Characterization of phosphorus in algae from a eutrophic lake by solution ^{31}P nuclear magnetic resonance spectroscopy

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Abstract

The identification and quantification of phosphorus (P) compounds derived from algal biomass are crucial for a better understanding of algal P dynamics in lake ecosystems. Quantity and species of P in algae collected from Chao Lake (a typical ultra-eutrophic lake) in China were analyzed by chemical analysis and ^{31}P NMR. Total P (TP) in algae biomass ranged from 2671 to 5385 mg kg^{-1} of dry matter. Proportion of organic P (P_o) accounted for $78.3 \pm 2.6\%$ in algae biomass collected from the western part of Chao Lake, which was higher than that ($64.7 \pm 1.4\%$) in the eastern part of the lake. Eight P species including inorganic P species (orthophosphate and pyrophosphate) and P_o species (five monoesters P and diesters P) were identified in NaOH–EDTA extracts of algal samples. Monoesters P accounted for 48.4% in extracted TP, which was the main component of P_o. β-glycerophosphates were the largest component of monoesters P, which accounted for 22.6% in extracted TP. This study improved knowledge on the mechanism of the cycling of endogenous P in the aquatic system and would be helpful in developing a strategy for control of repeated algae blooms in eutrophic Chao Lake.

Keywords ^{31}P NMR · Algae · Organic phosphorus · Lake · Eutrophication

Introduction

Algae blooms are serious environmental problems around the world, especially in developing countries (Kagaloua et al. 2008; Pernet-Coundrier et al. 2012). Algae blooms occur in an aquatic environment if too much phosphorus (P) enters the system (Giles et al. 2015). For a eutrophic lake, release of P from dead algae is an important nutrient source that will support continuous algal blooms in lakes (Li et al. 2009; Feng et al. 2016a). Algae are not usually collected by humans because they are not valuable, so that the debris is allowed to decompose in situ. The decomposition of algal residues affects the bio-cycling and release of P, increasing the risk of resurgence of algal blooms (Feng et al. 2016b; Lehman et al. 2017). Decaying algal debris releases both inorganic P (P_i) and P_o. The P_o constituents need to be hydrolyzed to bioavailable P_o by various enzymes (Feng et al. 2016b). Therefore, the forms and concentrations of P in algae shall be evaluated while algae debris is decomposing. However, until now, studies of the species, concentrations, and effects of P_o in algae of eutrophic lakes have been limited because of the complexity and limitations of analytical methods (Turner et al. 2005; Bell et al. 2017).

Typical analytical approaches, such as enzymatic reactivity, high performance liquid chromatography (HPLC), and mass spectrometry are based on operational definitions so that they cannot discern P classes at a molecular level (Suzumura 2005; Baldwin 2013; Karl 2014). Phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P

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NMR) is a non-destructive, non-invasive technique for identifying chemical forms in various environmental samples. Solid-state ^{31}P NMR is focused on inorganic P compounds, solution ^{31}P NMR is mainly used to determine organic P compounds (Turner et al. 2005; He et al. 2011; Abdi et al. 2014; Sørensen et al. 2014). Several P compounds have been detected by solution ^{31}P NMR, including phosphonates, orthophosphate,monoesters P, diesters P, pyrophosphates and polyphosphates (He et al. 2007; Turner et al. 2012; Zhu et al. 2013). Generally, monoesters P represent a wide range of important P_o compounds, such as inositol phosphate and sugar phosphates (He et al. 2007; Doolette et al. 2009; Jarosch et al. 2015). Therefore, it is an ideal technique for analyzing P_o species in algae of eutrophic lakes, as it would not only provide important information pertaining to P biogeochemical cycling in lake ecosystems, but also yield abundant insight into identities of specific P compounds.

Chao Lake (31°25′28″–31°43′28″N, 117°16′54″–117°51′46″E) is one of the five largest freshwater lakes in China. It is situated on the flood plains between the Yangtze River and Huai River in the central Anhui Province of eastern China (Zan et al. 2010; Tang et al. 2015). Chao Lake is a typical shallow lake with a mean depth of 3 m and a surface area of 780 km^2 and drainage area of

13,350 km^2 (Wang et al. 2013). Due to the rapid increase in anthropogenic activities in the lake's watershed over recent decades, the lake has suffered from serious pollution, eutrophication and algae blooms (Xu et al. 2005). As a matter of fact, since the mid 1980s, algae blooms have occurred each year in Chao Lake (Chen and Liu 2014). In order to assess the environmental risks of algae on the eutrophic lake, this study analyzed the P species in algae by solution ^{31}P NMR, and based on the knowledge obtained, predicted the P bioavailability of the algal biomass in aquatic environments.

Materials and methods

Study sites and sample collection

Samples of algae were collected from six sites in Chao Lake in September 2015 (Fig. 1). These sampling sites were located in different eutrophic areas. Generally, Chao Lake is divided into two parts: the western part (samples C_1 and C_2) and the eastern part (samples C_3, C_4, C_5 and C_6) along the line of Zhongmiao–Mushan–Qitouzui, as shown in Fig. 1. The quality of water was worst in the western part of the lake and gradually became better from west to east (Zhu et al.
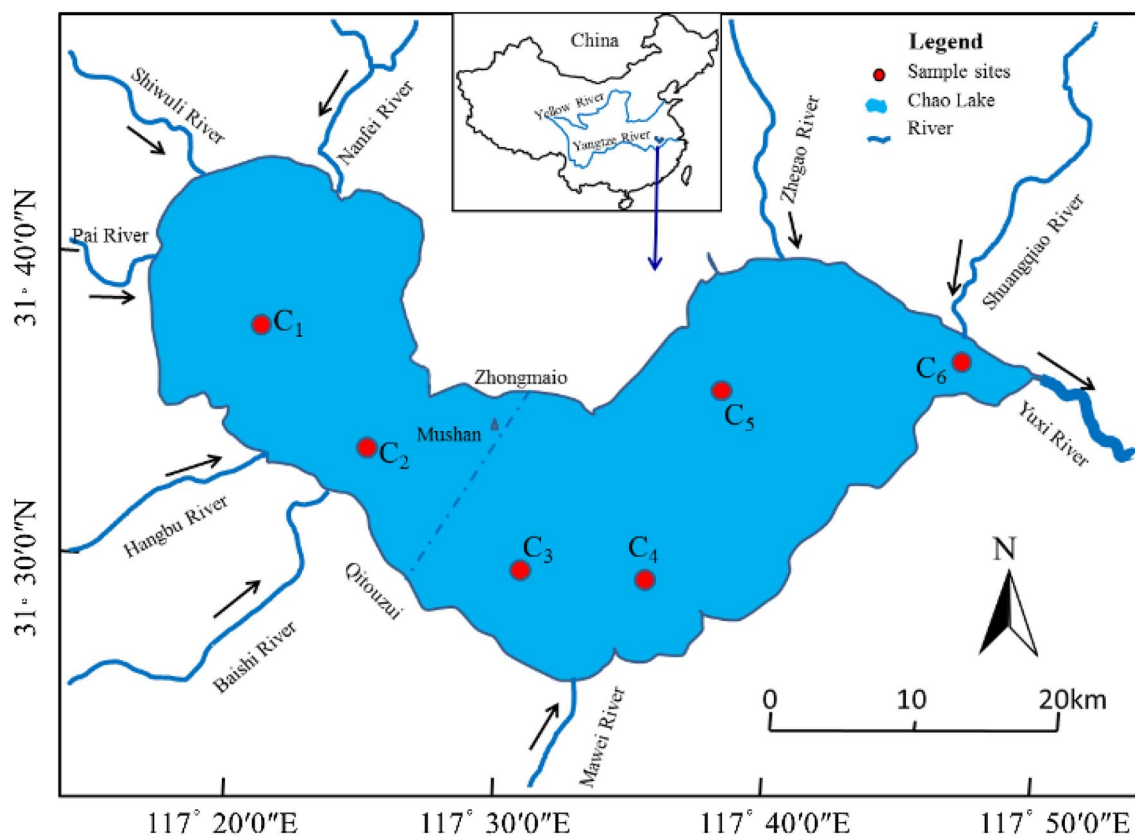

Fig. 1 Major inlets and outlets of Chao Lake with algal sampling sites

2006; Tang et al. 2015). The lake's annual mean concentrations of total nitrogen (TN) and TP approached 2.85 and 0.26 mg l^{-1}, respectively, and the annual mean chlorophyll-*a* reached up to 25.6 µg l^{-1} (Li et al. 2015). Algae were collected by use of a plankton collector (HB403-BWS). Samples were placed in sealed bags and put in ice boxes immediately. These algal samples were freeze-dried, ground, then passed through a 2-mm sieve before being stored at −20 °C (Feng et al. 2016a). The dominant species in Chao Lake was *Microcystic aeruginosa* with an appearance frequency of 90.9% (Yang et al. 2011).

Extraction of P and chemical analysis

Samples of algae were extracted by use of optimized NaOH–EDTA extracting agent (mixtures of 0.5 mol l^{-1} NaOH and 25 mmol l^{-1} EDTA) with a ratio of 150:1 (ml g^{-1}), and the mixtures were shaken for 18 h at room temperature (Cade-Menun and Preston 1996; Feng et al. 2016b). The extracting solutions were centrifuged (8000 × *g*) for 30 min, and filtered through 0.45-µm glass-fiber filters (Whatman GF/C). Extractable total phosphorus (ETP) after digestion and free molybdate reactive phosphorus (MRP) were measured using the molybdenum blue method (He and Honeycutt 2005). Extractable organic phosphorus (EOP) was calculated by the difference between ETP and MRP. The remaining extracts were freeze-dried for solution ^{31}P NRM spectroscopy analysis.

Percentages of carbon (C) and nitrogen (N) in algae were determined by use of an elemental analyzer (Elementarvario macro EL, Germany). Total phosphorus (TP) and inorganic phosphorus (P_i) were determined by the SMT method described by Ruban et al. (2001). Organic phosphorus (P_o) in algae samples was calculated by the difference between TP and P_i. There were three replications for extraction of P and chemical analysis. Data were checked for deviations from normality and homogeneity of variance before performing statistical analyses.

^{31}P NMR analysis

A 100-mg sample of freeze-dried algal extracts was ground, and then redissolved in 1 ml 1 mol l^{-1} NaOH + 0.1 mol l^{-1} EDTA and 0.2 ml D$_2$O. After ultrasonication for 30 min and equilibration for 5 min, 2% (v/v) of bicarbonate buffered dithionite (0.11 mol l^{-1} NaHCO$_3$ + 0.11 mol l^{-1} Na$_2$S$_2$O$_4$) was added to the extract to reduce interference from paramagnetic ions, such as Fe and Mn (He et al. 2009; Giles and Cade-Menun 2014). The pH of the supernatant solution was adjusted using 10 mol l^{-1} NaOH to ensure a pH > 12. The supernatant solution was centrifuged (8000 × *g*) for 30 min and transferred to a 5-mm NMR tube. Solution ^{31}P NMR spectra were acquired at 24 °C on a Bruker AVANCE

400 MHz spectrometer at a ^{31}P frequency of 161.98 MHz, using a 90° pulse, a 5 s relaxation delay and a 0.21 s acquisition time, similar to the parameters used in Feng et al. (2016b). The scan time for each sample was more than 15 h. Peak areas were calculated by integration and completed using MestrelabMNova v.10.

Spiking experiments

The peak of specific monoesters P forms (i.e., glucose 6-phosphate, riboncleotides, α-glycerophosphate, β-glycerophosphate, myo-inositol hexaphosphate) needs to be confirmed with spiking experiments (Fig. 4). Methods for identifying specific P forms in NMR spectra of soil and other environmental samples are well-established and have been used for many years (Smernik and Dougherty 2007; He et al. 2011; McDowell and Hill 2015), combined with P compound libraries developed by Turner et al. (2003); Doolette et al. (2009) and Cade-Menun (2015). Standard samples of monoesters P were purchased from Sigma-Aldrich. Spiked samples were analyzed by ^{31}P NMR as described above. Monoesters P compounds were identified by their chemical shifts, with the orthophosphate peak in all spectra standardized to 6.0 ppm. Spectral processing was done using MestReNove software version 9.0.1 (MestReNove Research SL).

Results and analysis

Nutrients (C, N and P) in debris of algae

Composition of C, N and their ratios in debris of algae are shown in Table 1. Percentages of C ranged from 31.5 to 50.3% with a mean value of 41.9% in the algae samples from Chao Lake. Content of N was 5.2–7.8% with a mean value of 6.4%. Both contents of C and N of algae was greater than those of aquatic macrophytes, which has been widely reported previously (Zhong et al. 2012; Qu et al. 2013, Feng et al. 2016a). The ratio of C:N was a good predictor of degradation in algae and aquatic macrophytes with a lower ratio of C:N for material more readily degradation (Reitzel et al. 2006). In this study, the ratios of C:N in algae from the western lake (7.1 ± 0.3) were higher than those of the eastern lake (6.4 ± 0.3), suggesting higher lability of the algal debris of the eastern lake. The ratio of C:N of aquatic macrophytes (average 12.9) (Feng et al. 2016a) was greater than that of algae in Chao Lake (average 6.7) (Table 1). Therefore, the algae decomposed more easily than aquatic macrophytes in the same lake. This was also consistent with the results of previous studies (Liu et al. 2016).

Contents of TP in debris of algae ranged from 2671 to 5385 mg kg^{-1} dry mass (dm) with a mean value of

Table 1 Contents of C, N, and P in algae and their NaOH–EDTA extraction efficiency in Chao Lake

Samples	Coordinates	Original algae powders						NaOH–EDTA extract algae samples	
		C (%)	N (%)	C:N	TP(mg kg^{-1})	P_o(mg kg^{-1})	P_o/TP(%)	ETP(mg kg^{-1})	EOP(mg kg^{-1})
C_1	31°38′3.52″E, 117°21′16.74″N	44.8 ± 2.5[a]	6.7 ± 0.5	6.7 ± 0.3	4173 ± 516	3372 ± 126	80.8 ± 1.6	$1864 \pm 126(44.7)$[b]	$785 \pm 124(23.3)$[b]
C_2	31°34′3.50″E, 117°24′51.80″N	39.5 ± 0.6	5.3 ± 0.8	7.5 ± 0.2	4059 ± 125	3072 ± 432	75.7 ± 3.5	$1757 \pm 214(43.3)$	$464 \pm 52(15.1)$
C_3	31°30′9.64″E, 117°28′56.52″N	38.3 ± 1.8	5.4 ± 0.2	7.1 ± 0.4	3787 ± 256	2536 ± 59	67.0 ± 2.5	$1572 \pm 56(41.5)$	$351 \pm 98(13.8)$
C_4	31°27′56.84″E, 117°34′50.00″N	31.5 ± 3.5	5.2 ± 0.1	6.1 ± 0.1	2671 ± 198	1476 ± 123	55.3 ± 1.4	$1292 \pm 89(48.4)$	$567 \pm 15(38.4)$
C_5	31°33′27.70″E, 117°36′46.18″N	50.3 ± 2.9	7.8 ± 0.8	6.5 ± 0.4	3543 ± 112	2107 ± 78	59.5 ± 0.9	$2774 \pm 21(78.3)$	$1141 \pm 215(54.2)$
C_6	31°36′10.25″E, 117°47′38.55″N	46.9 ± 4.6	7.8 ± 0.7	6.0 ± 0.2	5385 ± 290	4136 ± 19	76.8 ± 0.8	$2183 \pm 51(40.5)$	$1066 \pm 164(25.8)$

ETP extractable total phosphorus, *EOP* extractable organic phosphorus

[a]Mean $\pm$ standard deviation ($n = 3$)

[b]Values in brackets show the percentage of ETP, EOP in NaOH–EDTA extracts to TP and P_o in the unextracted algae powders, respectively

3936 mg kg^{-1}. The greatest concentration of TP in debris of algae was observed in sample C_6. This may be because of two major inflowing rivers (e.g., the Zhe gao river and Shuang qiao river) with heavy nutrition inputs (Tang et al. 2015). Contents of TP in the surface sediments from Chao Lake only ranged from 420 to 1090 mg kg^{-1} with a mean value of 687 mg kg^{-1} (Zhang and Xing 2013). Content of TP in debris of algae from Chao Lake was approximately 5 times higher than TP in surface sediments. Therefore, dead algae-derived P might be an important source of bioavailable P for repeated algal blooming in eutrophic lakes such as Chao Lake. Concentration of P_o in these algal samples ranged from 2107 to 4136 mg kg^{-1}with a mean value of 2783 mg kg^{-1}. The greatest concentration of P_o was also observed in the heavily polluted region (i.e., sample C_6). The proportion of P_o in algae of the western lake ($78.3\% \pm 2.6\%$) was higher than that in the eastern lake ($64.7\% \pm 1.4\%$). The mean ratio of P_o/TP of the six algal samples was 69.2% in Chao Lake (Table 1). This value was greater than that of algae from Tai Lake (mean ratio of P_o/TP 57.8%) (Feng et al. 2016b). The previous studies reported that P_o could be converted to bioavailable P (e.g., HPO_4^{2-}) for algae blooming through a series of redox-driven solubilization reactions and phosphatase-mediated hydrolytic processes (Wang and Pant 2010; Zhu et al. 2015). Thus, we believed that P_o in debris of algae from Chao Lake possessed larger bioavailability potential than algae from Tai Lake per their difference in P_o/TP ratios.

NaOH–EDTA extractable P from algae

In this study, contents of NaOH–EDTA extractable TP ranged from 1292 to 2774 mg kg^{-1}, with an extraction efficiency of 40.5–78.3%, and contents of extractable P_o from algae ranged from 351 to 1141 mg kg^{-1}, with an extraction efficiency of 13.8–54.2% (Table 1). The recoveries of P_o in debris of algae from Chao Lake were lower than those with pure algae such as *Microcystis*, *Chlorella vulgaris*, and *Sprilinaplatensis* (Feng et al. 2016a). However, the extraction efficiencies of P_o of algae from Chao Lake were similar to those of particulate P from Tai Lake (23–56%) (Bai et al. 2017) and of sediments from Haihe River (30–73%) (Zhang et al. 2017). The extraction efficiency of TP from algae from Chao Lake was similar to that of soils and sediments (49–83%) (Xu et al. 2005; Tang et al. 2015). Multiple-step extractions (e.g., additional or sequential HCl extraction) (He et al. 2008; Cade-Menun 2015; Zhu et al. 2016; Liu et al. 2017) seem needed to increase the P recovery from these algal samples.

Solution ^{31}P NMR spectra of NaOH–EDTA extracts of algae

Eight main P species including inorganic P species (orthophosphate and pyrophosphate) and P_o species (five monoesters P and diesters P) were identified in the NaOH–EDTA extracts of the six algal samples by solution ^{31}P NMR (Fig. 2; Table 2). The peak of orthophosphate was at 6.00 ppm in the ^{31}P NMR spectra, monoesters P was at 3.33–5.49 ppm, diesters P was at −0.69 to −0.31 ppm, and pyrophosphate was at −4.21 to −4.12 ppm (Fig. 2). The sum of orthophosphate and monoesters P in ETP accounted for more than 93% of ETP (Table 1; Fig. 3b). With the peak of orthophosphate as the largest signal in these ^{31}P NMR spectra. The content of orthophosphate was between 615.1 and 1331.7 mg kg^{-1}, and accounted
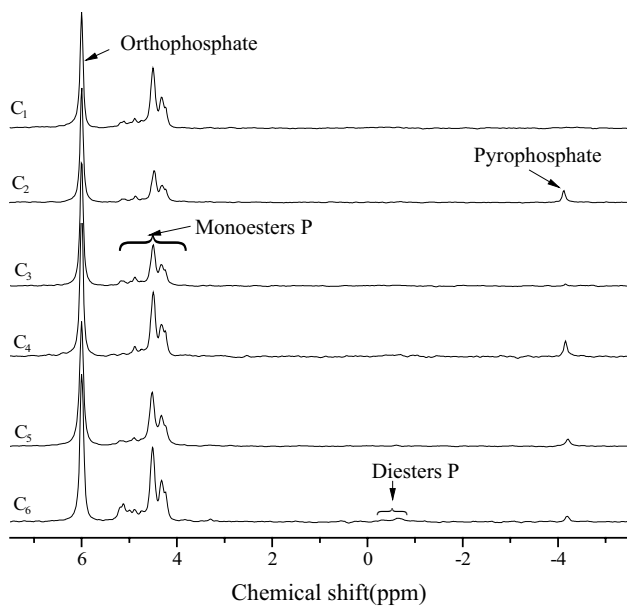
Fig. 2 Solution ^{31}P NMR spectra of NaOH–EDTA extracts of algae in Chao Lake

for 41.5–54.0% of ETP (Table 1; Fig. 3). Polyphosphates were not detected in any algae of Chao Lake.

Monoesters P comprised the largest P_o fraction with NaOH–EDTA extracts, and accounted for 48.4% (average) in ETP (Table 2; Fig. 3b). Through the spiking experiments (Fig. 4), the peak at 4.88 ± 0.02 ppm was assigned to α-glycerophosphates and the peak at 4.50 ± 0.02 ppm was assigned to β-glycerophosphates, based on Turner and Richardson (2004) and Doolette et al. (2009); the percentages of α- and β-glycerophosphates in ETP were 3.4% (65.5 mg kg^{-1}) and 22.6% (432.7 mg kg^{-1}), respectively. β-glycerophosphates were the largest component of monoesters P. The peak at 4.32 ± 0.01 ppm was assigned to ribonucleotides (He et al. 2011) and the percentage of ribonucleotides in ETP was 10.6% (218.2 mg kg^{-1}). The peak at 5.12 ± 0.01 ppm was assigned to glucose 6-phosphate (Cade-Menun 2015), the percentage of which in ETP was 1.9% (38.7 mg kg^{-1}). In addition, a few of the peaks in the monoesters P region were unidentified, because chemical shifts were strongly influenced by subtle differences among samples for viscosity, pH, salts and paramagnetic ions (Young et al. 2013; Abdi et al. 2014; He et al. 2011; Giles et al. 2015). Unidentified monoesters P are defined as 'other monoesters P' in this study. These other monoesters P accounted for 9.9% of ETP in algae of Chao Lake (Table 2).

Pyrophosphate was detected in most samples except the sample C_1, which is consistent with a number of other studies (Bedrock et al. 1995; Mahieu et al. 2000; He et al. 2011). In other literature, polyphosphates and pyrophosphate were

Table 2 Concentrations of P compounds in NaOH–EDTA extracts of the algae determined by solution ^{31}P NMR

Algae	P_i (mg kg^{-1})		P_o (mg kg^{-1})							
			Monoesters P						Diesters P	Total P_o
	Orthophosphate	Pyrophosphate	Glucose 6-phosphate	Ribonucleotides	α-glycerophosphate	β-glycerophosphate	Other monoesters P	Total monoesters P		
C_1	818.2(43.9)^a	n.d	32.7(1.8)	204.6(11.0)	60.6(3.2)	526.9(28.3)	220.9(11.9)	1045.7(56.1)	n.d	1045.7(56.1)
C_2	938.1(53.4)	115.4(6.6)	42.2(2.4)	144.5(8.2)	63.8(3.6)	322.7(18.4)	130.4(7.4)	703.5(40.0)	n.d	703.5(40.0)
C_3	849.1(54.0)	15.8(1.0)	n.d	137.6(8.7)	49.2(3.1)	325.2(20.5)	201.5(12.7)	714.1(45.0)	n.d	714.1(45.0)
C_4	615.1(47.6)	65.8(5.1)	15.4(1.2)	131.2(8.8)	44.9(3.5)	297.7(23.0)	131.0(10.1)	604.4(46.6)	9.2(0.7)	611.4(47.3)
C_5	1331.7(48.0)	87.9(3.2)	49.3(1.8)	487.4(17.6)	103.9(3.7)	636.6(22.9)	67.9(2.4)	1346.1(48.5)	9.3(0.3)	1354.4(48.8)
C_6	905.6(41.5)	32.6(1.5)	92.4(4.2)	203.8(9.3)	70.6(3.2)	487.2(22.3)	329.6(15.1)	1184.0(54.2)	61.6(2.8)	1245.2(57.0)

P_i inorganic P, P_o organic P, *Total P_o* the sum of monoesters P and diesters P, *n.d* not detected

[a] Values in parentheses are percentages of individual P compounds in NaOH–EDTA extracts TP
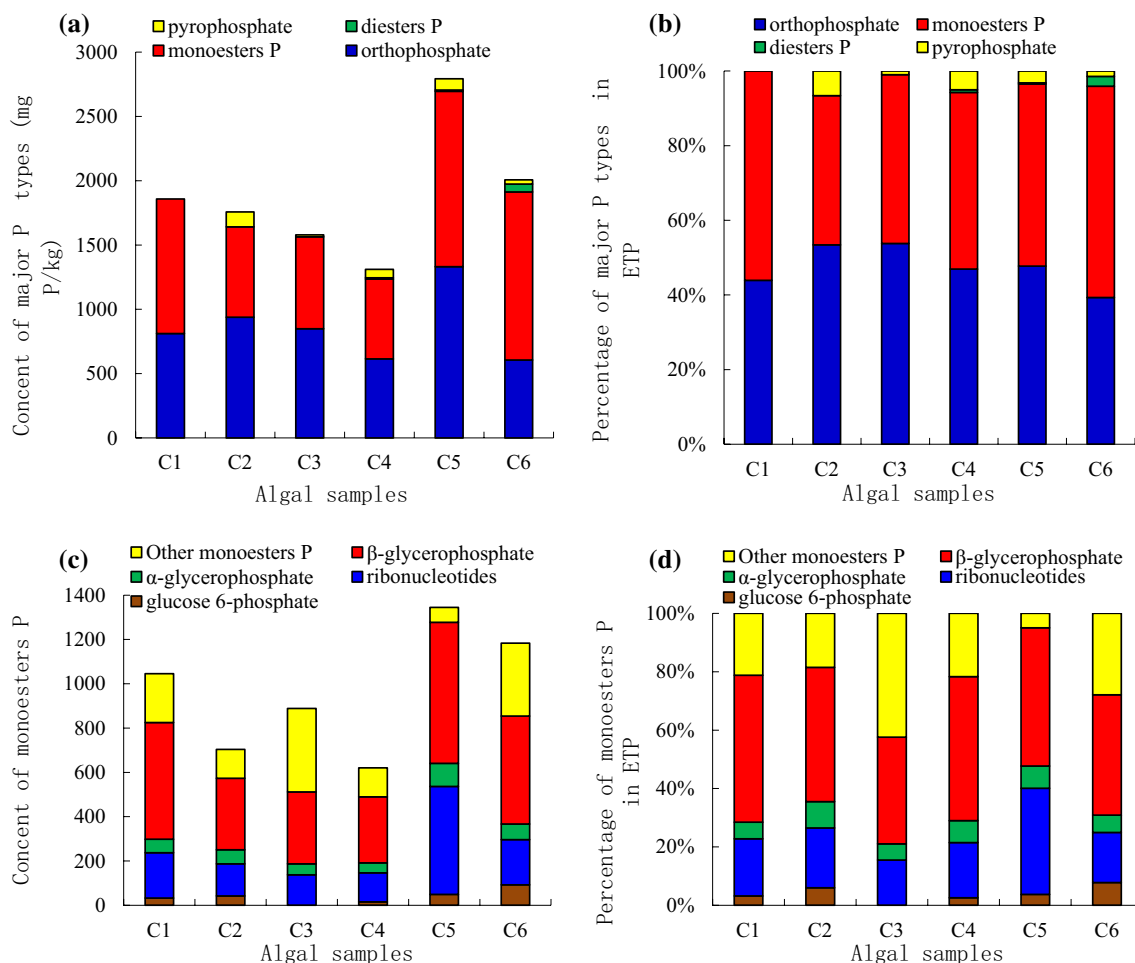
Fig. 3 Content and percentage of major P types (**a**, **b**) and monoesters P compound forms (**c**, **d**) in algae collected from six sites (C_1–C_6) of Chao Lake

detected in some samples, but not necessarily (Busato et al. 2005; Feng et al. 2016b).

Discussion

Identification of inositol hexaphosphate (IHP) in algae of Chao Lake

The inositol hexaphosphate (IHP) stereoisomers (scyllo-, myo-, chiro-, neo-IHP) were important monoesters P components in many environmental samples (Turner et al. 2012; Cade-Menun 2015). Each of these compounds contains six phosphates, and the conformation of those phosphate groups causes them to have multiple peaks in a single spectrum, in an arrangement specific to each compound. The only exception is scyllo-IHP, which has one peak for the six phosphates; the peak at 3.55 ± 0.02 ppm was assigned to scyllo-IHP, based on Turner and Richardson (2004) and Doolette et al. (2009). None of the spectra

shown in Fig. 2 have a peak at 3.55 ppm, so the scyllo-IHP was not present in algae of Chao Lake. Myo-IHP has four peaks in a 1:2:2:1 arrangement (with respect to peak areas), with peaks at 5.79 ± 0.01, 4.88 ± 0.01, 4.55 ± 0.01 and 4.42 ± 0.01 ppm (in Fig. 4e). Three of these peaks were not present in algae samples in Chao Lake (Fig. 2), so myo-IHP was not present in algae of Chao Lake. The fact that myo-IHP had not been observed in algae in a previous study is consistent with the observation of this study (Feng et al. 2016a). For chiro-IHP [in either the 4 equatorial/2 axial (4e/2a) conformation or the 2e/4a conformation], three peaks must be clearly visible, in a 2:2:2 arrangement, and the diagnostic peaks for each are at 6.2–6.5 ppm (Cade-Menun 2015); however, none of the spectra shown in Fig. 2 have any peaks between 6.0 and 7.0 ppm, so these compounds were not present in algae of Chao Lake. In addition, neo-IHP requires two peaks to be present, in a 4:2 arrangement, so that the peak at 6.4 ± 0.01 ppm is twice as large as the one at 4.3 ± 0.01 ppm. Given that there were no peaks between 6.0 and 7.0 ppm, it can be

Fig. 4 Solution ^{31}P NMR spectra of monoesters P standard compounds (a–e) and C_1 algal sample in Chao Lake (f)
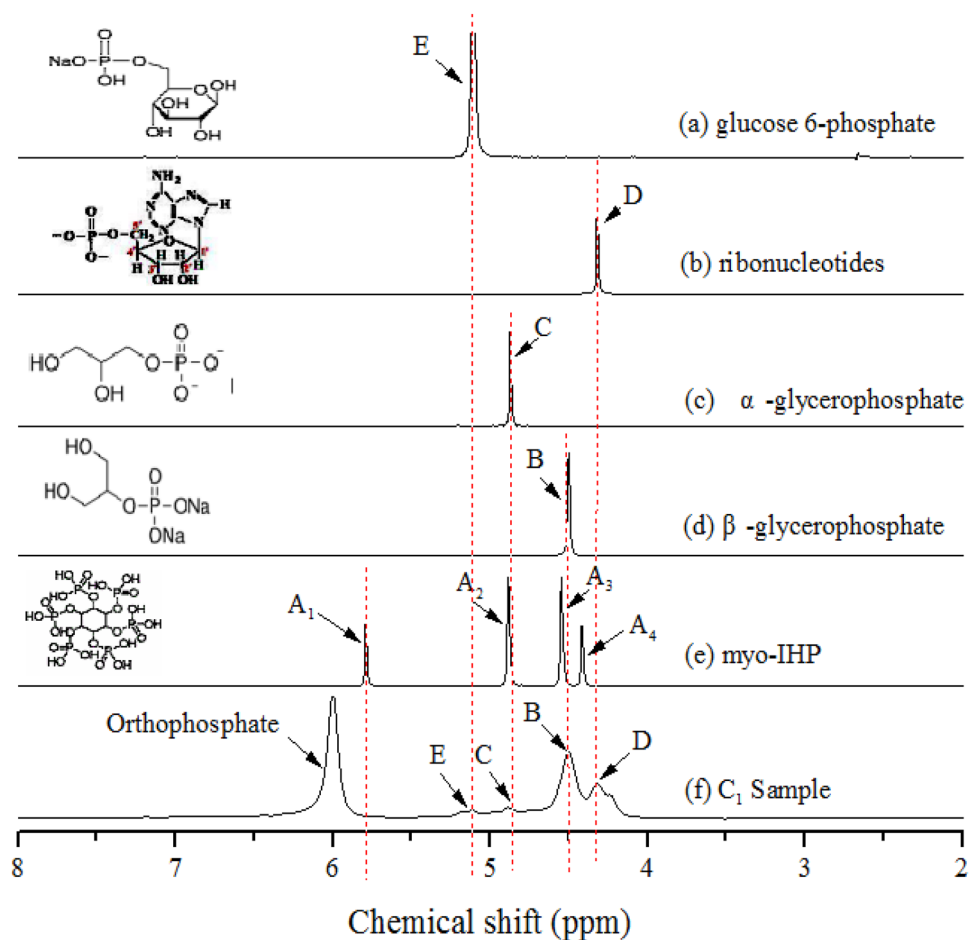
Degradation behaviors of diesters P in algae of Chao Lake

Apart from monoesters P, other important P_o compounds, diesters P, were detected in some algae samples. The concentration of diesters P was generally low (mean 0.63% of ETP), compared to other P_o fractions, and only detected in samples C_4, C_5 and C_6 (9.2–61.6 mg kg^{-1}).

It is well established that some diesters P such as phospholipids and RNA can degrade to monoesters P [e.g., α-,β-glycerophosphates (phospholipids) and various monophosphates (e.g., nucleotides) when analyzed at the high pH required for good peak separation in ^{31}P NMR spectra] (Turner et al. 2003; Doolette et al. 2009; He et al. 2011; Schneider et al. 2016). The degree of degradation will vary depending on the length of NMR experiment and other factors (Cade-Menun and Liu 2014; Cade-Menun 2015; Feng et al. 2018). It was essential that these degradation peaks were identified and quantified, in order to determine the correct concentrations of monoesters P and diesters

P (Young et al. 2013; Vincent et al. 2013). Therefore, the corrected total monoesters P and corrected total diesters P were those corrected by moving the percentages of α- and β-glycerophosphates and nucleotides from monoesters P to diesters P. When uncorrected, the total monoesters P were significantly higher than the total diesters P, but the reverse was true for the corrected values.

Eutrophication and algal blooming versus biogeochemical cycling of algae-derived P in Chao Lake

Based on the results in this study, we were able to think further about the biogeochemical cycling of P driven by algal blooming in Chao Lake. The TP of algae-derived biomass loading was 10.94×10^3 kg in Chao Lake (Li et al. 2015). With the P_o content in algae determined in this study (Table 1), we estimated the P_o biomass of algae to be approximately 7.57×10^3 kg in Chao Lake. In previous research (Feng et al. 2016b) with Tai Lake samples, we estimated that approximately 32.7–41.3% of extractable P_o from algae has the potential for phosphatase hydrolysis to soluble orthophosphate which can be released into the water body.

Thus, in the case of Chao Lake, this bioavailable P would be 2475–3126 kg in algae and would be released into the water and promote repeated algal blooms in Chao Lake if not appropriately removed naturally or artificially. This conclusion indicated that decomposition of algal debris would be a key factor in regeneration of bioavailable P for life in eutrophic lakes, even when external P is excluded. It is therefore necessary to remove algae debris from eutrophic lakes to control the release of P from internal P cycling and the phenomenon of eutrophication of lakes.

Conclusion

This research used solution ^{31}P NMR to provide insights into the P species and distribution of P in algae of the heavily polluted Chao Lake. Data derived from this study showed that the eutrophic lake algae have accumulated remarkable amounts of P_i and P_o. The proportion of P_o in algae ranged from 55.3 to 80.8% with a mean of 69.2% in Chao Lake.

Eight compounds P were detected in the NaOH–EDTA extracts of algal samples by ^{31}P NMR. The sum of orthophosphate and monoesters P in ETP was greater than 93% of algal P. Our observations implied that the release of P induced by the decomposition of algae debris could be a potential source of bioavailable P in aquatic systems of Chao Lake even without any more external P input. Thus, recycling of the potential bioavailable P in algae might be the mechanism of repeated algae blooming in eutrophic Chao Lake. Remediation of the lake requires a strategy to remove the algal biomass P effectively.

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