Environ Sci Pollut Res (2016) 23:7288–7297
DOI 10.1007/s11356-015-5913-5

RESEARCH ARTICLE

Characterization of phosphorus forms in lake macrophytes and algae by solution ^{31}P nuclear magnetic resonance spectroscopy

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Received: 23 July 2015 / Accepted: 2 December 2015 / Published online: 17 December 2015

Abstract Debris from aquatic macrophytes and algae are important recycling sources of phosphorus (P), which can result in continuing blooms of algae by recycling bioavailable P in the eutrophic lakes. However, knowledge of forms of P in aquatic macrophytes and algae and their contribution to internal loads of P in lakes is limited. Without such knowledge, it is difficult to develop appropriate strategies to remediate and or restore aquatic ecosystems that have become eutrophic. Therefore, in this work, P was extracted from six types of aquatic macrophytes and algae collected from Tai Lake of China and characterized by use of solution ^{31}P-nuclear magnetic resonance (NMR) spectroscopy. When extracted by 0.5 M NaOH-25 mM EDTA, extraction recovery of total P(TP) and organic P(P_o) exceeded 90 %. Concentrations of P_o in algae and aquatic macrophytes were 5552 mg kg^{-1} and 1005 mg kg^{-1} and accounted for 56.0 and 47.2 % of TP, respectively. When P_o, including condensed P, was characterized by solution ^{31}P-NMR P_o in algae included orthophosphate monoesters (79.8 %), pyrophosphate (18.2 %), and orthophosphate diester (2.0 %), and P_o in aquatic macrophytes included orthophosphate monoesters (90.3 %), pyrophosphate (4.2 %), and orthophosphate diester (5.5 %). Additionally, orthophosphate monoesters in algal debris mainly included β-glycerophosphate (44.1 %), α-glycerophosphate (13.5 %), and glucose 6-phosphate (13.5 %). Orthophosphate monoesters in aquatic macrophytes mainly included β-glycerophosphate (27.9 %), α-glycerophosphate (24.6 %), and adenosine 5′ monophosphate (8.2 %). Results derived from this study will be useful in better understanding nutrient cycling, relevant eutrophication processes, and pollution control for freshwater lakes.

Keywords Aquatic macrophytes · Algae · Organic phosphorus · ^{31}P-NMR · Lake · Orthophosphate monoesters

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Introduction

Eutrophication of lakes is a threat to water quality and ecosystem heath worldwide. In China, approximately 53 % of lakes suffer from eutrophication or hyper-eutrophication (Zhou et al. 2001; Tao et al. 2013; Zhu et al. 2013b; Zhu et al. 2015). The phenomenon of nutrient over enrichment has also caused problems in Europe and North America leading to dramatic deterioration of lake water quality and unfavorable secondary internal nutrient enrichment (Smith et al. 1999; Søndergaard et al. 2003).

For most lakes, phosphorus (P) is the limiting factor that contributes to eutrophication (Baldwin 2013; Giles et al. 2015). Though external loading of nutrients from agricultural and urban sources contributes to eutrophication, release of nutrients from internal loading to surface waters has also been shown to be an important factor maintaining eutrophication of lakes. Sediments, debris derived from aquatic macrophytes and algae, could be sources for internal recycling of P. Recently, forms and bioavailability of P in sediments has been widely reported; however, forms and bioavailability of P in aquatic macrophytes and algae have rarely been reported. Inorganic P (P_i) is generally considered to be the most mobile and bioavailable form of P in lakes, but P_o is also an important constituent of P, and can account for 10 to 70 % of total P(TP) in sediments of lakes. Through a series of redox-driven solubilization reactions and enzyme-mediated hydrolytic processes, P_o can be degraded and release phosphate that is available to phytoplankton (Turner et al. 2006; Monbet et al. 2009; Da-Peng and Yong 2010; Wang and Pant 2010). Enzymatic hydrolysis can be mediated by extracellular microbial and plant phosphatases (Cembella et al. 1982). Forms of P_o can account for a substantial proportion of extracellular P found in aquatic environments (Bai et al. 2009; Paerl et al. 2011), but understanding of the role of P_o in biogeochemical and ecological processes is less advanced than that of the role of forms of P_i in lakes. One of the main causes for this is the limited availability of suitable techniques to easily and routinely quantify and characterize P_o in aquatic macrophytes and algae.

Recently, solution ^{31}P-nuclear magnetic resonance (NMR) spectroscopy has been used to characterize species of P_o, such as soils, sediments, and manures (Turner and Richardson 2004; Cade-Menun 2005; He et al. 2009; Zhu et al. 2015). The ^{31}P-NMR spectrum provides information on relative abundances of specific P compounds. For example, orthophosphate, orthophosphate monoesters, orthophosphate diester, pyrophosphates in aqueous, and alkaline extracts of sediments, manures, or soils have been identified and quantified by this technique (Newman and Tate 1980; Cade-Menun et al. 2002; He et al. 2009). However, the composition, sources, bioavailability, and biogeochemical cycling of P_o derived from debris of aquatic macrophytes and algae in lakes are not well understood. Therefore, in this work, we investigated the characteristics of P_o derived from debris of aquatic macrophytes and algae collected from a eutrophic lake, Tai Lake (Ch: *Taihu*), by solution ^{31}P-NMR analysis.

Materials and methods

Aquatic macrophytes and algae material collection

Tai Lake, located in the Yangtze River delta, Jiangsu Province (30°55′ 40.58″~31°32′ N, 119°52′32″~120°36′ 10″ E), is the third largest freshwater lake in China. It is a typical large, shallow, eutrophic lake. The surface area is 2250 km^2 with a mean water depth of 1.9 m and maximum depth of 2.6 m (Bai et al. 2009). Due to the rapidly increase in the intensity of human activities, algal blooms have occurred each year since the 1990s. In last two decades or so, annual mean total nitrogen (TN) and TP concentrations have approached 3.0 and 0.1 mg·L^{-1}, respectively. The greatest mean annual concentration of TN was 5.34 mg L^{-1} in 2004, while greatest mean annual concentration of TP was 0.21 mg L^{-1} and occurred in 2000 (Ye et al. 2011). In May 2007, a huge algal bloom broke out and significantly impacted the supply of fresh water to about 6.1 million people in Wuxi City (Ye et al. 2011). Large amounts of toxins were formed by cyanobacteria which damaged animals and plants during 2009–2010 (Otten et al. 2012).

Submerged and emerged plants, cyanobacteria, and green algae are all present in large proportions in Tai Lake. Thus, six representative aquatic plants (two submerged plants and one emergent plant) and algae (two cyanobacteria and one green alga) were selected to be investigated. Aquatic macrophytes, including the foxtail (*Myriophyllum spicatum Linn.*), common reed (*Phragmites australis trin.gramieae*), and black plant (*Hydrilla verticillata*), were collected in the region of Tai Lake during late October 2010. Algae samples were the toxin-producing species (*Microcystis*), ordinary chlorella (*Chiorella vulgaris*), dunding spirulina (*Spirulina platensis*) provided by Institute of Hydrobiology, Chinese Academy of Sciences. Whole aquatic macrophytes were took back to laboratory as soon as possible quickly and stored at 4 °C, then they were completely dried at 60 °C, ground, and sieved through a 2-mm screen (He et al. 2011; Qu et al. 2013). The resulting powders were stored at −20 °C until use.

Chemical composition of phosphorus in macrophyte and algae

TP, P_i, and P_o in aquatic macrophytes and algae samples were determined by use of previously published methods (Ruban et al. 1999; Zhu et al. 2015). Briefly, 0.2 g of powdered aquatic macrophytes and algae (each of three parallel), were calcined for 3 h at 450 °C in a muffle furnace. The resulting powders were cooled to room temperature and then transferred to centrifuge tubes. Then, 20 mL of 3. 5 M HCl was added, and these samples were shaking at 22 °C for 16 h. After centrifugation, concentrations of TP in the supernatant were measured by the methods in literature (Murphy and Riley 1962). To determine concentrations of P_i and P_o, 0.2 g of powdered samples were extracted with 20 mL 1 M HCl, for 16 h on an end-over-end shaker, then centrifuged for 20 min. Concentration of P_i was determined, and then P_o were calculated as the difference between concentrations of TP and P_i. Percentages of carbon (C), nitrogen (N), sulfur (S), and hydrogen (H) were determined by use of an elemental analyzer (Elementar vario macro EL, Germany).

Phosphorus extraction and determination

Aquatic macrophytes (0.5 g) or algae (0.2 g) samples were extracted with 30 mL of 0.5 M NaOH and 25 mM Na_2EDTA at 22 °C for 16 h on an end-over-end shaker. After extraction, samples were centrifuged at 10000 × *g* for 30 min. Supernatants were filtered through 0.45-μm membrane filters, then concentrations of TP and P_i in extracts were quantified. TP in extracts was determined after digestion with potassium persulfate ($K_2S_2O_8$) in an autoclave at 121 °C for 30 min. Concentrations of P_o in extracts were then calculated as the difference between concentrations of TP and P_i in extracts. Phosphate was analyzed by the molybdenum blue/ascorbic acid method as describer above. The remainder of the samples was freeze-dried and kept in a freezer at −20 °C until redissolution.

Solution ^{31}P-NMR analysis

Freeze-dried NaOH-EDTA extracts were dissolved in 1 mL 1 M NaOH-0.1 M EDTA, adding 0.1 mL D_2O, and allowed to stand for 30 min at room temperature. Samples were then centrifuged for 30 min at 10000 × *g*, transferred to NMR tubes, and stored at 4 °C before analysis within 24 h. Solution ^{31}P-NMR spectra were acquired at 161.98 MHz by use of a Bruker AVANCE 400 MHz spectrometer equipped with a 5-mm broadband probe, using a 90° pulse, 0.21 s acquisition, 4.32 s pulse delay, and the number of points was 8192. For each sample, the total NMR experiment lasted about 15 h, collecting 24000 scans.

Peaks were identified by spiking experiments and previously published spectra (He et al. 2011; Cade-Menun 2015). Lyophilized NaOH-EDTA blanks spiked with orthophosphate, phytic acid dipotassium salt, DL-α-glycerol phosphorus, β-glycerophosphate, adenosine 5′ monophosphate, D-glucose 6-phosphate, and α-D-glucose 1-phosphate were all purchased from Sigma-Aldrich. Spiked samples were analyzed by ^{31}P-NMR as described above. P compounds were identified by their chemical shifts, with the orthophosphate peak in all spectra standardized to 6 ppm. Peak areas were calculated by integration on spectra processed with 5-Hz line broadening, using MestReNove software 9.0.

Results and discussion

Properties of debris derived from aquatic macrophytes and algae

Mean percentages of C, N, S, H, and P in aquatic macrophytes from Tai Lake were 40.0, 3.3, 0.4, 3.3, and 0.2 %, respectively, while percentages in algae were 45.1, 9.3, 0.5, 9.3, and 1.1 %, respectively (Table 1). Nutrients containing C, N, and P in these debris were important for recycling and population expansion of aquatic macrophytes and algae (Lorenz et al. 2004; Zhong et al. 2012). In Tai Lake, contents of C of aquatic macrophytes were greater than those of algae. In another lake in China, Lake Dianchi, contents of C (40.3 %) in aquatic macrophytes were also less than those in algae (Qu et al. 2013). The percent C in algae could be approached to 59.0 % in Tai Lake, which was greater than that in aquatic macrophytes (Zhong et al. 2012). Contents of C in aquatic macrophytes and algae were greater than those of sediments. For example, mean content of C was only 5.7 % in sediments from Lake Dianchi (Qu et al. 2013), which is significantly less than that in aquatic macrophytes. Contents of N and P in algae were greater (about 5.0 and 0.8 %, respectively) than those in aquatic macrophytes from Tai Lake. Contents of N in aquatic macrophytes from Tai Lake were similar to those from Lake Dianchi (Qu et al. 2013). Mean concentrations of N and P were lower in sediment (Zhang et al. 2014), thus the general trend of C, N, and P levels in Tai Lake were algae > aquatic macrophytes > sediment > water. C, N, and P at the observed levels would provide sufficient nutrients to serve as the basis for blooms of algae so that aquatic macrophytes and algae, especially algae, were likely to contribute to endogenous nutrients and support eutrophication of lakes even if external sources are controlled. Thus, without intervention to control internal cycling of nutrients, the eutrophic conditions in the lake would last longer than predicted from on allochthonous loadings.

Mean mass ratios of C/N, C/P, and N/P in aquatic macrophytes were 12.9, 205.8, and 15.0 for Tai Lake, respectively, while those in algae were 4.9, 15.3, and 9.2, respectively. Redfield ratios for C/N, C/P, and N/P of single-celled algae were 6.6, 106.0, and 16.0, respectively (Redfield et al. 1963). When 92 kinds of marine plants from different habitats were studied, the C/N ratio was approximately 18.3, while C/P was approximately 550, and N/P was approximately 30 (Atkinson and Smith 1983). The C/N ratios for terrestrial plants were generally greater than 20 (Meyers and Ishiwatari 1993). In this study, C/N and C/P of aquatic macrophytes from Tai Lake were between those of single-celled algae and those of marine benthonic plants. Ratios of C/N, C/P, and N/P in the algae studied here were less than those of aquatic macrophytes from Tai Lake, single-celled algae, and marine plants. Ratios of C/N or C/P could be used as preliminary estimates to determine sources or rates of degradation of organic matter in lakes. In general, lower values of C/N, C/P resulted from the endogenous sources with greater amounts of protein, indicating these sources are as a reduced carbon source by microorganisms. Both values of C/N, C/P in algae were lower than those in aquatic macrophytes from Tai Lake, indicating that the rate of degradation for algae was generally greater than that of aquatic macrophytes.

Table 1 Selected elements in aquatic macrophytes, algae, and relevant data in literature

Code	Sample	C%	N%	S%	H%	P%	C/N[d]	C/P[d]	N/P[d]
A_1	Foxtail algae	34.7	2.7	0.4	3.8	0.2	12.9	157.8	12.2
A_2	Common reed	44.2	2.6	0.6	5.2	0.1	17.1	347.3	19.9
A_3	*Hydrilla verticillata*	41.3	4.7	0.3	2.3	0.4	8.7	112.3	12.8
B_1	*Microcystis*	44.5	8.8	0.5	2.3	0.8	5.0	58.5	11.6
B_2	*Chiorella vulgaris*	51.1	9.2	0.6	3.9	11	5.5	46.8	8.5
B_3	*Spirulina platensis*	39.6	9.8	0.5	3.2	1.3	4.0	30.7	7.6
	Plants in Dianchi[a]	40.3	3.4	0.8	5.5	0.8	14.1	140.6	4.1
	Water in Taihu[b]	–	1.8×10^{-4}	–	–	7.3×10^{-6}	–	–	25.1
	Sediments in Dianchi[c]	5.7	0.8	–	–	0.2	7.6	28.7	3.8

[a] From the literature (Qu et al. 2013), the average of aquatic plants (sago pondweed, water lettuce, water hyacinth, parrot feather, water oats, common reed, oriental pepper) in lake Dianchi

[b] From the literature (Ye et al. 2011), long-term data of annual mean of N% and P% from 1980 to 2008 in Tai Lake

[c] From the literature (Zhu et al. 2013a), the average of 18 sediments in lake Dianchi

[d] C/N, C/P, N/P refers to the mass ratio

NaOH-EDTA extractable P from aquatic macrophytes and algae

In this study, for aquatic macrophytes 0.1, 0.25, 0.5 M NaOH and 10, 25, 50 mM EDTA were found to give recoveries of TP between 66.8 and 88.6 % and recoveries of P_o were between 47.8 and 84.7 % (Table 2). In contrast, mean recovery of TP and P_o with 0.5 M NaOH+25 mM EDTA was 91.4 and 88.1 %, respectively. Therefore, 0.5 M NaOH-25 mM EDTA would be the optimal extraction of P_o from aquatic macrophytes and algae, which was applied to P extraction here. After confirming the optimal extraction, macrophytes and algae were extracted by use of the optimal extraction. Recoveries of TP from aquatic macrophytes and algae were between 82.9 and 99.3 %, with a mean of 93.2 %, while recoveries of P_o form aquatic macrophytes and algae were between 80.1 and 96.2 %, with a mean recovery of 91.5 % with 0.5 M NaOH+25 mM EDTA (Table 3).

A range of extractants have been used to extract P_o from environmental samples, such as soils and sediments. These extractants have included NaAs-SD, H_2O, weak acid, $NaHCO_3$, H_2SO_4, NaOH, and NaOH-EDTA. Recoveries varied among these extractants (Makarov et al. 2002; He et al. 2009; Noack et al. 2014; Read et al. 2014; Turner et al. 2012; Zhang et al. 2014). In general, recovery was approximately 5~8 % with 50 mM H_2SO_4 and 500 mM $NaHCO_3$ as the extraction agent in fresh microbial cells (Makarov et al. 2002). Recoveries P_o from crops residues were between 56 and 63 % by water and between 61 and 64 % by weak acid (Noack et al. 2014). EDTA eliminates interferences of ions due to its strongly binding ability, thus improving the extraction efficiency and resolution of ^{31}P-NMR spectra. Therefore, NaOH-EDTA has been widely applied to extract P from environmental samples (Turner et al. 2003). Also concentrations of NaOH and EDTA in these mixtures are varied slightly among studies depending upon their objectives. Recoveries ranged from 57 to 90 % with 0.25 MNaOH-50 mM EDTA extraction of soil (Makarov et al. 2002) and sediment (Zhu et al. 2013a; Zhang et al. 2014). In this study, the optimal extraction of P_o from aquatic macrophytes and algae was achieved by use of 0.5 M NaOH-25 mM EDTA (Table 2).

Characterization of P compounds in aquatic macrophytes and algae

Solution ^{31}P-NMR spectra of all the aquatic macrophytes and algae samples are shown in Fig. 1, while right of each spectrum expanded to provide more detail of the orthophosphate monoesters. The results showed that aquatic macrophytes and algae contained orthophosphate (6.00 ppm), orthophosphate monoesters (3.89 to 5.76 ppm), orthophosphate diester (0.31 to 1.37 ppm), and pyrophosphate (4.43 to 4.51 ppm) (Fig. 1). There was no phosphonate or polyphosphate detected in these samples. It is possible that the contents of phosphonate and polyphosphate were less than the limit of quantification. It is also possible that some polyphosphate could have been hydrolyzed during alkaline extraction and then converted to pyrophosphate (He et al. 2011).

Integration of ^{31}P-NMR spectra peaks of algae showed that P_o including condensed P of algae was that orthophosphate monoesters (79.8 %, 4885 mg kg^{-1}) > pyrophosphate (18.2 %, 1114 mg kg^{-1}) > orthophosphate diester (2.0 %, 126 mg kg^{-1}). However, P_o and condensed P from aquatic macrophytes were that orthophosphate monoesters (90.3 %, 819 mg kg^{-1}) > orthophosphate diester (5.5 %, 50 mg kg^{-1}) > pyrophosphate (4.2 %, 38 mg kg^{-1})

Table 2 Recovery of P in aquatic macrophytes by extraction with different combinations of NaOH and EDTA

Sample	TP (%)	P_o (%)	TP (%)	P_o (%)	TP (%)	P_o (%)
	(1)0.1MNaOH+10mMEDTA		(2)0.1MNaOH+25mMEDTA		(3)0.1MNaOH+50mMEDTA	
Foxtail algae	73.3	56.9	70.0	52.8	42.0	41.7
Reed	101.0	100.1	94.9	81.5	84.3	51.4
Hydrilla verticillata	61.0	57.0	69.8	66.4	69.9	50.2
Average	78.1	71.3	78.2	66.9	75.4	47.8
	(4)0.25MNaOH+10mMEDTAA		(5)0.25MNaOH+25mMEDTA		(6)0.25MNaOH+50mMEDTA	
Foxtail algae	67.1	63.7	69.4	61.5	70.8	77.3
Reed	70.9	52.3	77.9	62.5	82.4	89.4
Hydrilla verticillata	62.3	78.2	78.0	75.2	73.0	70.8
Average	66.8	64.7	75.1	66.4	75.4	79.2
	(7)0.5MNaOH+10mMEDTA		(8)0.5MNaOH+25mMEDTA		(9)0.5MNaOH+50mMEDTA	
Foxtail algae	92.5	79.5	98.2	91.4	77.2	90.1
Reed	102.0	101.0	93.2	92.9	85.3	79.8
Hydrilla verticillata	73.4	66.4	82.9	80.1	85.4	84.2
Average	88.6	82.0	91.4	88.1	82.7	84.7

(Table 4). The composition of P_o and condensed P in the debris from aquatic macrophytes and algae was different, though orthophosphate monoesters were the major component of P_o from both. For sediments, contents of orthophosphate monoesters were only between 7.9 and 78.3 mg kg^{-1} and accounted for 3.7 to 18.9 % of total NaOH-EDTA extractable P determined by ^{31}P-NMR in Tai Lake (Zhu et al. 2015). Orthophosphate monoesters in sediments were mainly inositol phosphates, mononucleotides, sugar phosphates, and decomposition products of RNA. Their biological availability is less than that of orthophosphate diester. In general, orthophosphate monoesters can be quickly turned into phosphate. The proportion of orthophosphate diester in extracts was less than 3.2 % of TP in both aquatic macrophytes and algae. This result was similar to that in sediments, where concentrations of orthophosphate diester were between 5.2 and 22.9 mg kg^{-1} and accounted for between 0.9 and 5.5 % of TP extracted from sediments of Tai Lake (Zhu et al. 2015). Although the proportion was small, its stability was less than that of orthophosphate monoesters and can be decomposed and mineralized easily by bacteria and other microorganisms (He et al. 2011). The chemical shift of orthophosphate diesters in ^{31}P-NMR spectra from aquatic macrophytes and algae was about −0.35 ppm, which indicates that the orthophosphate diester here was mainly DNA. There were also few other orthophosphate diesters, such as phospholipids, in the spectra from algae. The mean content of orthophosphate diester in aquatic macrophytes was 50.0 mg kg^{-1} while that in algae was 125.5 mg kg^{-1}. Even though the content of orthophosphate diester was small, it was still an important source of endogenous P in lakes due to their mobility and bioavailability (Zhu et al. 2013a). In addition, pyrophosphate was also an important component in aquatic macrophytes and algae (Table 4, Fig. 1). In this research, the percentage of P that occurred as pyrophosphate was 1.61 % in aquatic macrophytes but 10.3 % in algae. This might be due to algae blooms that are due to increase nutrients in the lake from activities of humans. Orthophosphates are more easily absorbed and used by algae, but when orthophosphates are insufficient to meet the need of a growing population of phytoplankton, to maintain growth and energy supply, algae and cyanobacteria can use other forms of P such as pyrophosphate. Therefore, pyrophosphate was one of the important components of P in algae. This pyrophosphate would be precipitated to the sediments of lakes. However, pyrophosphates in sediments are easily hydrolyzed by alkaline phosphatase, and converted to orthophosphates, which are released to the overlying water in more available forms for accumulation by phytoplankton (Ahlgren et al. 2006; Reitzel et al. 2006). Therefore, pyrophosphate components of aquatic macrophytes and especially phytoplankton are potential sources of endogenous P in lakes.

Table 3 Concentration of P (mg kg^{-1}) and recovery (%) of aquatic macrophytes and algae with 0.5 M NaOH+25 mM EDTA

Code	Sample	TP	P_o	P_o/TP
A_1	*Foxtail algae*	2159 (98.2)	1339 (91.4)	66.6
A_2	*Reed*	1184 (93.2)	487 (92.9)	41.3
A_3	*Hydrilla verticillata*	3048 (82.9)	1188 (80.1)	40.4
B_1	*Microcystis*	7549 (99.3)	5120 (96.2)	70.0
B_2	*Chiorella vulgaris*	9779 (89.4)	6044 (95.9)	57.7
B_3	*Spirulina platensis*	12418 (96.2)	5494 (92.6)	46.0

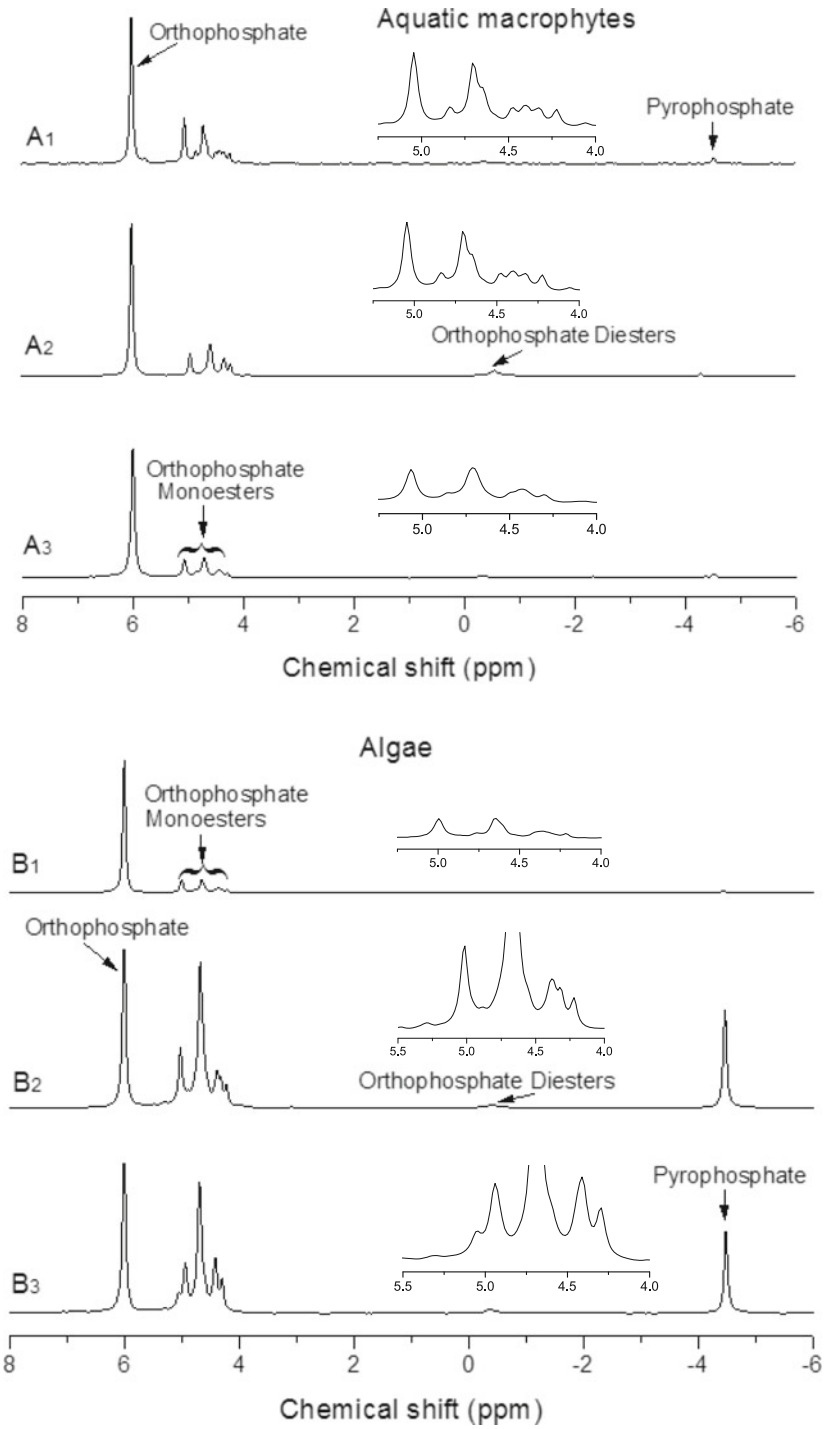


Fig. 1 Solution ^{31}P-NMR spectra of NaOH-EDTA extracts of aquatic macrophytes (**a**) and algae (**b**) collected from Tai Lake. Right of each spectrum expanded to provide more detail of the orthophosphate monoesters. A_1, A_2, A_3: foxtail algae, reed, and *hydrilla verticillata*, respectively; B_1, B_2, B_3: *Microcystis*, *Chiorella vulgaris*, and *spirulina platensis*, respectively

Orthophosphate monoesters and diester in aquatic macrophytes and algae

In order to determine chemical structure of orthophosphate monoester, six model P compounds were used in spiking experiments. Their ^{31}P-NMR spectra and chemical structures are presented in Fig. 2. Among the model P chemicals, *myo*-inositol hexakisphosphate showed four peaks, with chemical shifts at 5.79, 4.88, 4.55, and 4.42 ppm, respectively, and the relative proportions of these four peaks were 1:2:2:1. The chemical shift of -glycerophosphate, β-glycerophosphate, adenosine 5′ monophosphate, glucose 6-phosphate, and glucose 1-phosphate were 5.06, 4.73, 4.61, 5.34, and 3.24 ppm, respectively (Fig. 2).

Myo-inositol hexaphosphate (*Myo*-IHP) is the major storage form for P in terrestrial plants and commonly in seeds

Table 4 Distribution (mg kg^{-1}) of P forms in 0.5 M NaOH+25 mM EDTA extracts of aquatic macrophytes and algae samples

Sample	Orth	Pyro	Mono						Dies
			-Glyc	β-Glyc	Ade	G6P	Unidentified mono	Tot mono	lipids + DNA
A_1	1006.97	44.91	280.89	356.46	n.d.	n.d.	436.77	1073.90	33.25
A_2	656.83	10.78	n.d.	n.d.	201.81	n.d.	236.16	437.97	78.76
A_3	2005.25	58.22	322.78	328.87	n.d.	n.d.	294.74	946.39	38.10
B_1	5188.32	119.27	705.82	144.94	n.d.	n.d.	318.03	2168.78	72.47
B_2	2644.37	1487.46	923.18	2844.84	n.d.	n.d.	1730.96	5498.99	148.65
B_3	3540.48	1734.85	350.20	3475.91	n.d.	155.23	3006.53	6987.83	155.23

n.d. stand for did not detect, *Orth* orthophosphate, *Mono*, orthophosphate monoesters, *Dies* orthophosphate diesters, *Pyro* pyrophosphate, *Tot* P_o total organic phosphorus, *-Glyc* -glycerophosphate, *β-Glyc* β-glycerophosphate, *Ade* adenosine 5′ monophosphate, *G6P* glucose 6-phosphate, *Unidentified mono* unidentified orthophosphate monoesters, *Tot mono* total orthophosphate monoesters, *lipids + DNA* phospholipids + DNA

(Cade-Menun 2005). As an example, *Myo*-IHP spiked into the sample of *Hydrilla verticillata* (A_3), appeared as small peaks at 4.88 and 4.42 ppm with their proportions as 2.8 and 5.4 %, respectively (Fig. 3a). In phytoplankton sample represented by *Spirulina platensis* (B_3), a peak of 4.42 in chemical shift appeared (Fig. 3b) and its proportion integral was 10.23 %. As there were not overlaps between the indigenous peaks of the samples and the *Myo*-IHP, we concluded that both macrophytes and algae samples contained no *Myo*-IHP. The fact that *Myo*-IHP has been not observed in phytoplankton and algae in a previous study is consistent with the observation of this study; by spiking, *Myo*-IHP is reported not present in numeral humic acid samples (He et al. 2011).

Based on the spiking experiments, details of other orthophosphate monoesters from aquatic macrophytes and algae were identified and quantified (Table 4). The percentage of *β*-glycerophosphate in total orthophosphate monoesters was 27.9 % (228 mg kg^{-1}) in aquatic macrophytes, while the percentage of *α*-glycerophosphate was 24.6 % (201 mg kg^{-1}). In reeds, 17.0 % (202 mg kg^{-1}) was adenosine 5′ monophosphate, but it was not detected in samples of algae here. *β*-glycerophosphate accounted for 44.1 % of total orthophosphate monoesters and could be approached to 2155 mg kg^{-1} in algae, which was the largest component of orthophosphate monoesters. Followed by *α*-glycerophosphate that accounted for 13.5 % (660 mg kg^{-1}) of total orthophosphate monoesters in the debris from algae here.

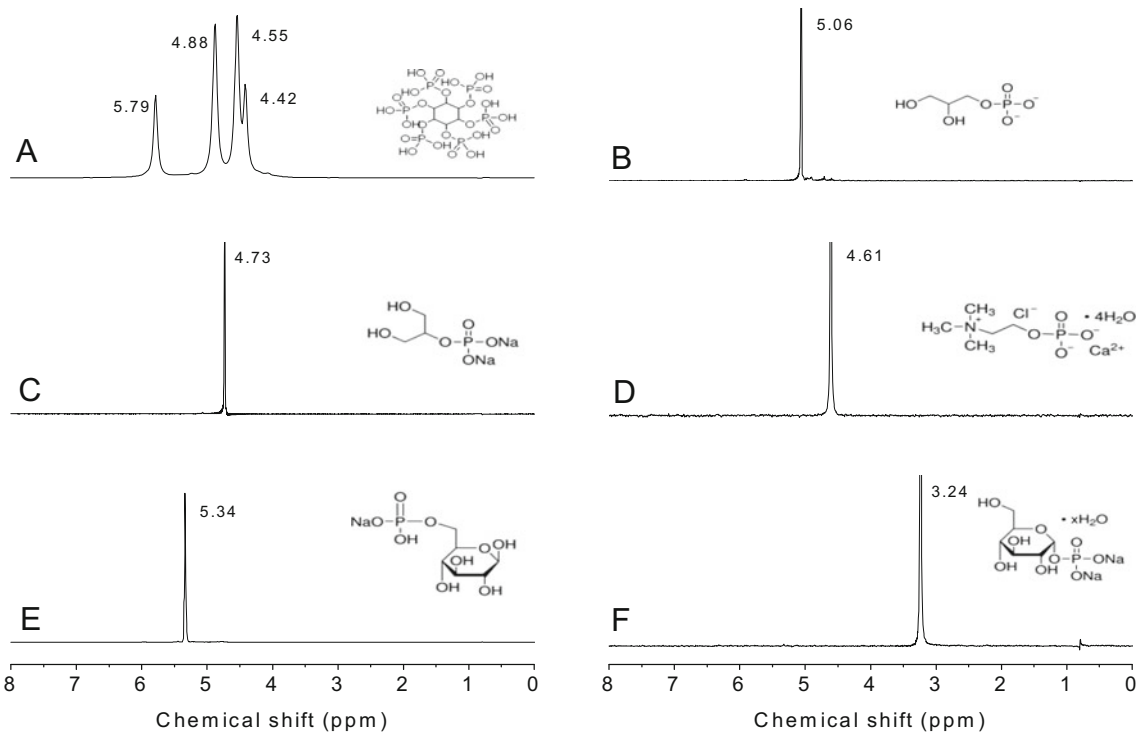
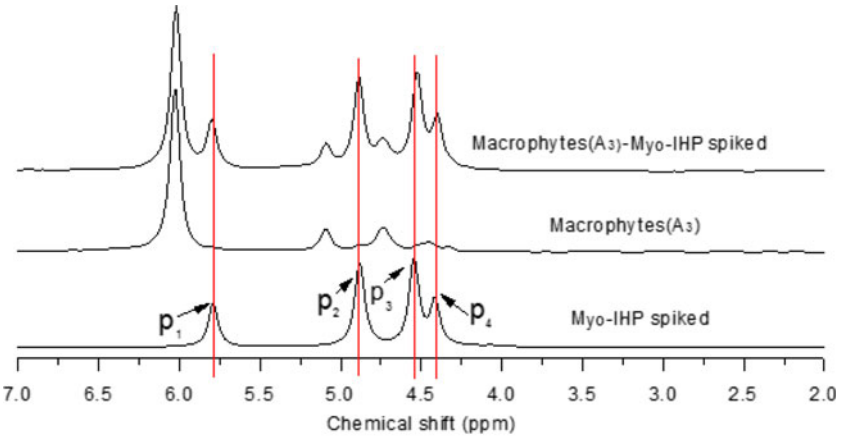
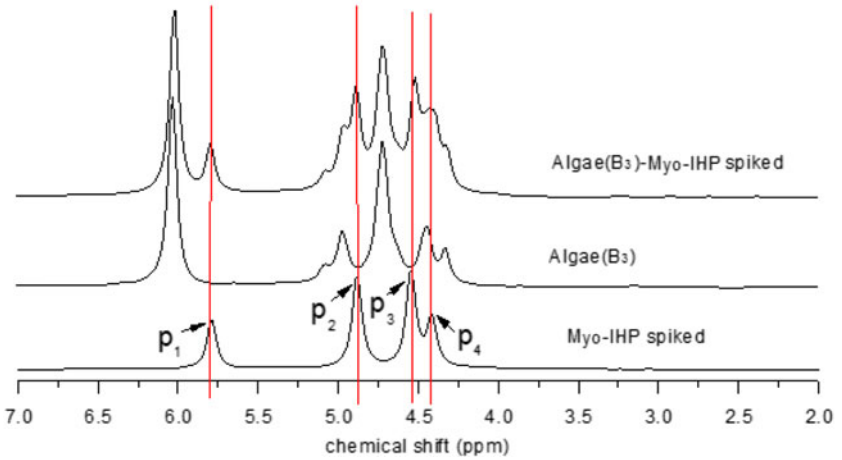


Fig. 2 Solution ^{31}P-NMR spectra of orthophosphate monoesters standard compounds. **a** *Myo*-IHP, **b** -glycerophosphate, **c** β-glycerophosphate, **d** adenosine 5′ monophosphate, **e** glucose 6-phosphate, and **f** glucose 1- phosphate



(a) Aquatic macrophytes



(b) Algae

Fig. 3 Comparison of solution ^{31}P-NMR spectra with and without *Myo*-IHP spiked. Aquatic macrophytes (A_3, *hydrilla vertillata*). Upper curve, A_3 with *Myo*-IHP spiked; middle curve, A_3 without *Myo*-IHP spiked, lower curve, *Myo*-IHP spiked. Panel 2. Same treatments with alga *spirulina platensis*(B_3). Chemical shifts of P_1, P_2, P_3, P_4 and 5.79, 4.88, 4.55 and 4.42 ppm, respectively

In algae such as S*pirulina platensis* glucose 6-phosphate accounted for 1.3 %, and the amounts was 155 mg kg^{-1}. Glucose 6-phosphate, which is common in cells, is involved in biochemical reactions of pentose phosphate and glycolysis. Additionally, there was a series of weak peaks appeared between 4.0 and 4.5 ppm of the ^{31}P-NMR spectra (Fig. 1). It has been shown that they represent orthophosphate diester degradation products and some unknown orthophosphate monoesters, which defined as "unidentified orthophosphate monoesters " here (He et al. 2007). These unidentified orthophosphate monoesters accounted for 28.9 and 39.3 % of total orthophosphate monoesters in aquatic macrophytes and algae, respectively.

Orthophosphate diesters are easily chemically hydrolyzed to monoesters during the alkaline extracts, the phenomenon is also appeared to other environmental samples and previous reports (Cade-Menun et al. 2015; He et al. 2011; Zhu et al. 2013b), the concentration of orthophosphate monoesters were usually overestimated, and the concentration of orthophosphate diester were usually underestimated. Orthophosphate diester mainly included phospholipids, DNA and RNA. ^{31}P-NMR results showed that the small content of phospholipids was detected (Fig. 1). This might be due to a portion of phospholipids being hydrolyzed to α- and β-glycerophosphate during the alkaline extracts. It has been previously reported that glycerophosphate (α-glyc and β-glyc) were degradation products of phospholipids (Doolette et al. 2009). Phospholipids are major components of plankton which was a part of the particulate fraction. For example, it has been reported that phospholipid accounted for as much as 22.0 % of TP in plankton from Tokyo Bay (Heath 2005). It has also been reported that phospholipids occur in sediments of lakes (Zhou et al. 2001; Søndergaard et al. 2003) and river (Wang and Pant 2010). Phospholipids of in sediments were likely to be derived from decomposition of aquatic macrophytes and algae and were eventually incorporated into biomass of microorganisms in sediments. Phospholipids contributed to reciprocal transformation of P in sediment, and migration from sediments was important for the P cycle of lakes.

Significance of aquatic macrophytes and algae in internal loading of P for eutrophication

Organic P could be one of the major fractions of P in many aquatic ecosystems (Baldwin 2013). Aquatic macrophytes and algae were sources of four categories of components of P in Tai Lake, including orthophosphate, orthophosphate monoesters, orthophosphate diester, and pyrophosphate, but not phosphonate or polyphosphate (Fig. 1), though other researchers have suggested that polyphosphate and trace amounts of phosphonate were found in most sediments and were an important source of endogenous P in lakes (Read et al. 2014). Most P_o was contributed by aquatic macrophytes and algae, which were significantly greater sources for bioavailable P than those contributed by sediments. Also, P_o in sediments would be mainly derived from decomposition of aquatic macrophytes and algae, though some P_o was contributed by microorganisms in sediments (Zhu et al. 2013b).

Based on ^{31}P-NMR analysis, the P_o from debris of aquatic macrophytes and algae was potentially bioavailable and released to the overlying water of lakes. Of these components, some orthophosphate diester was not stable and easily hydrolyzed to orthophosphate monoesters (e.g., RNA to α- and β-glycerophosphate). DNA was the main component of orthophosphate diester in aquatic macrophytes and algae in Tai Lake. DNA also could be an important source of P in aquatic environments especially when orthophosphate concentrations were small (Pinchuk et al. 2008). They were degraded less than DNA, polyphosphate, and phospholipids, but they were rapidly mineralized under anaerobic conditions. Degradation of phospholipids was likely generated when aquatic macrophytes and algae died or precipitated into the sediments. Components of P were transferred to dissolved P when plants died. Debris from aquatic macrophytes should be removed, otherwise, they might promote the growth of algae, thus accelerates cycling of nutrients and promotes eutrophication. The portion of P_o, such as - and β-glycerophosphate in aquatic macrophytes and algae, were converted into biologically available P in Lakes.

Conclusions

The chemical composition of P_o in aquatic macrophytes and algae was significantly different, even though orthophosphate monoesters were the major component of P_o from both aquatic macrophytes and algae. The bioavailable P_o was an important internal source of P to support growth of algae to form blooms when external sources of P have been controlled, at the same time, the biogeochemical cycle of P_o of aquatic macrophytes and algae in lakes might be an important process to self-regulate the nutrient status for eutrophic lakes and maintain their eutrophic status in a long time scale.

Acknowledgments This present study was supported in part by the National Natural Science Foundation of China (41403094, 41403743, 41261140337). Prof. Giesy was supported by the Canada Research Chair program, the Einstein Professor Program of the Chinese Academy of Sciences, and the Great-Tier Foreign Scholar program (#GDT20143200016), funded by the State Administration of Foreign Experts Affairs, the P. R. China to Nanjing University.

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