediment by identifying and comparing the P compounds in suspended particles and surface sediment in Lake Kasumigaura, Japan, over four months in 2011.

MATERIALS AND METHODS

Study Site and Sample Collection. Lake Kasumigaura is the second largest lake in Japan, with an area of 171 km^2, a mean depth of approximately 4 m, and a maximum depth of 7.4 m. The average water residence time is approximately 200 days.

More than 900 000 people live in the lake's watershed (1577 km^2). The lake is the source of raw drinking water for about 660 000 people. Land use in the watershed is 30% forest, 25% paddy field, 25% plowed field, 10% residential, and 10% other. Because of extremely high loads of organic matter and nutrients, this lake is well-known for eutrophication, with mean concentrations of total nitrogen (TN) and total phosphorus (TP) of 1.1 mg L^{-1} and 0.12 mg L^{-1}, respectively, measured at the center of the lake in 2010. These nutrient concentrations are much higher than the environmental standards (Environmental standard, category III: TN < 0.4 mg L^{-1}; TP < 0.03 mg L^{-1}). Recently, the P concentration in particular has been increasing in the lake, with the particulate P concentration higher on average than the dissolved P (DP) concentration (mean values in 2010: particulate P, 0.090 mg L^{-1}; DP, 0.028 mg L^{-1}).

We conducted sampling in summer (July and August) and winter (November and December) in 2011 at the center of the lake (140°24′25″E, 36°01′57″N), where we have been conducting routine sampling monthly for more than 30 years (GEMS/Water Trend Monitoring Project). The water depth at the sampling site is approximately 6 m. Wind speeds were less than 3 m s^{-1} on all sampling occasions. We collected surface water samples (about 20 L) to determine the P compounds in suspended particles. Core samples of sediment were collected from the same site by using a gravity core sampler (Φ = 4 cm; Rigo Co. Tokyo, Japan).

Preparation of Suspended Particles and Sediment Samples. Suspended particles were collected for ^{31}P NMR measurements by filtration of lake water (about 14 L) through precombusted GF/F glass-fiber filters (Whatman; nominal pore size 0.7 μm). Additional samples of lake water (200 mL) were also filtered through precombusted GF/F glass-fiber filters for particulate P analysis.

Surface sediment samples were collected by slicing the top of the core into sections at 0−1 cm and 1−2 cm in an N_2-purged environment. Sediment and sediment pore water were separated by centrifugation (RCF, 2278*g*) for 15 min at 4 °C. The collected sediment was immediately frozen at −30 °C and lyophilized for several days. The lyophilized sediment was thoroughly homogenized and then stored in a vial at room temperature until analysis.

Sample Preparation and Solution ^{31}P NMR Spectroscopy. We used solution-state ^{31}P NMR with NaOH-EDTA extraction because solution ^{31}P NMR can identify more P compounds than solid-state ^{31}P NMR.[15] We used the same extraction procedure for all samples. Briefly, a sufficient amount of sample (about 3 g of sediment, or suspended particles from about 14 L of lake water collected on a glass-fiber filter) was extracted with a NaOH-EDTA solution (0.25 mol L^{-1} NaOH, 0.05 mol L^{-1} EDTA; sample:solution =1:3 [v/v]) for 16 h.[29] The samples were centrifuged (RCF, 3080*g*) for 15 min after the extraction. The supernatants were then filtered through a GF/F glass-fiber filter. Duplicate samples of supernatant (about 1 mL) were collected for measurement of P concentrations. The filtrates were then frozen (−30 °C) and lyophilized for several days.

Just before analysis by ^{31}P NMR spectroscopy, the lyophilized extracts were redissolved in NaOH (2.7 mL, 1 mol L^{-1}) with D_2O (0.3 mL) to lock the signals. The samples were transferred into a 5 mm NMR tube. Solution ^{31}P NMR spectra were obtained using a JEOL ECA 500 MHz spectrometer (Jeol, Tokyo, Japan) operating at 202.5 MHz for ^{31}P. Samples were analyzed using a 0.64 s (90°) pulse and a relaxation delay of 10 s, with broadband proton decoupling. We checked the relaxation delay and used 10 s to accurately quantify each P compound. Approximately 20 000 scans (requiring approximately 60 h) were acquired for each sample. The long extraction time and analytical requirements could induce the degradation, but we observed only a slight difference in composition of P groups (<1.5%) as compared with that by a procedure with 4 h extraction + 24 h analysis (methods recommended by Turner et al. (2008, 2011)[30,31]). A D_3PO_4 standard (Sigma-Aldrich Japan K.K., Tokyo, Japan) was diluted by a factor of approximately 10 (8.5%) as an external standard (δ = 0 ppm). Several other studies have used H_3PO_4 (85%) as an external standard; our chemical shifts appear to differ by about 0.5 ppm from these previous reports.[27]

Each P group appears in a range of chemical shifts as follows:[15] orthophosphate, 5−7 ppm; orthophosphate monoesters, 3−6 ppm; orthophosphate diesters including phospholipids and DNA-P, 2.5 to −1 ppm; pyrophosphate, −4 to −5 ppm; and polyphosphate, −20 ppm. Each P group was quantified by multiplying its proportion in the ^{31}P NMR results by the TP concentration in the NaOH-EDTA extract. To measure TP in NaOH-EDTA extracts, the extracts were diluted by a factor of 100,[32] digested by autoclaving with persulfate solution,[33] and then analyzed spectrophotometrically

Table 1. Proportions of Each Group of P Compounds in NaOH-EDTA Extracts[a]

sediment	TP mg g^{-1}/mg L^{-1}	extraction rate % of TP	ortho-P %	monoester-P %	diester-P %	pyro-P %	poly-P %	biogenic P %
August 0−1 cm	1.05	61.3	73.0	12.4	14.0	0.6	0.0	27.0
August 1−2 cm	1.01	55.0	73.6	12.8	13.2	0.3	0.0	26.4
September 0−1 cm	1.02	42.7	70.0	16.1	13.5	0.4	0.0	30.0
September 1−2 cm	0.99	50.2	76.0	10.6	12.9	0.4	0.0	24.0
November 0−1 cm	0.92	47.3	74.3	12.6	12.6	0.4	0.0	25.7
November 1−2 cm	0.96	51.9	76.0	12.5	11.0	0.5	0.0	24.0
December 0−1 cm	1.05	39.7	74.7	12.7	12.4	0.2	0.0	25.3
December 1−2 cm	0.97	48.3	77.4	9.3	13.3	0.0	0.0	22.6
average	1.00	50.4	74.4	12.4	12.9	0.4	0.0	25.6
suspended particles								
August	0.06	59.1	27.2	50	12.5	10.3	0	72.8
September	0.058	43.6	24.2	51.1	16.0	8.7	0	75.8
November	0.044	80.6	32.3	44.5	14.5	8.7	0	67.7
December	0.04	42.4	19.2	47	17.9	13.6	2.3	80.8
average	0.051	56.4	25.7	48.2	15.2	10.3	0.6	74.3
ANOVA P value		$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P > 0.05$	$P < 0.01$

[a]The results of one-way analysis of variance (ANOVA) between suspended particles ($n = 4$) and sediment ($n = 8$) are also shown as the probability (ANOVA P value). TP, total phosphorus in sediment or suspended particles; ortho-P, orthophosphate; mono-P, orthophosphate monoesters; diester-P, orthophosphate diesters; pyro-P, pyrophosphate; poly-P, polyphosphate; biogenic P, all biogenic P groups combined. Most monoester-P forms are originated from orthophosphate diesters, phospholipids and RNA-P.

as orthophosphate by the molybdenum blue method[34] (Shimadzu UV2500PC, Kyoto, Japan). Compounds other than orthophosphate were considered biogenic P.[29]

We verified the chemical shifts of P compounds by carrying out spiking experiments using known compounds to assign peaks.[35−37] Briefly, β-glycerophosphate and α-glycerophosphate were diluted by a factor of 20−50, and then about 0.1 mL of each was spiked into the NMR tube just after sample analysis was finished. The samples were then analyzed by the same procedure as described above. Since RNA is easily degraded in the alkaline extract, we carried out the preparation procedure using an NaOH-EDTA extraction to check the chemical shifts of the peaks. All chemicals used were purchased from commercial sources: β-glycerophosphate disodium salt hydrate and DL-α-glycerol phosphate[37] from Sigma-Aldrich (catalog numbers G1150 and 17766, respectively) and ribonucleic acid from yeast from Wako (catalog number 185-00202).

P Analysis. Concentrations of P in suspended particles and sediment were measured with a combustion method.[38] Briefly, sediment samples and samples on filters were combusted at 550 °C for 2 h, and then P was extracted using HCl (1 mol L^{-1}) for 16 h. The extracts were analyzed spectrophotometrically as orthophosphate by using the molybdenum blue method.

Data Analysis. We used one-way analysis of variance (ANOVA) to test for differences between suspended particles and sediment. Probability values (P) less than 0.05 were regarded as significant.

RESULTS

P Compounds in Suspended Particles and Sediment. Concentrations of P in sediments and particulate material on the samples dates were similar to those found in a previous investigation[6] (Table 1). The extraction rate (calculated as [TP in NaOH-EDTA extract] ÷ [TP in sediment]) with NaOH-EDTA (>40%; Table 1) is within the range of other reports (e.g., Ahlgren et al., 2006;[23] >16%). The nonextractable P could be Ca-bound and refractory organic P, which might not be bioavailable.[5,39]

Our analysis using ^{31}P NMR successfully determined mainly orthophosphate, orthophosphate monoesters, orthophosphate diesters, and pyrophosphate (Figure 1). Polyphosphate was detected only in December.

In descending order of concentration, the P groups in suspended particles were orthophosphate monoesters > orthophosphate > orthophosphate diesters > pyrophosphate (Figure 1, Table 1). In suspended particles, biogenic P accounted for more than 67.7% of the NaOH-EDTA-extractable P. In sediment, the relative concentrations of P groups were different from those in suspended particles: orthophosphate > orthophosphate diesters > orthophosphate monoesters > pyrophosphate. Biogenic P accounted for an average of 25.6% of NaOH-EDTA-extractable P (Table 1).

The concentration of biogenic P in suspended particles exhibited wide fluctuations during the observation period, but that in sediment remained constant, with only a slight difference between the sediment depths (ANOVA, $P > 0.05$; Supporting Information (SI), Figure S1). Orthophosphate in sediment from both depths of 0−1 cm and 1−2 cm decreased from August to September. The decrease of orthophosphate was much greater than that of biogenic P.

Among orthophosphate monoesters, we observed multiple peaks within the chemical shift range from approximately 2.5 to 5.5 ppm (Figure 2). A detailed look at the chemical shifts showed similar shifts in the orthophosphate monoester peaks of suspended particles and sediment (Figure 2; SI, Figure S2). There were particularly large peaks at approximately 4.37 and 4.73 ppm. The peak at 4.73 ppm in suspended particles was overlapped with some compounds, including α-glycerophosphate.[40] For the sediment, the peak was only α-glycerophosphate. The peak at 4.37 ppm was identified as β-glycerophosphate.[41] The other peaks were indicative of mononucleotides; degradation products of RNA-P. A small peak was sometimes observed at approximately 5.3 ppm, an indication of the C2 position of *myo*-inositol hexakisphosphate.

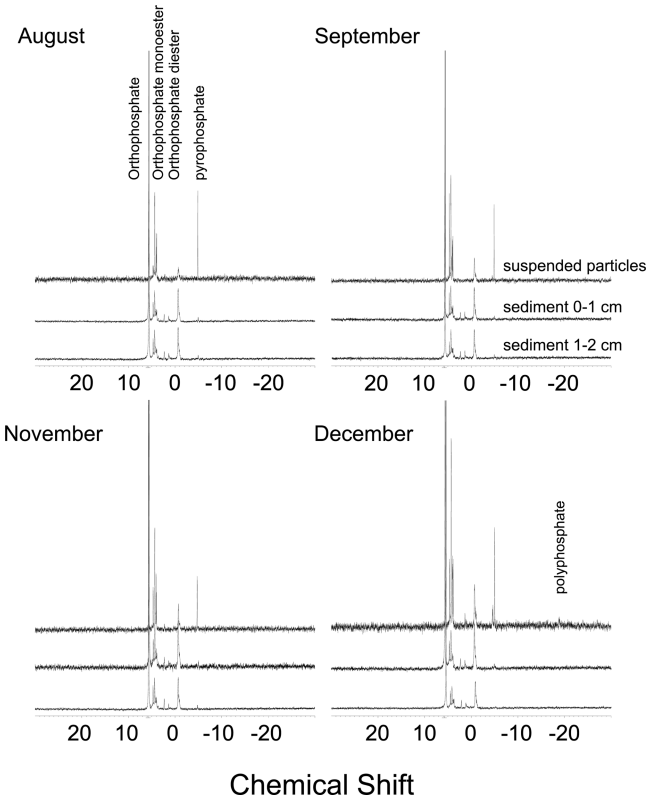


Figure 1. ^{31}P NMR spectra. Peaks reflect phosphorus (P) compounds in suspended particles and surface sediment (0−1 cm and 1−2 cm sediment depths) in August, September, November, and December of 2011.

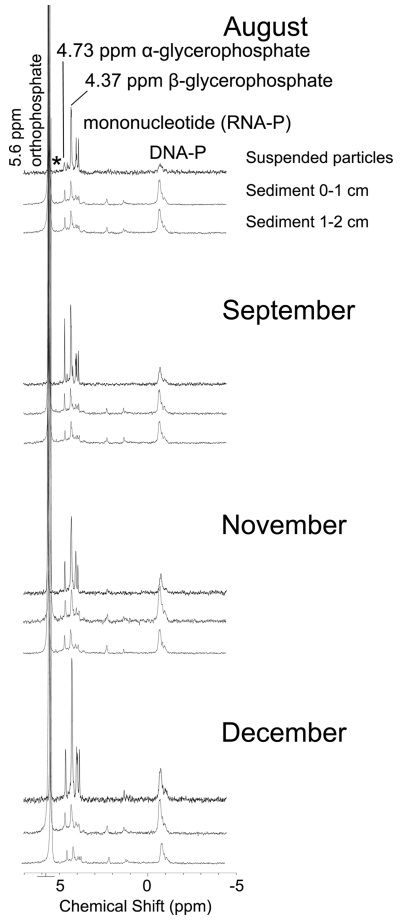


Figure 2. Comparison between chemical shift in ^{31}P NMR spectra from suspended particles and surface sediment (0−1 cm and 1−2 cm sediment depths) in August, September, November, and December of 2011 over the range that includes orthophosphate, orthophosphate monoesters, and orthophosphate diesters. The compounds associated with each peak are also shown. Asterisk (*) indicates peak location for the C2 position of *myo*-inositol hexakisphosphate.

However, this peak was too small to be included in further analysis. The greatest proportion of compounds within the orthophosphate diester range was attributable to DNA (DNA-P), which accounted for more than 80% of the orthophosphate diesters.

Comparison between Biogenic P in Suspended Particles and Sediment. There was a significant difference between the groups of P compounds in suspended particles and sediment (Table 1). The most notable difference was in orthophosphate. This difference might also reflect the composition of biogenic P groups in suspended particles and sediment.

Pyrophosphate made a greater contribution to the biogenic P in suspended particles than in sediment, as shown by the orthophosphate monoester:diester:pyrophosphate ratios (Table 2; 5:2:1 for suspended particles and 34:34:1 for sediment). There was also a substantial difference in orthophosphate monoesters and diesters between suspended particles and sediment. The orthophosphate diester:monoester ratios ranged from about 0.25 to 0.38 for suspended particles and from about 0.84 to 1.4 for sediment, a significant difference (ANOVA: $P < 0.01$). The orthophosphate diester:pyrophosphate ratio also exhibited a significant difference (ANOVA: $P < 0.01$). The ratio was much higher in sediment than in suspended particles.

These differences between the ratios in suspended particles and sediment indicate that pyrophosphate was relatively lower in sediment than in suspended particles in spite of its low concentration in surface water (approximately 0.003 mg L^{-1}). Orthophosphate monoester and diester concentrations were more than four times those of pyrophosphate in suspended particles (monoesters, 0.008−0.018 mg L^{-1}; diesters, 0.003−0.004 mg L^{-1}).

DISCUSSION

Concentrations of particulate and sediment P have been measured in many shallow eutrophic lakes, and our results for these components are similar to those previously reported for Lake Kasumigaura. By using ^{31}P NMR, our study is the first to our knowledge to determine in detail the P composition in suspended particles and sediment, and their differences. Our results provide clear evidence of the P compounds responsible for altering the respective proportions in sediment and suspended particles.

P Compounds in Suspended Particles and Sediment. We ascribe a biogenic origin to the major constituents of NaOH-EDTA-extractable P in suspended particles in Lake Kasumigaura (Table 1). The presence of the same P compounds in suspended particles and sediment might be the result of sedimentation (Figure 2, SI Figure S2), but the sediment, with only a small amount of biogenic P (about 25.9%), exhibits a composition significantly different from that of suspended particles.

Table 2. Average Proportions among the Groups of Biogenic P in NaOH-EDTA Extracts, And the Ratios between the Biogenic P Groups (Orthophosphate Monoesters, Orthophosphate Diesters, And Pyrophosphate)[a]

	mono-P	diester-P	pyro-P	poly-P	diester:monoester	diester:pyro-P	monoester:diester:pyro-P
unit	%	%	%	%			
sediment	48.2	50.4	1.4	0.0	1.05	35.9	34:34:1
suspended particles	65.0	20.4	13.8	0.71	0.31	1.48	5:2:1
ANOVA	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P > 0.05$	$P < 0.01$	$P < 0.01$	

[a]The results of one-way analysis of variance (ANOVA) between suspended particles ($n = 4$) and sediment ($n = 8$) are also shown as the probability (ANOVA P value). Mono-P, orthophosphate monoesters; diester-P, orthophosphate diesters; pyro-P, pyrophosphate; poly-P, polyphosphate. Most monoester-P forms are originated from orthophosphate diesters, phospholipids and RNA-P.

This difference in composition can also be seen in the ratios among components of biogenic P (Table 2). The substantially lower proportion of pyrophosphate in sediments suggests that pyrophosphate could be released during or after sedimentation. However, the low concentration of pyrophosphate in suspended particles (approximately 0.003 mg L^{-1} in surface water) indicates that orthophosphate monoesters and diesters might be more important to P loading (SI Figure S1). In contrast, the significantly higher orthophosphate diester:monoester ratio in sediment, an indicator of P degradation, suggests that the compounds identified as orthophosphate monoesters and orthophosphate diesters could undergo substantial transformation during or after sedimentation.

Several compounds with the phosphodiester linkage have been detected by NMR within the orthophosphate monoester range as a result of alkaline extraction.[21,22,41] We detected α-glycerophosphate and β-glycerophosphate derived from phospholipids (phosphatidylcholine),[40] as well as RNA-P derived mononucleotides, within the orthophosphate monoester range.[41] Consequently, the peaks detected within the orthophosphate monoester range, both in suspended particles and sediment, mostly indicate the presence of orthophosphate diesters, particularly phospholipids, with a small amount of an important organic P compound, *myo*-inositol hexakisphosphate, evident as a peak at 5.35−5.40 ppm (5.85 ppm in Turner et al. (2003) [27]) that is indicative of the C2 position on the inositol ring (Figure 2).

Information on the P groups and the different orthophosphate diester-to-monoester ratios provides strong evidence for transformation during or after sedimentation. Specifically, higher orthophosphate diester:monoester ratios in sediment indicate that phospholipids detected within the orthophosphate monoester range could be degraded, whereas the compound in the orthophosphate diester range, DNA-P, could be produced during or after the sedimentation.

The degradation of phospholipids is a reasonable explanation for the shifting ratios, because phospholipids might be easy to degrade.[42] Our measurements suggest that this degradation happens within suspended particles and sediment in a shallow, freshwater lake. Alternatively, an increase in DNA-P during sedimentation is also consistent with a previous report by Reitzel et al. (2012);[43] they measured P compounds in settling matter using ^{31}P NMR and concluded that bacterial activity increases orthophosphate diester concentrations. Similarly, bacteria contain large amounts of orthophosphate diesters,[44] which might alter the composition of P groups in sediment. We cannot quantitatively evaluate these explanations because of the absence of settling flux data in the current study, yet our conclusion is certainly supported by multiple lines of evidence that orthophosphate diesters, particularly phospholipids, RNA-P, and DNA-P, are important for the P cycle in Lake Kasumigaura.

Importance of Orthophosphate Diester. For internal P loading, the release of sediment P has commonly been acknowledged as an important source.[45,46] The biogenic P in sediment is derived from that in suspended particles; we herein report a significant difference in the relative abundance of several groups of organic P compounds in suspended particles and sediment. In particular, orthophosphate diesters are responsible for the difference, which was observed consistently in four months both in summer and winter.

Overall, we derived a conceptual model to explain the biogenic P cycle in Lake Kasumigaura described above (Figure 3). The most notable finding is the different composition of orthophosphate diester and monoester groups, as evidenced by their respective ratios in suspended particles and sediment. Microbial activity might be responsible for this difference, as this change of ratios is consistent with an increase in orthophosphate diesters as a result of microbial activity.[43] In

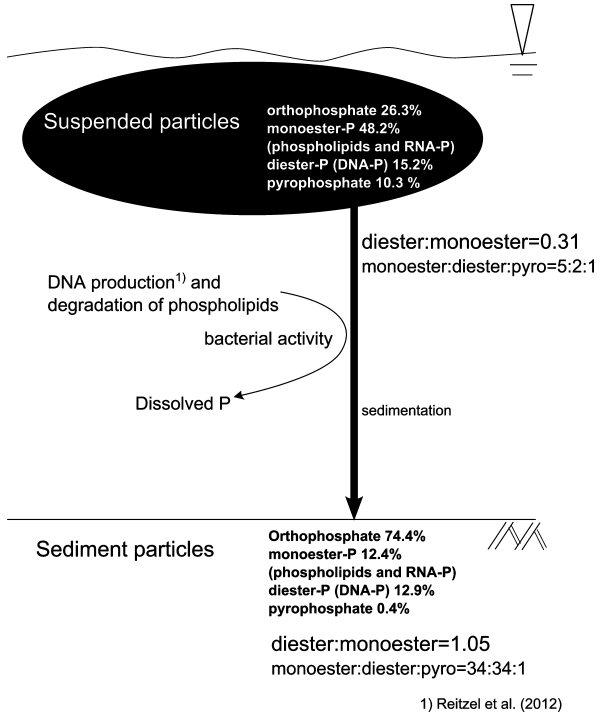


Figure 3. Conceptual model of P groups in suspended particles and sediment. Percentages indicate the proportions of NaOH-EDTA extractable P in suspended particles and sediment. Most orthophosphate monoesters are originated from orthophosphate diesters, phospholipids and RNA-P.

this study, we did not examine the transformation as it happened, although we found definite differences in the relative abundance of several groups of biogenic P in sediment and suspended particles. More information about other reactions involving P, including those linking particulate and dissolved P via bacteria, is needed to clarify organic P dynamics in lakes.

Because orthophosphate diesters are labile compounds,[21] our results show that the organic P in both suspended particles and sediment is quite labile. Reitzel et al. (2007)[24] reported that depth attenuation rate of orthophosphate diesters is faster than that of orthophosphate monoesters. If this attenuation through decomposition also happens in Lake Kasumigaura, then the production of an orthophosphate diester, DNA-P, during or after sedimentation, is responsible for the release of sediment P to sediment pore water through microbial activity. On the other hand, the release of Fe-bound P is also important to dissolved orthophosphate in Lake Kasumigaura,[6] and could explain the decrease in sediment orthophosphate concentration from August to September (SI, Figure S1). Because orthophosphate predominates in surface sediment, the transformation from biogenic P to orthophosphate in sediment needs further investigation.

Sampling strategies such as the use of sediment traps[47] will help clarify the transformation of P during sedimentation[48,49] and the resuspension rate.[50,51] We noticed little change in sediment P during the observation period regardless of the change in particulate P concentration (SI Figure S1). Sediment P could be affected by multiple factors, such as the settling P flux, the flux of resuspended P, and degradation of P in particles during settling. To better understand the P cycle in Lake Kasumigaura, future studies should include quantitative evaluations of potential mechanisms that could change the composition of P during the transition from suspended particles to sediment. These studies should include assessment of bioavailability by using the activities of enzymes such as phosphodiesterase that degrade orthophosphate diesters.[52,53]

■ ASSOCIATED CONTENT

Supporting Information

Figure S1 shows the concentrations of P groups in suspended particles and sediment. Figure S2 shows detailed ^{31}P NMR spectra in the orthophosphate monoester range. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +81-29-850-2785; e-mail: r-shino@nies.go.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was financially supported by a "Grant-in-Aid for Research Activity Start-up" from the Japan Society for the Promotion of Science (No. 23860066). The sampling was financially supported by the GEMS/Water Trend Monitoring Project at Lake Kasumigaura. We thank the members of our laboratory for their assistance. We also thank Mrs. Mariko Watanabe for her analytical support in using nuclear magnetic resonance spectroscopy. We thank anonymous referees for their valuable comments.

■ REFERENCES

(1) Graneli, W. Internal phosphorus loading in Lake Ringsjon. *Hydrobiologia* **1999**, *404*, 19−26.
(2) Boström, B.; Andersen, J. M.; Fleischer, S.; Jansson, M. Exchange of phosphorus across the sediment-water interface. *Hydrobiologia* **1988**, *170*, 229−244.
(3) Shinohara, R.; Isobe, M. Effects of temperature-induced sea breezes on phosphorus dynamics in a shallow eutrophic lake. *Mar. Freshwater Res.* **2012**, *63*, 119−127.
(4) Feuillade, M.; Dorioz, J. M. Enzymatic release of phosphate in sediments of various origins. *Water Res.* **1992**, *26*, 1195−1201.
(5) Reynolds, C.; Davies, P. Sources and bioavailability of phosphorus fractions in freshwaters: A British perspective. *Biol. Rev.* **2001**, *76*, 27−64.
(6) Ishii, Y.; Harigae, S.; Tanimoto, S.; Yabe, T.; Yoshida, T.; Taki, K.; Komatsu, N.; Watanabe, K.; Negishi, M.; Tatsumoto, H. Spatial variation of phosphorus fractions in bottom sediments and the potential contributions to eutrophication in shallow lakes. *Limnology* **2010**, *11*, 5−16.
(7) Mortimer, C. The exchange of dissolved substances between water and mud in lakes. *J. Ecol.* **1941**, *29*, 280−329.
(8) Rydin, E. Potentially mobile phosphorus in Lake Erken sediment. *Water Res.* **2000**, *34*, 2037−2042.
(9) Hupfer, M.; Gloess, S.; Grossart, H. P. Polyphosphate-accumulating microorganisms in aquatic sediments. *Aquat. Microb. Ecol.* **2007**, *47*, 299−311.
(10) Hupfer, M.; Rube, B.; Schmieder, P. Origin and diagenesis of polyphosphate in lake sediments: A ^{31}P-NMR study. *Limnol. Oceanogr.* **2004**, *49*, 1−10.
(11) Golterman, H.; Paing, J.; Serrano, L.; Gomez, E. Presence of and phosphate release from polyphosphates or phytate phosphate in lake sediments. *Hydrobiologia* **1997**, *364*, 99−104.
(12) Tornblom, E.; Rydin, E. Bacterial and phosphorus dynamics in profundal Lake Erken sediments following the deposition of diatoms: A laboratory study. *Hydrobiologia* **1997**, *364*, 55−63.
(13) Goedkoop, W.; Pettersson, K. Seasonal changes in sediment phosphorus forms in relation to sedimentation and benthic bacterial biomass in Lake Erken. *Hydrobiologia* **2000**, *431*, 41−50.
(14) Hupfer, M.; Lewandowski, J. Oxygen controls the phosphorus release from Lake Sediments-a long lasting paradigm in Limnology. *Int. Rev. Hydrobiol.* **2008**, *93*, 415−432.
(15) Cade-Menun, B. J. Characterizing phosphorus in environmental and agricultural samples by ^{31}P nuclear magnetic resonance spectroscopy. *Talanta* **2005**, *66*, 359−371.
(16) Cade-Menun, B. J.; Navaratnam, J. A.; Walbridge, M. R. Characterizing dissolved and particulate phosphorus in water with ^{31}P nuclear magnetic resonance spectroscopy. *Environ. Sci. Technol.* **2006**, *40*, 7874−7880.
(17) Wang, J.; Pant, H. K. Identification of organic phosphorus compounds in the Bronx River bed sediments by phosphorus-31 nuclear magnetic resonance spectroscopy. *Environ. Monit. Assess.* **2010**, *171*, 309−319.
(18) Reitzel, K.; Ahlgren, J.; Gogoll, A.; Jensen, H. S.; Rydin, E. Characterization of phosphorus in sequential extracts from lake sediments using ^{31}P nuclear magnetic resonance spectroscopy. *Can. J. Fish. Aquat. Sci.* **2006**, *63*, 1686−1699.
(19) Turner, B. L.; Weckström, K. Phytate as a novel phosphorus-specific paleo-indicator in aquatic sediments. *J. Paleolimn.* **2009**, *42*, 391−400.
(20) Suzumura, M.; Kamatani, A. Mineralization of inositol hexaphosphate in aerobic and anaerobic marine sediments: Implications for the phosphorus cycle. *Geochim. Cosmochim. Acta* **1995**, *59*, 1021−1026.
(21) Makarov, M.; Haumaier, L.; Zech, W. Nature of soil organic phosphorus: An assessment of peak assignments in the diester region of ^{31}P NMR spectra. *Soil Biol. Biochem.* **2002**, *34*, 1467−1477.
(22) Paytan, A.; Cade-Menun, B. J.; McLaughlin, K.; Faul, K. L. Selective phosphorus regeneration of sinking marine particles: Evidence from ^{31}P-NMR. *Mar. Chem.* **2003**, *82*, 55−70.

(23) Ahlgren, J.; Reitzel, K.; Tranvik, L.; Gogoll, A.; Rydin, E. Degradation of organic phosphorus compounds in anoxic Baltic Sea sediments: A ^{31}P nuclear magnetic resonance study. *Limnol. Oceanogr.* **2006**, *51*, 2341−2348.
(24) Reitzel, K.; Ahlgren, J.; DeBrabandere, H.; Waldeback, M.; Gogoll, A.; Tranvik, L.; Rydin, E. Degradation rates of organic phosphorus in lake sediment. *Biogeochemistry* **2007**, *82*, 15−28.
(25) Sondergaard, M.; Jensen, J.; Jeppesen, E. Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* **2003**, *506*, 135−145.
(26) Cade-Menun, B.; Preston, C. A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. *Soil Sci.* **1996**, *161*, 770−785.
(27) Turner, B. L.; Mahieu, N.; Condron, L. M. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. *Soil Sci. Soc. Am. J.* **2003**, *67*, 497−510.
(28) Zhang, R.; Wu, F.; He, Z.; Zheng, J.; Song, B.; Jin, L. Phosphorus composition in sediments from seven different trophic lakes, China: A phosphorus-31 NMR study. *J. Environ. Qual.* **2009**, *38*, 353−359.
(29) Ahlgren, J.; Reitzel, K.; Danielsson, R.; Gogoll, A.; Rydin, E. Biogenic phosphorus in oligotrophic mountain lake sediments: Differences in composition measured with NMR spectroscopy. *Water Res.* **2006**, *40*, 3705−3712.
(30) Turner, B. L. Soil organic phosphorus in tropical forests: An assessment of the NaOH−EDTA extraction procedure for quantitative analysis by solution ^{31}P NMR spectroscopy. *Eur. J. Soil Sci.* **2008**, *59*, 453−466.
(31) Turner, B. L.; Engelbrecht, B. M. J. Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry* **2011**, *103*, 297−315.
(32) Turner, B. L.; Mahieu, N.; Condron, L. M. The phosphorus composition of temperate pasture soils determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy. *Org. Geochem.* **2003**, *34*, 1199−1210.
(33) APHA, Standard methods for the examination of water and wastewater; American Public Health Association: Washington, DC, 1995.
(34) Murphy, J.; Riley, J. P. A modified single solution method for determination of phosphate in natural waters. *Anal. Chim. Acta* **1962**, *26*, 31−36.
(35) Smernik, R. J.; Dougherty, W. J. Identification of phytate in phosphorus-31 nuclear magnetic resonance spectra: The need for spiking. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1045−1050.
(36) McDowell, R.; Cade-Menun, B.; Stewart, I. Organic phosphorus speciation and pedogenesis: Analysis by solution 31P nuclear magnetic resonance spectroscopy. *Eur. J. Soil Sci.* **2007**, *58*, 1348−1357.
(37) He, Z.; Olk, D. C.; Cade-Menun, B. J. Forms and lability of phosphorus in humic acid fractions of hord silt loam soil. *Soil Sci. Soc. Am. J.* **2011**, *75*, 1712−1722.
(38) Aspila, K.; Agemian, H.; Chau, A. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *The Analyst* **1976**, *101*, 187−197.
(39) Pacini, N.; Gachter, R. Speciation of riverine particulate phosphorus during rain events. *Biogeochemistry* **1999**, *47*, 87−109.
(40) Doolette, A. L.; Smernik, R. J.; Dougherty, W. J. Spiking improved solution phosphorus-31 nuclear magnetic resonance identification of soil phosphorus compounds. *Soil Sci. Soc. Am. J.* **2009**, *73*, 919−927.
(41) Turner, B. L.; Newman, S. Phosphorus cycling in wetland soils: The importance of phosphate diesters. *J. Environ. Qual* **2005**, *34*, 1921−1929.
(42) Turner, B. L.; Frossard, E.; Baldwin, D. S. *Organic Phosphorus in the Environment*; CABI Publishing: New South Wales, Australia, 2005.
(43) Reitzel, K.; Ahlgren, J.; Rydin, E.; Egemose, S.; Turner, B. L.; Hupfer, M. Diagenesis of settling seston: Identity and transformations of organic phosphorus. *J. Environ. Monit.* **2012**, *14*, 1098−1106.
(44) Reddy, K. R.; DeLaune, R. D. *Biogeochemistry of Wetlands: Science and Applications*; CRC Press: Boca Raton, FL, 2008.
(45) Kamiya, H.; Ohshiro, H.; Tabayashi, Y.; Kano, Y.; Mishima, K.; Godo, T.; Yamamuro, M.; Mitamura, O.; Ishitobi, Y. Phosphorus release and sedimentation in three contiguous shallow brackish lakes, as estimated from changes in phosphorus stock and loading from catchment. *Landscape Ecol. Eng.* **2011**, *7*, 53−64.
(46) Sondergaard, M.; Jensen, J. P.; Jeppesen, E. Internal phosphorus loading in shallow Danish lakes. *Hydrobiologia* **1999**, *409*, 145−152.
(47) Bloesch, J. Mechanisms, measurement and importance of sediment resuspension in lakes. *Mar. Freshwater Res.* **1995**, *46*, 295−304.
(48) Pettersson, K. Phosphorus characteristics of settling and suspended particles in Lake Erken. *Sci. Total Environ.* **2001**, *266*, 79−86.
(49) Hupfer, M.; Gachter, R.; Giovanoli, R. Transformation of phosphorus species in settling seston and during early sediment diagenesis. *Aquat. Sci* **1995**, *57*, 305−324.
(50) Niemisto, J.; Holmroos, H.; Pekcan-Hekim, Z.; Horppila, J. Interactions between sediment resuspension and sediment quality decrease the TN: TP ratio in a shallow lake. *Limnol. Oceanogr.* **2008**, *53*, 2407−2415.
(51) de Vicente, I.; Cruz-Pizarro, L.; Rueda, F. Sediment resuspension in two adjacent shallow coastal lakes: Controlling factors and consequences on phosphate dynamics. *Aquat. Sci. -Research Across Boundaries* **2010**, *72*, 21−31.
(52) Turner, B. L.; Cade-Menun, B. J.; Westermann, D. T. Organic phosphorus composition and potential bioavailability in semi-arid arable soils of the western United States. *Soil Sci. Soc. Am. J.* **2003**, *67*, 1168−1179.
(53) Wang, J.; Pant, H. K. Enzymatic hydrolysis of organic phosphorus in river bed sediments. *Ecol. Eng.* **2010**, *36*, 963−968.