Received: 17 January 2018 | Revised: 9 July 2018 | Accepted: 13 July 2018 | Published on: 21 December 2018

DOI: 10.1111/1440-1703.1006

WILEY ECOLOGICAL RESEARCH

ORIGINAL ARTICLE

Characterization and sources of dissolved and particulate phosphorus in 10 freshwater lakes with different trophic statuses in China by solution ^{31}P nuclear magnetic resonance spectroscopy

Chen Zhang[1] | Weiying Feng[1] | Haiyan Chen[1] | Yuanrong Zhu[1] | Fengchang Wu[1] | John P. Giesy[1,2] | Zhongqi He[3] | Hao Wang[1] | Fuhong Sun[1]

[1]State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing, China

[2]Department of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

[3]USDA-ARS, Southern Regional Research Center, New Orleans, Louisiana

Correspondence
Weiying Feng, State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China.
Email: feng_weiying@126.com

Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 41703115, 41573126, 41630645

Abstract

Information on dissolved phosphorus (DP) and particulate phosphorus (PP) is essential to evaluate the P dynamics and control eutrophication. In this work, DP and PP in 10 freshwater lakes representing various trophic statuses were analyzed by solution ^{31}P nuclear magnetic resonance spectroscopy. The results indicated that the predominant forms of DP and PP were orthophosphate (Ortho-P) and monoester phosphate (Mono-P). There was a greater concentration of Ortho-P and Mono-P in the water and particulate matter of medium-eutriphic lakes than in lightly eutriphic or mesotrophic lakes. α-Glycerophophate (2.7–32.5%), β-glycerophosphate (1.3–23.4%), guanosine 2′Mono-P (20.2–29.3%), inositol hexakisphosphate (IHP) (8.3–36.4%) and nicotinamide adenine dinucleotide (NAD) (17.3–35.9%) were identified as the major chemical forms of Mono-P in water and particulate matter, which originate mainly from the degradation of labile diesters, aquatic/microbial sources and a combination of terrestrial and aquatic/microbial sources. Diester phosphate (Di-P) was dominated by teichoic acid (0.5–14.8%) and deoxyribonucleic acid (DNA) (0.6–17.7%), which originated from aquatic/microbial sources. Moreover, correlation analysis showed that dissolved Mono-P in water and particulate matter had a positive correlation with chlorophyll-*a* (Chl-*a*), which indicated their potential bioavailability for algal activity. pH was a crucial parameter to control Di-P, pyrophosphate (Pyro-P) and polyphosphate (Poly-P) in water. Mono-P/PPs showed a positive correlation with Chl-*a* (R^2 = 0.459), total phosphorus (TP) (R^2 = 0.586) and the trophic status index (R^2 = 0.588), which suggested that particulate Mono-P can potentially contribute to lake eutrophication. Both Ortho-P and Mono-P were major contributors of P nutrients for algae, and therefore source control and new techniques are needed for reducing eutrophication.

KEYWORDS

^{31}P-NMR, dissolved phosphorus, eutrophication, lake, particulate phosphorus

1 | INTRODUCTION

Phosphorus (P) is a major limiting nutrient for phytoplankton and algae, but excessive P will lead to lake eutrophication and water quality deterioration (Yang, Cao, Liu, & Zhang, 2015). Internal P loading plays an important role in causing seasonal nitrogen limitation for harmful algal blooms, which accounts for 54% of increased total phosphorus (TP) in the water column during the algal prebloom and bloom period (Ding et al., 2018). In lake waters, dissolved phosphorus (DP) and particulate phosphorus (PP) play an important role because they are the direct or indirect P sources of the primary product. Information on DP and PP chemical forms is beneficial for understanding the biogeochemical cycles of P in lake eutrophication (Cao, Wang, He, Luo, & Zheng, 2016). Phosphonate (Phos-P), orthophosphate (Ortho-P), monoester phosphate (Mono-P), diester phosphate (Di-P), pyrophosphate (Pyro-P) and polyphosphate (Poly-P) are the main forms of P in the aquatic environment. Among them, Ortho-P is the most bioavailable P form for phytoplankton, which is a direct source of P utilized by algae and aquatic macrophytes (Giles et al., 2015). Mono-P and Di-P are both important fractions of dissolved and particulate organic phosphorus (OP), which are potential P sources after being mineralized in water (Nash et al., 2014). Phos-P is a stable P fraction, and the C─P bond structure is responsible for making it hard to be hydrolyzed chemically and enzymatically. Both Pyro-P and Poly-P are the labile fractions of P because of their short half-life, whereas Pyro-P partially comes from esters, which can be hydrolyzed under alkaline conditions (Ahlgren et al., 2005). And Poly-P is easily transformed into other forms of P and combines with metal ions in surface waters (Hupfer, Ruübe, & Schmieder, 2004; Reitzel, Ahlgren, Gogoll, & Rydin, 2006).

In the aquatic environment, P can diffuse from sediments to the overlying water with a concentration gradient. Perhaps, P is also released from suspended particulate matter (i.e., PP fractions) if the concentration of DP is relatively low in the aquatic environment (Jensen & Andersen, 1992; Søndergaard, Kristensen, & Jeppesen, 1992). Recent research shows that Fe–P coupling is the mechanism for the P release from sediments, and the decomposition of algae is a dominant source of P release (Chen et al., 2018; Gu et al., 2016). In a shallow eutrophic lake, PP is generated by living or dead phytoplankton, bacterial cells and sediment resuspension (Shinohara et al., 2016). If the Dissolved inorganic phosphorus (DIP) level is too low to provide nutrition for algae, mineralization of organic phosphorus (OP) will occur in DP and PP, and this process makes the internal loading of P from DP and PP a key factor during eutrophication (Ding et al., 2013). Previous study has shown PP can be used as a proxy for internal P source in a cyanobacteria-dominated lake, which is a potential source of bioavailable P (Løvdal, Tanaka, & Thingstad, 2007; Read, Ivancic, Hanson, Cade-Menun, & McMahon, 2014). However, not all DP and PP fractions can be utilized by algae, leading to the eutrophication of a lake; the bioavailability largely depends on the detailed chemical forms of DP and PP. Therefore, comprehensive information of DP and PP forms is necessary for understanding their transformation and potential contribution for lake eutrophication.

Solution ^{31}P nuclear magnetic resonance spectroscopy (^{31}P-NMR) is a powerful method for the analysis P forms, but chemical forms of DP are difficult to detect because of their the low concentrations in freshwater. In the last decade, substantial progress has been made in the characterization of DP and PP in the aquatic environment (Lin, Guo, Chen, Tong, & Lin, 2012; Solórzano & Sharp, 1980; Zhang, Davison, Gadi, & Kobayashi, 1998). A lyophilization method to concentrate P in spiked and natural water samples makes the characterization possible (Cade-Menun, Navaratnam, & Walbridge, 2006; Read et al., 2014). For the sake of high enrichment, poly(aluminum chloride) ($AlCl_3$) was adopted as the flocculating agent to concentrate DP in 20 L of a water sample, whereupon P in $AlCl_3$ precipitated, which was followed by NaOH extraction and rotary evaporation (Bai, Zhou, Sun, Ma, & Zhao, 2015; Reitzel, Jensen, Flindt, & Andersen, 2009). However, concentrating the solvent containing P by rotary evaporation takes a long time, so a rapid and high enrichment method for concentrating DP is still needed. For the sake of efficiency of extraction, the methods of flocculation and lyophilization were combined for extracting DP in the present study.

To improve our knowledge on the role of organic P in the eutrophication of lakes, the characterization of internal dissolved and PP is the key. However, the forms of internal DP and PP in P sources and cycling in lakes with different trophic statuses are poorly understood. Therefore, we aimed at (a) investigating the major DP and PP fractions, the main forms of Mono-P and Di-P, in 10 lakes with different trophic statuses, for the first time, and discussing the differences of DP and PP forms and their levels in different trophic lakes, (b) exploring the effect of the environmental conditions of the lakes and the relationship between PP fractions and the trophic status by seeking a potential factor of limiting P; and (c) obtaining insight into the mechanisms of P biogeochemical cycling, as well as evaluating and providing a basis for eutrophication control in China.

2 | MATERIALS AND METHODS

2.1 | Study sites and sample collection

Ten freshwater lakes with different eutrophic statuses from the middle-lower reaches of the Yangtze River basin, the Yun-Gui Plateau, the Haihe Plain and the Beijing area were chosen for the study. These were four representative regions in China, which were in the south, south-west and north of China, respectively. The lake ecosystem not only provides water sources for plants and animals but also maintains the cycle of nutrients and primary productivity. However, eutrophication of lakes in China has become a serious environmental problem, especially in the lakes from the middle-lower reaches of the Yangtze River region. Specifically, Tai Lake is an important freshwater source for the Jiangsu Province in China and Meiliang Bay and Zhushan Bay are the most important eutrophic regions of Tai Lake. Chao Lake is an important freshwater lake located in Anhui Province. Dian Lake is the largest shallow lake in the Yun-Gui Plateau. Red Maple Lake and Aha Lake are deep lakes in the Yun-Gui Plateau, which are sources of drinking water and also used for generating electricity. Baiyangdian is the largest lake in the Haihe Plain, which is an important water source for local residents, but pollution problems have occurred in recent years. Lakes of the Ming Tombs, Shangzhuang and Shahe are typical water sources in Beijing, which are also troubled by problem of excessive P. To sum up, except for the Olympic Park Lake, the studied lakes were chosen as important drinking water sources for the local residents. In the last decade, algal bloom frequently occurred in Tai Lake, Chao Lake, Dian Lake and Baiyangdian Lake. Excessive algae lead to the eutrophication of the lake and deterioration of water. Once algal bloom occurs, the drinking water supply has to be broken off and people's health is threatened. The sampling time was chosen in May and June because phytoplankton is rapidly produced and accumulates during this period and water conditions obviously changes during the algal bloom. Detailed information of the study regions and lakes is given in Table 1 and Figure 1. The sampling number was 1 at a typical site in Chao Lake, Baiyangdian Lake, Red Maple Lake, Aha Lake, Olympic Park Lake, the Ming Tombs Lake, Shangzhuang Lake and Shahe Lake. For both Tai Lake and Dian Lake, sampling was done at two sites, because the Meiliang and Zhushan Bay of Tai Lake, the Caohai region and the coast of Dian Lake have been troubled by the eutrophication problem for long time. From each sampling site, we collected two samples for reproducible control.

In order to reflect the water properties accurately, water samples were collected at the depth of 0.5 m below the surface of water. Each sample was transferred into an acid-cleaned 25-L tank. Basic parameters of the water samples, including TP and inorganic P (IP), dissolved oxygen (DO), pH, total nitrogen (TN), chlorophyll-a (Chl-*a*), chemical oxygen demand (COD) and clarity, were determined. TP and IP were quantified by use of the molybdenum blue method, and TP in water was digested by potassium peroxy disulfate for 30 min at 120 °C (Murphy & Riley, 1962). TN, Chl-*a* and Secchi depth (SD) were analyzed using standard methods (EPA of China, 1989). DO, COD, pH and clarity were determined by a multi-parameter water quality monitor (HORIBA u-52, Japan) when collecting the samples.

2.2 | Enrichment and separation of DP and PP

In order to collect enriched DP samples quickly in batches, a modified method was developed for the enrichment of DP in the water samples. First, for specific operation, a water sample (20 L) was vacuum-filtrated by a GF/C filter (Whatman GF/C, 1822-150, UK), and then transferred into a container for flocculation and precipitation of DP. The particulates retained on the filter were transported to the laboratory in air-sealed plastic bags and stored at 25 °C after air-drying the PP.

Second, DP was enriched by precipitation combined with freeze-drying. A flocculating agent (4.0 g $AlCl_3$) (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) was added to the filtrated water sample for the adsorption and precipitation of DP. Quick flocculation was performed by the inverted conical bottom, and the precipitate was collected by centrifugation (Thermo Fisher Scientific, Waltham, USA) at 8000*g* for 20 min; 20 mL of 1 M NaOH + 0.05 M ethylenediaminetetraacetic acid (EDTA) was used for extracting DP for 16 h at 25 °C. The extracts were treated by freeze-drying for ^{31}P-NMR analyses. Third, PP in the particulate matter was extracted by 1 M NaOH + 0.05 M EDTA (20 mL) for 16 h. The samples were centrifuged at 8000*g* for 15 min, and the supernatants containing PP were collected. The extracts were also treated by freeze-drying for ^{31}P-NMR analyses. The freeze-dryer was purchased from Sim International Group Co. Ltd., China. NaOH and EDTA were purchased from Sinopharm Chemical Reagent Co.

Both lyophilization and rotary evaporation are effective preconcentration methods for P (Bai et al., 2015; Read et al., 2014). However, rotary evaporation can only treat a single sample at a time and has a low rate of water removal, leading to large time consumption. By contrast, lyophilization shows superiority in the preconcentration of P. Lyophilization is suitable for temperature-sensitive samples, and samples

TABLE 1 Geographic information of the studied lakes in four representative regions of China

Lake regions	Orientation	No.	Lakes	Positions	Sampling time
The middle-lower reaches of the Yangtze River basin	South of China	S1	Meiliang Bay of Tai Lake	31°25′N, 120°10′E	May 3, 2015
		S1	Zhushan Bay of Tai Lake	31°24′N,120°03′E	May 3, 2015
		S2	Chao Lake	30°56′–32°02′N, 117°00′ ~118°29′E	May 8, 2015
Yun-Gui Plateau	South-west of China	S3	Caohai of Dian Lake	25°01′N, 102°40′E	May 18, 2015
		S3	Coast of Dian Lake	24°55′N, 102°41′E	May 18, 2015
		S4	Red Maple Lake	38° 49′N, 116°04′E	May 20, 2015
		S5	Aha Lake	26°25″N, 106°20′E	May 21, 2015
Haihe Plain	North of China	S6	Baiyangdian Lake	26°30′N, 106°36′E	May 24, 2015
Beijing area	North of China	S7	Olympic Park Lake	40°44′N, 116°35′E	May 30, 2015
		S7	Ming Tombs Lake	40°05′N, 116°12′E	May 30, 2015
		S7	Shangzhuang Lake	40°00′N, 115°50′E	June 1, 2015
		S7	Shahe Lake	40°01′N, 116°23′E	June 2, 2015

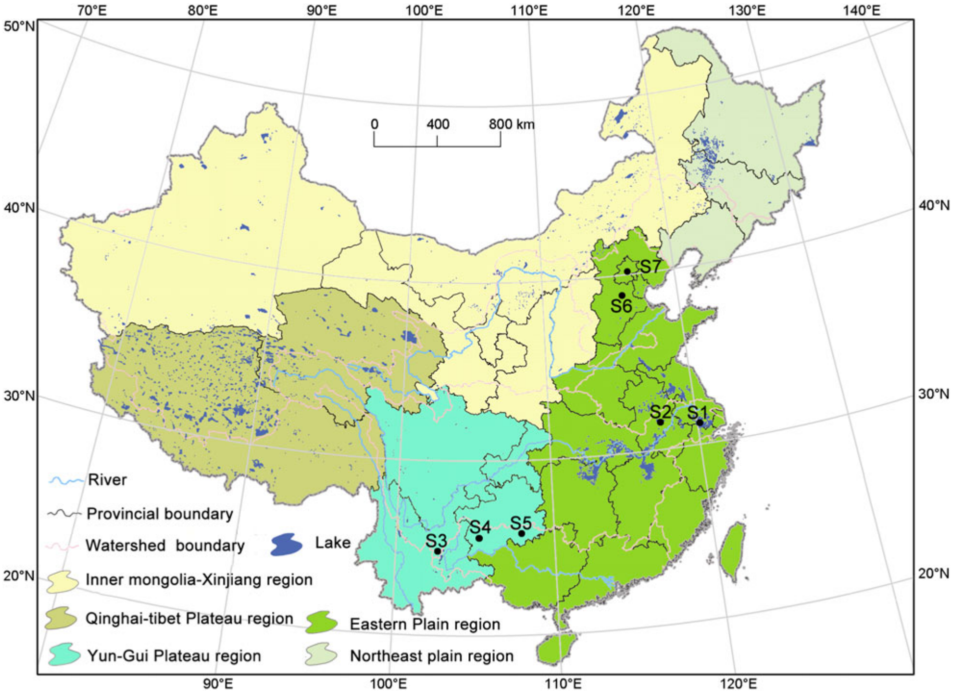

FIGURE 1 Geographic location of the sampling sites. S1: Tai Lake; S2: Chao Lake; S3: Dian Lake; S4: Red Maple Lake; S5: Aha Lake; S6: Baiyangdian Lake; S7: Lakes of Beijing

which could be kept completely and easily dissolved again. A potential negative effect could be the incomplete dissolution of the lyophilized powder, but in the present study the freeze-dried powder was dissolved 1 M NaOH + 0.01 M EDTA solution with ultrasonication for 30 min, and the samples were prepared and analyzed after most of the P dissolved in the solution.

2.3 | Solution ^{31}P-NMR analysis

Each lyophilized sample (300 mg powder) was redissolved in 1 mL of 1 M NaOH + 0.01 M EDTA solution with ultrasonication (Kunshan Ultrasonic Instrument Co. Ltd., Jiangsu, China) for 30 min. After the dissolution was complete, the solution was centrifuged at 8,000*g* for 30 min. The clear solution (0.5 mL) was transferred into a 5-mm NMR tube, and 0.1 mL of D_2O (Tokyo Chemical Industry, Tokyo, Japan) was added to the tube to lock the signal for NMR. All ^{31}P-NMR spectra were obtained on a Bruker AV 600 MHz spectrometer equipped with a 5-mm BBO probe (LabX Company, Mettler, Switzerland). The NMR parameters were as follows: 1.35 s acquisition time, 2.0 s relaxation delay, temperature 25°C and 242.96 MHz resonant frequency with no decoupling. The scan time was about 18 h for each sample. The spectra and integrations were analyzed by the MestReNova10.0 software (Tucson, University Libraries, Spain). About 85% of H_3PO_4 was used for recording the chemical shifts, and the assignment of the peaks was performed on the basis of literature values (Cade-Menun, 2015; Feng et al., 2015). For the waste disposal, the volume of each analysis sample was 0.6 mL in the 5-mm NMR tube, which is very small. Hence, the waste samples are usually diluted more than 1,000 times and uniformly reclaimed by the Beijing Nuclear Magnetic Resonance Center.

2.4 | Dissolved organic matter sources analysis

Dissolved organic matter (DOM) in the water samples from Tai Lake and Lakes Chao, Red Maple, Aha and Ming Tombs were analyzed by their three-dimensional fluorescence spectrum (3DEEM). Fluorescence was measured with 3D excitation/emission matrix spectroscopy (HITACHI F-7000, Hitachi High-Technologies Corporation, Japan) by a fluorescence spectrophotometer. The wavelength range was 200–400 nm for excitation and 250–500 nm for emission. A blank sample was prepared, and the excitation/emission (E_x/E_m) maximum value was identified.

3 | RESULTS AND DISCUSSION

3.1 | Trophic classification and DOM sources of water in different lakes

The physicochemical properties of the water samples were investigated (Table 2). The concentrations of TP were in the range 0.02–0.32 mg/L. Our investigation showed that TP, TN, Secchi depth (SD), COD and Chl-*a* are all critical factors reflecting the eutrophication status of the lakes. Especially, Chl-*a* is an indicator of phytoplankton biomass in the lakes, and therefore Chl-*a* was used as the primary estimator of the algal biomass and the standard index in the assessment of classification standard on lake eutrophication (Carlson, 1977; Huo et al., 2013). In order to clarify the pollution status of the

TABLE 2 Limnological features and trophic statuses of water in 10 different lakes of China

No.	Lakes	TP (mg/L)	TN (mg/L)	COD (mg/L)	pH	DO (mg/L)	Chl-*a* (μg/L)	Secchi depth (cm)	TSI	Eutrophic status	Polluted status
S1	Meiliang Bay of Tai Lake	0.15	2.0	121.6	8.9	11	46	22	63.7	Medium eutrophic	Heavily polluted
S1	Zhushan Bay of Tai Lake	0.32	4.5	91.5	8.0	8.0	46	70	65.3	Medium eutrophic	Heavily polluted
S2	ChaoLake	0.44	1.1	5.9	9.5	9.1	44	15	62.0	Medium eutrophic	Heavily polluted
S3	Caohai region of Dian Lake	0.32	5.3	7.8	8.5	7.8	55	54	52.7	Light eutrophic	Medium polluted
S3	Coast of Dian Lake	0.08	2.4	11	8.6	8.0	32	46	46.8	Mesotrophic	Lightly polluted
S4	Red maple Lake	0.09	2.5	7.0	8.6	9.8	7.5	10	46.8	Mesotrophic	Lightly polluted
S5	Aha Lake	0.05	1.5	7.1	8.4	9.9	8.6	10	46.3	Mesotrophic	Lightly polluted
S6	Baiyangdian Lake	0.02	1.8	7.5	8.0	8.1	27	10	43.4	Mesotrophic	Lightly polluted
S7	Olympic Park Lake	0.10	0.11	6.0	7.1	9.3	7.0	8.0	43.1	Mesotrophic	Lightly polluted
S7	Ming Tombs Lake	0.09	0.96	7.9	7.3	9.9	7.0	12	54.5	Light eutrophic	Medium polluted
S7	Shangzhuang Lake	0.13	1.5	38	7.6	7.9	14	15	57.9	Light eutrophic	Medium polluted
S7	Shahe Lake	0.23	2.1	42	7.1	6.9	16	16	37.0	Mesotrophic	Lightly polluted

Chl-*a*: chlorophyll-*a*; COD: chemical oxygen demand; TP: total phosphorus; TN: total nitrogen; TSI: trophic status index.

studied lakes, the trophic status index (TSI) was first obtained according to an identical calculation method based on the parameters TP, TN, SD, COD and Chl-*a*. The relationships between the parameter values and the relevant TSI were expressed by the following Equations (1)–(5):

$$\mathrm{TLI}(chl) = 10(2.50 + 1.86\ \ln chl - a) \tag{1}$$

$$\mathrm{TLI}(TP) = 10(9.44 + 1.62\ \ln TP) \tag{2}$$

$$\mathrm{TLI}(TN) = 10(5.45 + 1.69\ \ln TN) \tag{3}$$

$$\mathrm{TLI}(SD) = 10(5.12 - 1.94\ \ln SD) \tag{4}$$

$$\mathrm{TLI}(COD_{Mn}) = 10(0.11 + 2.66\ \ln COD_{Mn}) \tag{5}$$

The comprehensive trophic level index is then given by

$$\mathrm{TSI} = \sum\nolimits_{j=1}^{m} W_j \cdot \mathrm{TLI}(j) \sum\nolimits_{j=1}^{m} W_j \cdot \mathrm{TLI}(j)$$

$$W_j = W_j = r_{ij}^2 / \sum_{j=1}^{m} r_{ij}$$

Here, TLI(*chl*), TLI(*TP*), TLI(*TN*), TLI(*SD*) and TLI (COD_{Mn}) are the relevant TSIs calculated on the basis of Chl-*a*, TP, TN, SD and COD, respectively. TLI(*j*) is the TSI of Chl-*a*, TP, TN, SD, or COD; W_j is the relative weight of TSI. Meanwhile, the classification details of the trophic statuses of lakes were obtained (Huo et al., 2013).

The pollution status of the lakes was also identified under various trophic statuses (Table 2). As can be inferred from Table 2, Tai Lake and Chao Lake were mid-trophic and heavily polluted, while the other lakes were medium-polluted and lightly polluted.

A previous study had suggested that DOM is a potential source of bioavailable organic P (OP) in lakes (Liu et al., 2016). In order to clearly understand the potential source of dissolved organic phosphorus (DOP), the constituents and sources of DOM in several lakes were investigated by 3DEEM, and the index $f_{450/500}$ was calculated (Table 3).

The sources of DOM of Meiliang Bay, Zhushan Bay and Chao Lake were dominated by a tryptophan-like compound, while the primary DOM of the Red Maple Lake, Aha Lake and Ming Tombs Lake was fulvic-acid-like. $f_{450/500}$ is an index reflecting the sources of organic matter (OM): an $f_{450/500}$ index of 1.4 indicates terrestrial sources of OM, while an $f_{450/500}$ index of 1.9 indicates aquatic/microbiota as the source of OM (Jaffé et al., 2004). Values of the $f_{450/500}$ index of the studied lakes ranged from 1.6 to 1.8, which showed that the sources of DOM originated from a combination of terrestrial and aquatic/microbial products (Wu, Kothawala, Evans, Dillon, & Cai, 2007). The $f_{450/500}$ values of Tai Lake, Chao Lake, Red Maple Lake and Aha Lake were closer to 1.9, which indicated that aquatic/microbial products made a higher contribution to the DOM source; for example, the primary productivity, metabolism of bacteria and residues of algae were all possible sources of DOM. A statistically significantly positive Pearson coefficient (R^2 = 0.80**, $p < .01$) between concentrations of DOM and DP was observed, which showed that the DP sources were closely related to DOM in lake waters and that the sources of DP in studied lakes were extremely likely the import of terrestrial and aquatic/microbial sources.

3.2 | Qualitative and quantitative analyses of dissolved phosphorous (DP) in various trophic lakes

Based on the ^{31}P-NMR spectra of the filtered water samples (Figure 2) and the chemical shifts reported previously (Cade-Menun, 2005; Cade-Menun, 2015; Feng et al., 2015), DP was classified as phosphonate (Phos-P, 19–24 ppm), Ortho-P (5.7–6.1 ppm), Mono-P (3–6 ppm), Di-P (1–2.5 ppm) and condensed P including Pyro-P (−4.5–5.0 ppm) and Poly-P (−19–21 ppm). The

TABLE 3 Types, sources and concentrations of dissolved organic matter (DOM) in the studied lakes

No.	Lakes	$\lambda_{Ex/Em}$	f_{max}	$f_{450/500}$	DOM (mg/L)
S1	Meiliang Bay	230/340 tryptophan-like	362	1.79	8.66
S1	Zhushan Bay	232/346 tryptophan-like	391	1.74	13.9
S2	Chao Lake	225/352 tryptophan-like	671	1.76	12.4
		280/313 tyrosine-like	478	1.76	12.2
S4	Red Maple Lake	257/431 fulvic acid-like	156	1.69	4.28
S5	Aha Lake	254/428 fulvic acid-like	142	1.69	2.47
S7	Ming Tombs Lake	249/437 fulvic acid-like	117	1.61	2.12

concentrations and relative abundances of DP were investigated in typical eutrophic lakes (Table 4). After the identification of P, for the first time, the major DP and PP fractions, the main forms of Mono-P and Di-P, in 10 lakes with different trophic statuses were investigated. The differences in the DP forms and levels in different trophic lakes were also discussed. The findings will benefit further research of P transformation between the overlying water, particulate matter, and phytoplankton as well as the potential contribution of P species to lake eutrophication.

Ortho-P was the main constituent of DP in Zhushan Bay, Chao, Shangzhuang, Red Maple, Aha Lake and Olympic Park Lake, which accounted for about 12–127 μg/L (57–97%), while in Meiliang Bay of Tai Lake, the coast of Dian Lake, Baiyangdian, the Ming Tombs and Shahe Lake the predominant form of DP was Mono-P, concentrations of which were 15–50 μg/L (45–88%). In the Caohai region of Dian Lake, DP was dominated by Ortho-P (48%) and Mono-P (48%) equally. Concentrations of Di-P in the 10 lakes were in the range 0.16–40 μg/L (0.39–28%). Phosphonate (R-PO$(OH)_2$, Phos-P) was a relatively stable fraction, which constituted 0.68–7.7% of DP. Pyro-P ((OH)2OP-O-PO$(OH)_2$) was detected in Tai Lake, and Lakes Chao, Dianchi, Baiyangdian and Shahe, with relative proportions of DP ranging from 0.12% to 13%, while Poly-P was detected only in Meiliang Bay of Tai Lake (0.08%). To further understand the differences in the relative proportions and concentrations of DP fractions among the 10 lakes, the relationship between the forms of DP and eutrophication statuses was investigated (Figure 3).

In this study, the eutrophic statuses were classified according to the method used previously (Huo et al., 2013). The results showed that DP was dominated by Ortho-P (48–79%) and Mono-P (48–70%) equally, and Di-P was the second form of DP (2.9–28%). The rank order of concentrations of Ortho-P in various lake waters was mid-eutrophic > light-eutrophic > mesotrophic, but the relative ratio (%) was almost the same in lakes with different trophic statuses. Ortho-P was the most direct nutrition source for algae and phytoplankton, but excessive amounts of Ortho-P inevitably led to algal bloom of the lake. The concentration of Mono-P was greater in mid-eutrophic (heavy polluted) and light-eutrophic (medium polluted) lakes. Mono-P was also an important component of DP, and labile Mono-P might be

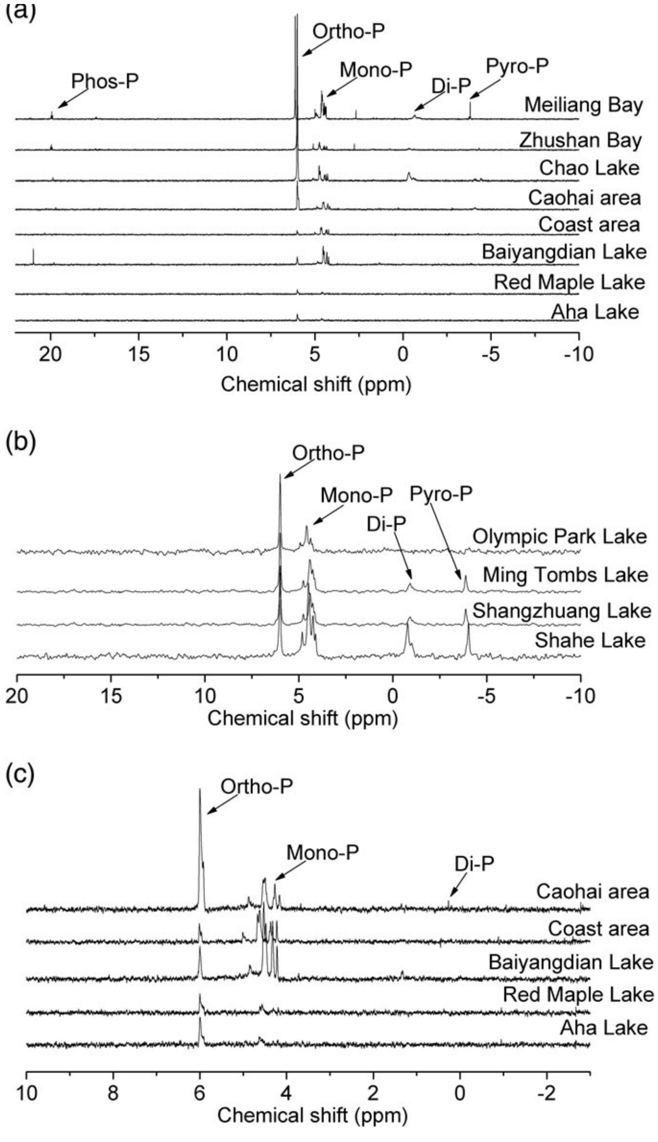

FIGURE 2 ^{31}P nuclear magnetic resonance (^{31}P-NMR) spectra of dissolved phosphorus (DP) in trophic lakes of China: the classes include phosphonate (Phos-P), orthophosphate (Ortho-P), monoester phosphate (Mono-P), diester phosphate (Di-P), pyrophosphate (Pyro-P) and polyphosphate (Poly-P). (a) DP forms of Meiliang Bay and Zhushan Bay of Tai Lake, Chao Lake, Caohai, and Coast area of Dian Lake, Baiyangdian Lake, Red Maple Lake and Aha Lake. (b) DP forms of the lakes in Beijing including Olympic Park Lake, Ming Tombs Lake, Shangzhuang Lake and Shahe Lake. (c) Magnified ^{31}P-NMR spectra of Ortho-P, Mono-P and Di-P in Caohai and the coastl area of Dian Lake, Baiyangdian Lake, Red Maple Lake and Aha Lake

TABLE 4 Concentrations of different DP fractions detected by ^{31}P-NMR and their ratios to TP in lake waters

			Phos-P		Ortho-P		Mono-P		Di-P		Pyro-P		Poly-P	
Lakes	**No.**	**Sites**	**µg/L**	**%**	**µg/L**	**%**	**µg/L**	**%**	**µg/L**	**%**	**µg/L**	**%**	**µg/L**	**%**
Tai Lake	S1	Meiliang Bay	1.5	2.6	8.6	15	50	70	5.0	8.5	2.3	3.9	0.050	0.085
	S1	Zhushan Bay	3.3	2.1	127	79	25	16	3.5	2.2	1.1	0.70	n.d.	n.d.
	S2	Chao Lake	0.97	0.68	77	54	21	15	40	28	3.5	2.4	n.d.	n.d.
Dian Lake	S3	Caohai region of Dian Lake	1.1	0.83	62	48	62	48	1.9	1.5	0.16	0.12	n.d.	n.d.
	S3	Coast of Dian Lake	0.54	1.4	2.9	7.2	35	88	0.16	0.39	0.91	2.3	n.d.	n.d.
	S4	Red Maple Lake	n.d.	n.d.	29	57	19	38	1.5	2.9	n.d.	n.d.	n.d.	n.d.
	S5	Aha Lake	n.d.	n.d.	12	73	4.1	24	0.30	1.8	n.d.	n.d.	n.d.	n.d.
	S6	Baiyangdian Lake	1.5	7.7	2.6	13	15	75	0.73	3.6	0.12	0.61	n.d.	n.d.
Artificial lakes in Beijing	S7	Ming Tombs Lake	n.d.	n.d.	19	32	31	52	9.8	16	n.d.	n.d.	n.d.	n.d.
	S7	Shangzhuang Lake	n.d.	n.d.	35	71	12	23	1.7	3.4	n.d.	n.d.	n.d.	n.d.
	S7	Shahe Lake	n.d.	n.d.	20	20	45	45	18	18	13	13	n.d.	n.d.
	S7	Olympic Park Lake	n.d.	n.d.	72	80	18	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Di-P: diester phosphate; Mono-P: monoester phosphate; n.d.: not detected; Ortho-P: orthophosphate; Phos-P: phosphonate; Poly-P: polyphosphate; Pyro-P: pyrophosphate.

hydrolyzed into Ortho-P in the presence of alkaline phosphatases. This result implied that the control of dissolved Ortho-P and Mono-P should be given more attention in eutrophic lakes, especially heavy polluted lakes. The abundance of Pyro-P and Poly-P was less than that of the other forms of DP. Both Pyro-P and Poly-P were the labile fractions of P, but Pyro-P came partially from esters, which could be hydrolyzed under alkaline conditions (Ahlgren et al., 2005). Poly-P was also very unstable, and its short half-life meant that it could be easily transformed into other forms of P and combined with metal ions in surface waters (Hupfer et al., 2004; Reitzel et al., 2006). It has been reported that Poly-P could be completely hydrolyzed into Pyro-P and partially into Ortho-P under alkaline condition and in the presence of metal ion catalysts (Ahlgren et al., 2005). Pyro-P and Poly-P are both easily utilized by the majority of phytoplankton, such as cyanobacteria, for primary storage of energy, but their small abundance in water limited their absorption by phytoplankton (Kromkamp, 1987).

DP forms showed obvious differences among the lakes from Yangtze River basin, Yun-Gui Plateau, Haihe Plain and Beijing area. First, geographical differences decided the different abundances of DP. Internal cycling of P is also dependent on the local climatic conditions and external nutrient loading. Second, the characterization of DP content in lakes was closely related to the eutrophic status and pollution degree, as well as human's activities such as the discharge of sewage and the use of farm fertilizers. Third, the mean depth of lakes was also a factor influencing the DP forms in a lake. Internal recycling of P in shallow lakes was especially high because of the transformation of nutrients at the sediment–water interface (Jensen & Andersen, 1992; Søndergaard et al., 1992), while the internal cycling in deeper lakes was less because of stratification, which could isolate sediments from the epilimnion even though P

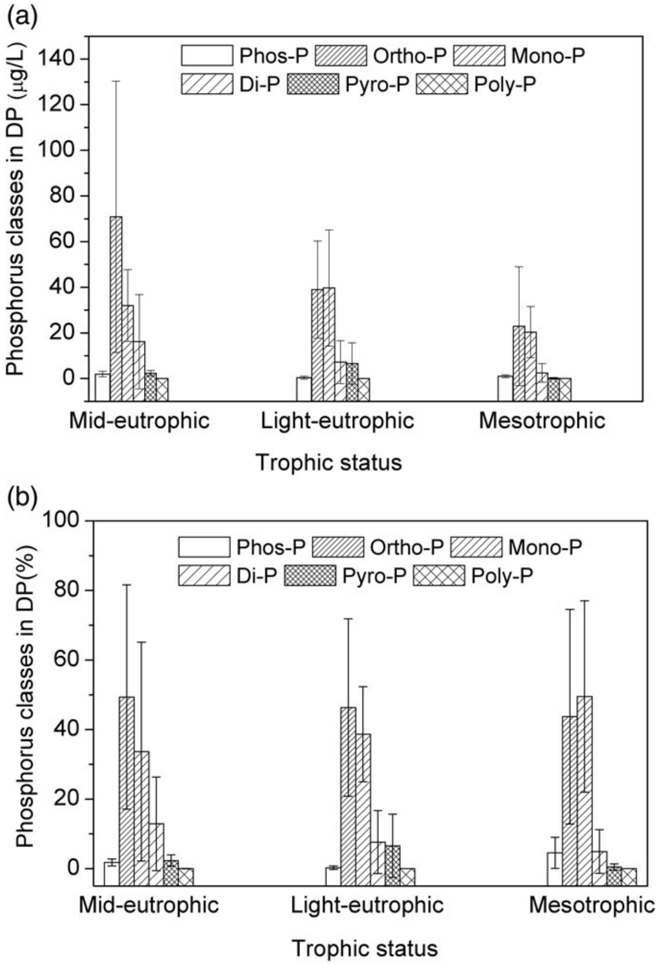

FIGURE 3 Differences in dissolved phosphorus (DP) forms in various lakes with different trophic statuses. The main forms of DP include phosphonate (Phos-P), orthophosphate (Ortho-P), monoester phosphate (Mono-P), diester phosphate (Di-P), pyrophosphate (Pyro-P) and polyphosphate (Poly-P). (a) Concentration (µg/L) variations of the main forms of DP with mid-eutrophic, light-eutrophic and mesotrophic statuses. (b) Relative ratio (%) variations of the main forms of DP with mid-eutrophic, light-eutrophic, and mesotrophic statuses

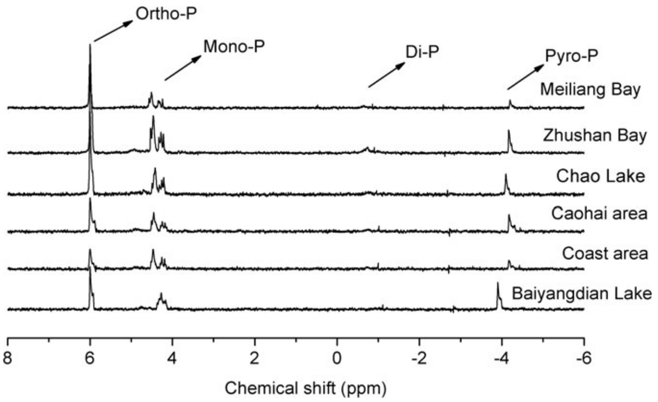

FIGURE 4 ^{31}P nuclear magnetic resonance (^{31}P-NMR) spectra of particulate phosphorus in typical lakes of Meiliang Bay and Zhushan Bay of Tai Lake, Chao Lake, Caohai and coastal area of Dian Lake and Baiyangdian Lake. The classes include orthophosphate (Ortho-P), monoester phosphate (Mono-P), diester phosphate (Di-P) and pyrophosphate (Pyro-P)

accumulates in the hypolimnion (Soranno, Carpenter, & Lathrop, 1997).

3.3 | Quantification of PP fractions in the various trophic lakes

We can infer from the ^{31}P-NMR spectra of PP (Figure 4) and chemical characterizations (Table 5) that Ortho-P is the predominant form of PP in Meiliang and Zhushan Bays of Tai Lake, Lakes Baiyangdian and Aha, with concentrations in the range 4.5–73 μg/L and relative proportions of 37–59%. PP in Chao Lake and the coast of Dian Lake was dominated by Mono-P, with concentrations of 21–35 μg/L and relative proportions of DP of 53–58%. Only in the Caohai region of Dian Lake was PP mainly consisted of Ortho-P and Mono-P, with concentrations and relative proportions of 73 μg/L (37%) and 79 μg/L (40%), respectively.

Concentrations of Phos-P, Ortho-P, Mono-P and Pyro-P followed the rank order of the trophic status in particulate matter: mid-eutrophic > light-eutrophic > mesotrophic (Figure 5).

PPs were found to be significantly correlated with Chl-*a* ($R^2 = 0.634$) in Pearson correlation. In eutrophic lakes, PP was controlled by biogenic P (P in living/dead phytoplankton and bacterial cells), which could be employed as a proxy for internal P sources of phytoplankton and might point to physiological nutrient status of a lake system (Read et al., 2014; Shinohara et al., 2016). This result suggested that the ratio of chemical forms of PP in lakes indirectly reflected the pollution status of the lakes. In mid-eutrophic and light-eutrophic lakes, the components of PP were equally dominated by Ortho-P and Mono-P, but in mesotrophic lakes Ortho-P was the predominant form of PP. The above result indicated that Ortho-P and Mono-P played an important role in the primary production in eutrophic lakes, but particulate Mono-P also tended to accumulate in mid-eutrophic (heavily polluted) lakes rather than mesotrophic (lightly polluted) lakes, as well as dissolved Mono-P. Mono-P was one of the primary components of PP, which was more stable than Di-P in particulate matter, but it could be mineralized swiftly to Ortho-P under anaerobic conditions.

3.4 | Analysis of the sources of main fractions of mono-P and Di-P

The concentrations and relative abundances of dissolved and particulate Mono-P and Di-P were also investigated in this study (Tables 6 and 7), and the results are compared with the spiking studies and chemical shifts previously reported (Cade-Menun, 2015).

α-Glycerophophate (α-Gly), β-glycerophosphate (β-Gly), guanosine 2′ Mono-P, and inositol hexakisphosphate (IHP) were the main forms of Mono-P in water and particulate matter. α-Gly (32.5%) and β-Gly (23.4%) were the predominant forms of dissolved Mono-P in the Meiliang Bay of Tai Lake and Baiyangdian Lake, respectively. Both α-Gly and β-Gly were active Mono-P and the main degradation products of labile diesters such as deoxyribonucleic acid (DNA) and phospholipids (Liu et al., 2016). There was transformation between Di-P and Mono-P: a large amount of bioavailable Di-P degraded into Mono-P by algae and phytoplankton, and then the degraded Mono-P was released into the water again in Meiliang Bay and Baiyangdian Lake during the algal bloom. IHP was an important component in Mono-P, which obviously tended to accumulate in the lake ecosystem. The results showed the IHP took up about 24.1–36.4% and 8.3–26.0% in surface water and particulate matter, respectively. IHP was the main storage form of P in

TABLE 5 Concentrations of different PP fractions detected by ^{31}P-NMR and their ratios to TP in lake water

		Phos-P		Ortho-P		Mono-P		Di-P		Pyro-P		Poly-P	
No.	DP	μg/L	%	μg/L	%	μg/L	%	μg/L	%	μg/L	%	μg/L	%
S1	Meiliang Bay	n.d.	n.d.	45	50	33	36	1.6	1.8	9.0	10	0.87	0.97
S1	Zhushan Bay	n.d.	n.d.	67	45	60	40	5.6	3.7	17	11	0.18	0.12
S2	Chao Lake	n.d.	n.d.	22	37	34	58	2.5	4.2	0.25	0.42	n.d.	n.d.
S3	Caohai region of Dian Lake	n.d.	n.d.	73	37	79	40	3.6	1.8	42	21	n.d.	n.d.
S3	Coast of Dian Lake	n.d.	n.d.	12	30	21	53	0.40	0.99	5.9	15	n.d.	n.d.
S6	Baiyangdian Lake	n.d.	n.d.	4.3	43	1.8	18	0.19	1.9	3.7	37	n.d.	n.d.

Di-P: diester phosphate; DP: dissolved phosphorus; Mono-P: monoester phosphate; n.d.: not detected; Ortho-P: orthophosphate; Phos-P: phosphonate; Poly-P: polyphosphate; Pyro-P: pyrophosphate.

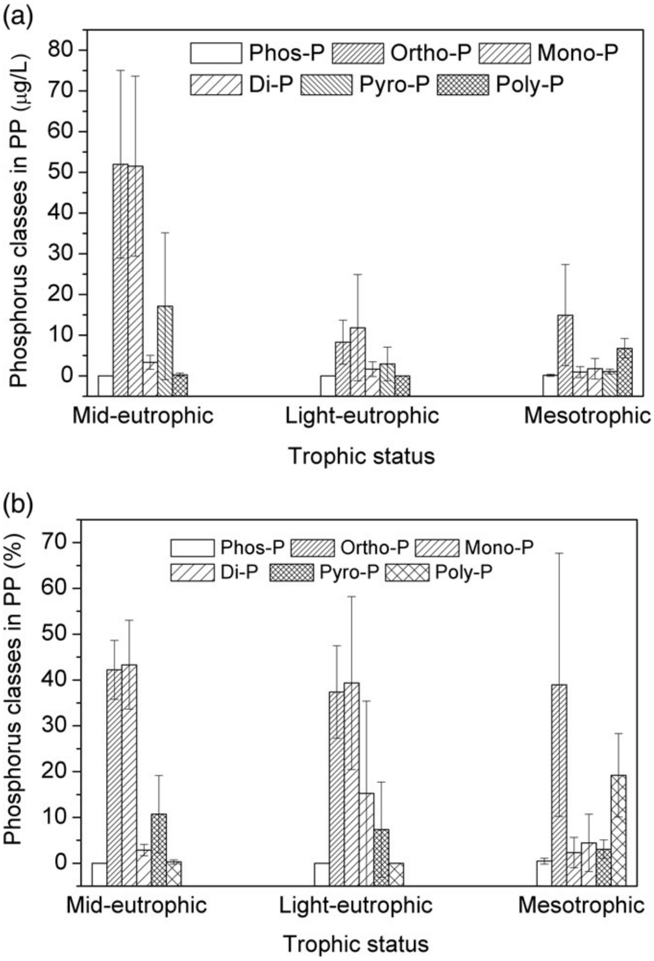

FIGURE 5 Differences in particulate phosphorus (PP) forms in various lakes with different trophic statuses. The main forms of dissolved phosphorus (DP) are phosphonate (Phos-P), orthophosphate (Ortho-P), monoester phosphate (Mono-P), diester phosphate (Di-P), pyrophosphate (Pyro-P) and polyphosphate (Poly-P). (a) Concentration (μg/L) variations of the main forms of PP with mid-eutrophic, light-eutrophic and mesotrophic statuses. (b) Relative ratio (%) variations of main forms of PP with mid-eutrophic, light-eutrophic and mesotrophic statuses

plant tissue (especially seeds), and some IHP (*neo*-IHP and *chiro*-IHP) existed in terrestrial and aquatic/microbial environments (Klamt, Jensen, Mortensen, Schreiber, & Reitzel, 2017; Liu et al., 2016; Zhu et al., 2015). The high ratio of IHP indicated that aquatic organisms and vegetation near the lakes grew prosperously. Hence, it was deduced that IHP in lakes mainly originated from terrestrial and aquatic/microbial sources. Nicotinamide adenine dinucleotide (NAD) was a coenzyme in the redox reaction, and guanosine 2′ and 3′ Mono-P would be the biodegradation products separated from the metabolites of cells. We can infer from the result that the particulate matter of Zhushan Bay, Caohai of Dian Lake and Coast of Dian Lake contained abundant NAD (17.3–30.3%). The metabolism of aquatic organisms was active in the ecosystem of Dian Lake, and algal bloom was happening due to the utilization of some phosphate forms. Di-P was a mixture of multiple P compounds such as phospholipids, DNA and teichoic acid. In the studied lakes, teichoic acid and DNA were the primary forms of Di-P and they were closely related to the amount of microorganism, which indicated that Di-P in the studied lakes was mainly an aquatic or microbial product. Meanwhile, DNA was usually from bacteria and decomposing phytoplankton, which also indicated the bacterial abundance in the aquatic environment.

3.5 | Relationship between DP fractions and characteristics of the water environment

In this study, in order to better understand the relationship between the forms of DP and the properties of water, a Pearson (pairwise) correlation analysis was conducted (Table 8).

Ortho-P, Di-P (teichoic acid and DNA), Pyro-P and Poly-P were significantly correlated with TDP, which indicated that the concentrations of Ortho-P, Di-P (teichoic acid and DNA), Pyro-P and Poly-P directly affected the amount of TDP in the studied lakes. The concentrations of Di-P (teichoic acid and DNA), Pyro-P and Poly-P were significantly influenced by the pH. This was because some species of Di-P such as DNA, Pyro-P and Poly-P were labile P forms and tended to hydrolyze under appropriate pH and alkaline conditions (Xu et al., 2013). For example, the hydrolysis of Di-P was pH-dependent in the range 7.0–9.0. Meanwhile, pH control is also a crucial method to reduce nitrogen and TP of water in the algae/bacteria system (Liang et al., 2013). We can infer from the above result that an appropriate pH value is an important condition to maintain a healthy ecosystem and relieve the problem of eutrophication. Concentrations of Phos-P and Mono-P were both significantly correlated with Chl-*a* of the lakes. Phos-P and Mono-P are closely related to aquatic organisms and have potential bioavailability under suitable conditions. The labile Mono-P might be hydrolyzed to Ortho-P by alkaline phosphatases, and Ortho-P could be absorbed by phytoplankton (Zhu, Wu, Feng, Liu, & Giesy, 2016). In natural lakes, acid phosphatase usually occurs in algal cells and adjusts the internal metabolism of P, whereas alkaline phosphatase is mainly focused on external synthesis and the excretion of P (Zhou et al., 2008; Zhu et al., 2013). Phos-P was a relatively stable fraction of DP, and the strength of the C—P bond in their structure makes their chemical and enzymatic hydrolysis difficult, but a recent study has shown that Phos-P can be an alternative source of P for marine microorganisms (Martinez, Gene, & Delong, 2010). It was deduced that Phos-P might be utilized as a source of P under special conditions, such as when the C—P bond is broken by unknown microorganisms.

In the lakes, the concentrations of Ortho-P and DNA were found to be significantly related to TOC. The abundance of aquatic organisms easily affected the DNA and the accumulation of TOC in lakes (Zhang, Wu, He, Zheng, & Song, 2009). Ortho-P was one of the primary forms of DP that was easily utilized by phytoplankton and algae, so the

TABLE 6 Concentrations (μg/L) and ratios (% of total dissolved phosphorus (DP)) of dissolved Mono-P and Di-P in the 10 lakes in China

No. P fractions		S1 Meiliang Bay	S1 Zhushan Bay	S2 Chao Lake	S3 Caohai of Dian Lake	S3 Coast of Dian Lake	S4 Red Maple Lake	S5 Aha Lake	S6 Baiyangdian Lake	S7 Ming Tombs Reservoir	S7 Shangzhuang reservoir	S7 Shahe reservoir	S7 Olympic Park Lake
Mono-P	α-Glyc	19.2(32.5)	n.d.	n.d.	7.9(6.1)	n.d.	n.d.	n.d.	0.5(4.9)	n.d.	2.5(5.0)	11.3(11.3)	n.d.
	β-Glyc	1.2(2.1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.3(23.4)	n.d.	n.d.	n.d.	n.d.
	Gyanosine 2′ Mono-P	8.7(14.7)	4.1(2.6)	n.d.	38.0(29.3)	n.d.	n.d.	n.d.	n.d.	n.d.	7.8(15.6)	20.2(20.2)	n.d.
	NAD	6.4(10.9)	1.3(0.8)	3.4(2.4)	n.d.	14.4(35.9)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Guanosine 3′ Mono-P	0.8 (1.3)	n.d.	n.d.	9.9 (7.6)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	IHP	n.d.	14.9 (9.3)	6.4 (4.5)	n.d.	11.7 (29.4)	16.6 (33)	4.1 (24.1)	3.2 (31.2)	21.9 (36.4)	0.3 (0.7)	7.6 (7.6)	18.1 (20.1)
	D-G6P	n.d.	3.9 (2.4)	2.0 (1.4)	1.5 (1.2)	1.3 (3.1)	n.d.	n.d.	1.0 (9.8)	n.d.	0.9 (1.9)	n.d.	n.d.
	D-F6P	n.d.	n.d.	n.d.	n.d.	6.0 (15.0)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Others	5.2 (8.9)	0.8 (0.5)	4.2 (2.9)	4.9 (3.8)	2.0 (5.0)	2.5 (5.1)	9.3 (15.5)	0.6 (5.9)	n.d.	n.d.	6.3 (6.3)	n.d.
Di-P	Teichoic acid	0.6 (1.0)	1.2 (0.7)	21.1 (14.8)	n.d.	n.d.	n.d.	n.d.	0.4 (3.6)	n.d.	1.1 (2.3)	n.d.	n.d.
	DNA	4.4 (7.5)	2.3 (1.4)	14.9 (10.4)	1.9 (1.5)	0.2 (0.4)	1.5 (2.9)	0.3 (1.8)	n.d.	9.8 (16.3)	0.3 (0.6)	17.7 (17.7)	n.d.
	Others	n.d.	n.d.	4.4 (3.1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1 (0.3)	n.d.	n.d.

%: the ratio of Mono-P and Di-P in DP; D-F6P: D-fructose 6-phosphoate; D-G6P: D-glucose 6-phosphate; DNA: deoxyribonucleic acid; gyanosine 2′ Mono-P: gyanosine 2′ monophosphonate; gyanosine 3′ Mono-P: guanosine 3′ monophosphonate; IHP: inositol hexakisphosphate; NAD: nicotinamide adenine dinucleotide; α-Glyc: α-glycerophophate; β-Glyc: β-glycerophophate.

concentration of Ortho-P in water was affected by the metabolic activities of phytoplankton under different conditions (Agawin, Duarte, & Agustí, 2000; Karlsson & Brunberg, 2004). This result suggests that there is a close relationship between the sources of TOC and P forms of Ortho-P and DNA and that aquatic/microbial products might be the primary sources of TOC, Ortho-P and DNA. This is in accordance with the result that the DOM source in some lakes was mainly from aquatic/microbial products.

3.6 | Relationship between PP fractions and the trophic status of lakes

PPs fractions are important components related to phytoplankton and algae. Our study indicated that PP might be used as a proxy for internal P sources in a cyanobacteria-dominated lake, which is a potential source of bioavailable P (Løvdal et al., 2007; Read et al., 2014). Hence, to further understand the relationship of PP fractions and the trophic status of lakes, correlation analyses between characteristics of PP and TP, Chl-*a* and TSI (Huo et al., 2013) in lakes were conducted (Table 9).

The result of correlation analysis showed that Mono-P/PP was significantly positive correlated with Chl-*a* ($R^2 = 0.459$), TP ($R^2 = 0.586$) and TSI ($R^2 = 0.588$), whereas Poly P/PP was negatively correlated with Chl-*a* ($R^2 = -0.447$). This result indicated the Mono-P and Poly P fractions had important contributions to the trophic state of lake waters. Previous knowledge had indicated that P could be present as nucleic acids in living algae cells, such as lipids, lipopolysaccharides and cytoplasmic solutes (Zhu et al., 2013). Mono-P was the main content of lipids and lipopolysaccharides, and Poly-P was contained in cytoplasmic solutes. Significant correlation between Mono-P/PP and Chl-*a*, Poly-P/PP and Chl-*a* and Mono-P/PP and TP showed that particulate Mono-P and Poly-P had potential bioavailability in surface waters.

According to the result of DP and PP in the lake waters, we can generalize the cycling of DP and PP in a eutrophic lake (Figure 6). Among the P forms of Phos-P, Ortho-P, Mono-P, Di-P, Pyro-P and Poly-P in water and particulate matter, Ortho-P was an important fraction of DP and PP in water, which was directly utilized by algae. Meanwhile, soluble OP in the sediment could be hydrolyzed by phosphatase and then Ortho-P was released into water, which also provided nutrition for phytoplankton and macrophytes. Dissolved and particulate Mono-P was more liable to hydrolyzing into IP during blooms of phytoplankton, especially in eutrophic lakes (Feng et al., 2018), which had a potential bioavailability for phytoplankton. For DP, both of Mono-P and Phos-P had potential bioavailability. Particulate Mono-P and Poly-P tended accumulate in living algal cells and provided the energy for cell's activity and metabolism, whereas Mono-P processed a potential contribution to lake eutrophication. In the decomposing debris of aquatic

TABLE 7 Concentrations (μg/L) and ratios (% of total PP) of particulate Mono-P and Di-P in the 10 lakes in China

	S1		S1		S2		S3		S3		S6	
	Meiliang Bay		Zhushan Bay		Chao Lake		Caohai of Dian Lake		Coast of Dian Lake		Baiyangdian Lake	
No. P fractions	μg/L	%	μg/L	%	μg/L	%	μg/L	%	μg/L	%	μg/L	%
Mono-P												
α-Glyc	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.1	2.7	1.1	2.7	n.d.	n.d.
β-Glyc	n.d.	n.d.	8.8	6.1	0.8	1.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gyanosine 2′ Mono-P	18.7	20.7	n.d.	n.d.	17.0	28.4	6.7	3.5	n.d.	n.d.	n.d.	n.d.
NAD	n.d.	n.d.	24.6	17.3	n.d.	n.d.	37.1	19.5	12.1	30.3	n.d.	n.d.
IHP	14.2	15.7	23.3	16.3	13.2	22.1	18.9	8.3	4.3	10.8	2.6	26.0
Others	n.d.	n.d.	n.d.	n.d.	3.1	5.2	9.1	4.8	2.9	7.2	n.d.	n.d.
Di-P												
Teichoic-acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	0.5	n.d.	n.d.
DNA	1.6	1.8	5.3	3.7	2.5	4.2	6.0	3.2	0.4	1.0	0.08	0.8
Others	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.1	2.6	n.d.	n.d.

DNA: deoxyribonucleic acid; Gyanosine 2′ Mono-P: Gyanosine 2′ monophosphonate; IHP: inositol hexakisphosphate; n.d.: not detected; NAD: nicotinamide adenine dinucleotide; α-Glyc: α-glycerophophate; β-Glyc: β-glycerophophate.

TABLE 8 Relationships between the concentration of different P species and parameters of lake water samples

	Water parameters											
Phosphorus forms	TP	TDP	Temperature	Conductivity	pH	Clarity	Chl-*a*	COD	DO	TOC	TN	NH_4^+-N
Phos-P	0.25	0.20	0.19	0.40	−0.07	0.16	**0.73***	0.65	−0.05	0.24	−0.14	0.18
Ortho-P	**0.84***	**0.74***	0.52	−0.66	0.56	0.13	0.14	−0.28	0.26	**0.82****	0.36	0.34
Mono-P	−0.23	0.37	0.33	−0.07	0.28	0.15	**0.75***	**0.75***	0.46	−0.29	−0.19	−0.07
Di-P	0.57	**0.84****	0.36	−0.54	**0.84***	−0.24	0.23	−0.14	0.56	0.68	−0.02	−0.15
Pyro + poly-P	0.45	**0.86****	0.51	−0.47	**0.81****	−0.09	0.64	0.53	0.70	0.44	−0.18	−0.09
α-Glyc	−0.06	−0.10	−0.17	0.32	0.03	−0.26	0.26	0.66	0.29	−0.07	−0.38	−0.29
β-Glyc	−0.26	0.25	0.29	−0.03	0.14	0.19	0.62	**0.78***	0.46	−0.40	−0.26	0.02
Gyanosine 2′ Mono-P	−0.29	−0.44	−0.58	**0.91****	−0.48	−0.30	−0.04	−0.06	−0.35	−0.16	−0.34	−0.26
NAD	0.01	0.04	0.44	−0.20	0.19	0.36	0.20	0.13	−0.23	0.42	0.67	−0.04
Guanosine 3′ Mono-P	−0.37	−0.45	−0.63	**0.92****	−0.46	−0.36	−0.11	−0.18	−0.37	−0.20	−0.35	−0.34
IHP	0.36	0.05	0.27	−0.43	−0.10	0.41	−0.27	−0.40	−0.31	0.29	0.66	0.50
D-G6P	0.67	0.26	0.54	0.00	−0.23	0.68	0.48	−0.03	−0.54	0.67	0.59	**0.80***
D-F6P	−0.22	−0.19	0.28	−0.05	−0.06	0.41	−0.01	−0.16	−0.51	0.22	**0.73***	−0.02
Teichoic-acid	0.58	**0.84***	0.39	−0.53	**0.80***	−0.18	0.23	−0.19	0.49	0.69	0.02	−0.09
DNA	0.64	**0.82***	0.34	−0.48	**0.79***	−0.25	0.26	−0.06	0.58	**0.72***	−0.04	−0.11

Chl-*a*: chlorophyll-*a*; D-F6P: D-fructose 6-phosphoate; D-G6P: D-glucose 6-phosphate; DNA: deoxyribonucleic acid; DO: dissolved oxygen; Gyanosine 2′ Mono-P: Gyanosine 2′ monophosphonate; gyanosine 3′ Mono-P: guanosine 3′ monophosphonate; IHP: inositol hexakisphosphate; NAD: nicotinamide adenine dinucleotide; TDP: total dissolved phosphorus; TN: total nitrogen; TOC: total dissolved carbon; TP: total phosphorus; α-Glyc: α-glycerophophate; β-Glyc: β-glycerophophate.
*Correlation is significant at the 0.05 level (two-tailed).
**Correlation is significant at the 0.01 level (two-tailed).

macrophytes and phytoplankton, a part of the OP was released into water and transformed into dissolved Ortho-P and a part of the OP precipitated into the sediments of lakes.

TABLE 9 Correlations of PPs fractions with Chl-*a*, TP and trophic status index (TSI)

	Ortho-P	Mono-P	Di-P	Pyro-P	Poly-P
Chl-*a*	−0.023	0.459*	0.090	0.224	0.447*
TP	−0.175	0.586**	−0.235	0.117	0.222
TSI	0.006	0.588**	0.281	0.262	0.389

Chl-*a*: chlorophyll-*a*; Di-P: diester phosphate; Mono-P: monoester phosphate; Ortho-P: orthophosphate; Poly-P: polyphosphate; Pyro-P: pyrophosphate; TP: total phosphorus; TSI: trophic status index.
*$p < 0.05$.; **$p < 0.01$.

Previous study had suggested that no matter what the mechanism of eutrophication, prevention of Poly-P accumulation was not an effective measure for avoiding algal blooms (Read et al., 2014). Therefore, the role of Mono-P in eutrophic lakes should be paid more attention to, especially in heavily polluted lakes. The above results also showed particulate Mono-P was a limiting factor for lake eutrophication, as important as dissolved Mono-P. Obvious reduction of P in a short time cannot improve the eutrophication of lakes, but it should be done over a long time for keeping the balance of the algae–bacteria system, for which the suitable pH is required (Liang et al., 2013).

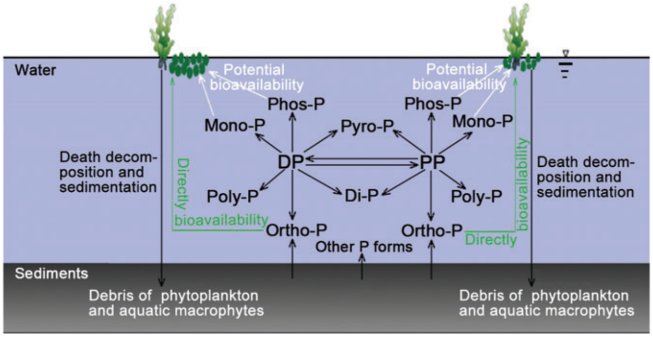

FIGURE 6 Cycling of dissolved phosphorus (DP) and particulate phosphorus (PP) fractions in water system of a eutrophic lake. The main classes of DP and PP are phosphonate (Phos-P), orthophosphate (Ortho-P), monoester phosphate (Mono-P), diester phosphate (Di-P), pyrophosphate (Pyro-P) and polyphosphate (Poly-P)

4 | CONCLUSIONS

Characterizations of DP and PP in 10 freshwater lakes with contrasting ecological and eutrophication statuses were investigated by ^{31}P-NMR. The following conclusions could be drawn: (a) In the 10 freshwater lakes studied, Ortho-P (43–79%) and Mono-P (40–88%) were the predominant forms of DP and PP. A greater level of Ortho-P and Mono-P was found in mid-eutriphic lakes than lightly eutriphic or mesotrophic lakes. α-Gly (2.7–32.5%), β-Gly (1.3–23.4%), guanosine 2′ Mono-P (20.2–29.3%), NAD (17.3–35.9%) and IHP (31.2–36.4%) were the primary compositions of Mono-P in water and particulate matter. Di-P was dominated by teichoic acid (0.5–14.8%) and DNA (0.6–17.7%). In the lake waters, dissolved Mono-P fractions originated from a combination of terrestrial and aquatic/microbial sources, whereas Di-P mainly originated from aquatic/microbial products. (b) In addition, correlation analysis of DP fractions and water conditions showed that pH was a crucial factor in controlling Di-P, Pyro-P, and Poly-P in the aquatic environment, and a suitable pH was necessary in a healthy ecosystem. Phos-P and Mono-P had potential bioavailability for algae and phytoplankton under appropriate conditions. (c) Mono-P/PPs was significantly positively correlated with Chl-*a* (R^2 = 0.459), TP (R^2 = 0.586) and TSI (R^2 = 0.588), which showed Mono-P was a potential nutrition source for algae. In other words, both of Ortho-P and Mono-P could contribute to lake eutrophication. The present study is beneficial for further exploring the circulation of DP and PP in the lake ecosystem and controlling the sources of eutrophic contributors. It also showed that new techniques are necessary to prevent lake eutrophication.

ACKNOWLEDGMENTS

The authors thank the Taihu Laboratory of Lake Ecosystem Research, Nanjing Institute of Geography & Limnology of the Chinese Academy of Sciences and the Institute of Geochemistry of the Chinese Academy of Sciences for assistance with sampling and experiments. The authors also wish to thank Prof. John P. Giesy and He Z for language help, which made the manuscript more readable and understandable. This work was supported by the National Natural Science Foundation of China (grant nos 41630645, 41573126 and 41703115).

REFERENCES

Agawin, N. S. R., Duarte, C. M., & Agustí, S. (2000). Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. *Limnology and Oceanography*, *45*, 1–600. https://doi.org/10.4319/lo.2000.45.8.1891a

Ahlgren, J., Tranvik, L., Gogoll, A., Waldebäck, M., Markides, K., & Rydin, E. (2005). Sediment depth attenuation of biogenic phosphorus compounds measured by ^{31}P NMR. *Environmental Science & Technology*, *39*, 867–872. https://doi.org/10.1021/es049590h

Bai, X., Zhou, Y., Sun, J., Ma, J., & Zhao, H. (2015). Classes of dissolved and particulate phosphorus compounds and their spatial distributions in the water of a eutrophic lake: A ^{31}P NMR study. *Biogeochemistry*, *126*, 227–240. https://doi.org/10.1007/s10533-015-0155-7

Cade-Menun, B. J. (2005). Characterizing phosphorus in environmental and agricultural samples by ^{31}P nuclear magnetic resonance spectroscopy. *Talanta*, *66*(2), 359–371. https://doi.org/10.1016/j.talanta.2004.12.024

Cade-Menun, B. J. (2015). Improved peak identification in ^{31} P-NMR spectra of environmental samples with a standardized method and peak library. *Geoderma*, *257-258*, 102–114. https://doi.org/10.1016/j.geoderma.2014.12.016

Cade-Menun, B. J., Navaratnam, J. A., & Walbridge, M. R. (2006). Characterizing dissolved and particulate phosphorus in water with ^{31}P nuclear magnetic resonance spectroscopy. *Environmental Science & Technology*, *40*, 7874–7880. https://doi.org/10.1021/es061843e

Cao, X., Wang, Y., He, J., Luo, X., & Zheng, Z. (2016). Phosphorus mobility among sediments, water and cyanobacteria enhanced by cyanobacteria blooms in eutrophic Lake Dianchi. *Environmental Pollution*, *219*, 580–587. https://doi.org/10.1016/j.envpol.2016.06.017

Carlson, R. E. (1977). A trophic state index for lakes. *Limnology and Oceanography*, *22*, 361–369. https://doi.org/10.4319/lo.1977.22.2.0361

Chen, M., Ding, S., Chen, X., Sun, Q., Fan, X., Lin, J., … Zhang, C. (2018). Mechanisms driving phosphorus release during algal blooms based on hourly changes in iron and phosphorus concentrations in sediments. *Water Research*, *133*, 153–164. https://doi.org/10.1016/j.watres.2018.01.040

Ding, S., Chen, M., Gong, M., Fan, X., Qin, B., Xu, H., … Zhang, C. (2018). Internal phosphorus loading from sediments causes seasonal nitrogen limitation for harmful algal blooms. *Science of the Total Environment*, *625*, 872–884. https://doi.org/10.1016/j.scitotenv.2017.12.348

Ding, S., Xu, D., Bai, X., Yao, S., Fan, C., & Zhang, C. (2013). Speciation of organic phosphorus in a sediment profile of Lake Taihu II. Molecular species and their depth attenuation. *Journal of Environmental Sciences*, *25*, 925–932. https://doi.org/10.1016/s1001-0742(12)60137-5

EPA of China. (1989). *The method of monitoring and analyzing water and wastewater*. Beijing, China: Environmental Science Press House.

Feng, W., Wu, F., He, Z., Song, F., Zhu, Y., Giesy, J. P., … Sun, F. (2018). Simulated bioavailability of phosphorus from aquatic macrophytes and phytoplankton by aqueous suspension and incubation with alkaline phosphatase. *Science of the Total Environment*, *616-617*, 1431–1439. https://doi.org/10.1016/j.scitotenv.2017.10.172

Feng, W., Zhu, Y., Wu, F., Meng, W., Giesy, J. P., He, Z., … Fan, M. (2015). Characterization of phosphorus forms in lake macrophytes and algae by solution ^{31}P nuclear magnetic resonance spectroscopy. *Environemental Science and Pollution Research*, *23*, 1–10. https://doi.org/10.1007/s11356-015-5913-5

Giles, C. D., Lee, L. G., Cade-menun, B. J., Hill, J. E., Isles, P. D., Schroth, A. W., & Druschel, G. K. (2015). Characterization of organic phosphorus form and bioavailability in Lake sediments using P nuclear magnetic resonance and enzymatic hydrolysis. *Journal of Environmental Quality*, *44*, 882–894. https://doi.org/10.2134/jeq2014.06.0273

Gu, S., Qian, Y., Jiao, Y., Li, Q., Pinay, G., & Gruau, G. (2016). An innovative approach for sequential extraction of phosphorus in sediments: Ferrous iron

P as an independent P fraction. *Water Research*, *103*, 352–361. https://doi.org/10.1016/j.watres.2016.07.058

Huo, S., Ma, C., Xi, B., Su, J., Zan, F., Ji, D., & He, Z. (2013). Establishing eutrophication assessment standards for four lake regions, China. *Journal of Environmental Sciences*, *25*, 2014–2022. https://doi.org/10.1016/s1001-0742(12)60250-2

Hupfer, M., Ruübe, B., & Schmieder, P. (2004). Origin and diagenesis of polyphosphate in lake sediments: A ^{31}P-NMR study. *Limnology and Oceanography*, *49*, 1–10. https://doi.org/10.4319/lo.2004.49.1.0001

Jaffé, R., Boyer, J. N., Lu, X., Maie, N., Yang, C., Scully, N. M., & Mock, S. (2004). Source characterization of dissolved organic matter in a subtropical mangrove-dominated estuary by fluorescence analysis. *Marine Chemistry*, *84*, 195–210. https://doi.org/10.1016/j.marchem.2003.08.001

Jensen, H. S., & Andersen, F. O. (1992). Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. *Limnology and Oceanography*, *37*, 577–589. https://doi.org/10.4319/lo.1992.37.3.0577

Karlsson, E., & Brunberg, A. K. (2004). The importance of shallow sediments in the recruitment of anabaena and aphanizomenon (cyanophyceae). *Journal of Phycology*, *40*, 831–836. https://doi.org/10.1111/j.1529-8817.2004.04070.x

Klamt, A., Jensen, H. S., Mortensen, M., Schreiber, F. N., & Reitzel, K. (2017). The importance of catchment vegetation for alkalinity, phosphorus burial and macrophytes as revealed by a recent paleolimnological study in a soft water lake. *Science of the Total Environment*, *580*, 1097–1107. https://doi.org/10.1016/j.scitotenv.2016.12.065

Kromkamp, J. (1987). Formation and functional significance of storage products in cyanobacteria. *New Zealand Journal of Marine and Freshwater Research*, *21*, 457–465. https://doi.org/10.1080/00288330.1987.9516241

Liang, Z., Liu, Y., Ge, F., Xu, Y., Tao, N., Peng, F., & Wong, M. (2013). Efficiency assessment and pH effect in removing nitrogen and phosphorus by algae-bacteria combined system of *Chlorella vulgaris* and *Bacillus licheniformis*. *Chemosphere*, *92*, 1383–1389. https://doi.org/10.1016/j.chemosphere.2013.05.014

Lin, P., Guo, L., Chen, M., Tong, J., & Lin, F. (2012). The distribution and chemical speciation of dissolved and particulate phosphorus in the Bering Sea and the Chukchi-Beaufort seas. *Deep Sea Research Part II*, *81–84*, 79–94. https://doi.org/10.1016/j.dsr2.2012.07.005

Liu, S. S., Zhu, Y. R., Meng, W., He, Z. Q., Feng, W. Y., Zhang, C., & Giesy, J. P. (2016). Characteristic and degradation of carbon and phosphorus from aquatic macrophytes in lakes: Insight from solid-state ^{13}C NMR and solution ^{31}P NMR spectroscopy. *Science of the Total Environment*, *543*, 746–756. https://doi.org/10.1016/j.scitotenv.2015.11.080

Løvdal, T., Tanaka, T., & Thingstad, T. F. (2007). Algal-bacterial competition for phosphorus from dissolved DNA, ATP, and orthophosphate in a mesocosm experiment. *Limnology and Oceanography*, *52*, 1407–1419. https://doi.org/10.4319/lo.2007.52.4.1407

Martinez, A., Gene, W. T., & Delong, E. F. (2010). Widespread known and novel phosphonate utilization pathways in marine bacteria revealed by functional screening and metagenomic analyses. *Environmental Microbiology*, *12*, 222–238. https://doi.org/10.1111/j.1462-2920.2009.02062.x

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*, 31–36. https://doi.org/10.1016/s0003-2670(00)88444-5

Nash, D. M., Haygarth, P. M., Turner, B. L., Condron, L. M., McDowell, R. W., Richardson, A. E., … Heaven, M. W. (2014). Using organic phosphorus to sustain pasture productivity: A perspective. *Geoderma*, *221–222*, 11–19. https://doi.org/10.1016/j.geoderma.2013.12.004

Read, E. K., Ivancic, M., Hanson, P., Cade-Menun, B. J., & McMahon, K. D. (2014). Phosphorus speciation in a eutrophic lake by ^{31}P NMR spectroscopy. *Water Research*, *62*, 229–240. https://doi.org/10.1016/j.watres.2014.06.005

Reitzel, K., Ahlgren, J., Gogoll, A., & Rydin, E. (2006). Effects of aluminum treatment on phosphorus, carbon, and nitrogen distribution in lake sediment: A ^{31}P NMR study. *Water Research*, *40*, 647–654. https://doi.org/10.1016/j.watres.2005.12.014

Reitzel, K., Jensen, H. S., Flindt, M., & Andersen, F. Ø. (2009). Identification of dissolved nonreactive phosphorus in freshwater by precipitation with aluminum and subsequent ^{31}P NMR analysis. *Environmental Science & Technology*, *43*, 5391–5397. https://doi.org/10.1021/es900994m

Shinohara, R., Akio, I., Kohzu, A., Tomioka, N., Furusato, E., Satou, T., … Shimotori, K. (2016). Dynamics of particulate phosphorus in a shallow eutrophic lake. *Science of the Total Environment*, *563–564*, 413–423. https://doi.org/10.1016/j.scitotenv.2016.04.134

Solórzano, L., & Sharp, J. H. (1980). Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnology and Oceanography*, *25*, 754–758. https://doi.org/10.4319/lo.1980.25.4.0754

Søndergaard, M., Kristensen, P., & Jeppesen, E. (1992). Phosphorus release from resuspended sediment in the shallow and wind-exposed lake arreso, Denmark. *Hydrobiologia*, *228*, 91–99. https://doi.org/10.1007/bf00006480

Soranno, P. A., Carpenter, S. R., & Lathrop, R. C. (1997). Internal phosphorus loading in Lake Mendota: Response to external loads and weather. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*, 1883–1893. https://doi.org/10.1139/cjfas-54-8-1883

Wu, F. C., Kothawala, D. N., Evans, R. D., Dillon, P. J., & Cai, Y. R. (2007). Relationships between DOC concentration, molecular size and fluorescence properties of DOM in a stream. *Applied Geochemistry*, *22*, 1659–1667. https://doi.org/10.1016/j.apgeochem.2007.03.024

Xu, D., Ding, S., Li, B., Bai, X., Fan, C., & Zhang, C. (2013). Speciation of organic phosphorus in a sediment profile of Lake Taihu I: Chemical forms and their transformation. *Journal of Environmental Sciences*, *25*, 925–932. https://doi.org/10.1016/s1001-0742(12)60136-3

Yang, B., Cao, L., Liu, S., & Zhang, G. (2015). Biogeochemistry of bulk organic matter and biogenic elements in surface sediments of the Yangtze River estuary and adjacent sea. *Marine Pollution Bulletin*, *96*, 471–484. https://doi.org/10.1016/j.marpolbul.2015.05.003

Zhang, H., Davison, W., Gadi, R., & Kobayashi, T. (1998). In situ measurement of dissolved phosphorus in natural waters using DGT. *Analytica Chimica Acta*, *370*, 29–38. https://doi.org/10.1016/s0003-2670(98)00250-5

Zhang, R., Wu, F., He, Z., Zheng, J., & Song, B. (2009). Phosphorus composition in sediments from seven different trophic lakes, China: A phosphorus-31 NMR study. *Journal of Environmental Quality*, *38*, 353–359. https://doi.org/10.2134/jeq2007.0616

Zhou, Y., Song, C., Cao, X., Li, J., Chen, G., Xia, Z., & Jiang, P. (2008). Phosphorus fractions and alkaline phosphatase activity in sediments of a large eutrophic Chinese Lake (Lake Taihu). *Hydrobiologia*, *599*, 119–125. https://doi.org/10.1007/s10750-007-9185-z

Zhu, Y., Wu, F., Feng, W., Liu, S., & Giesy, J. P. (2016). Interaction of alkaline phosphatase with minerals and sediments: Activities, kinetics and hydrolysis of organic phosphorus. *Colloids and Surfaces, A: Physicochemical and Engineering Aspects*, *495*, 46–53. https://doi.org/10.1016/j.colsurfa.2016.01.056

Zhu, Y., Wu, F., He, Z., Giesy, J. P., Feng, W., Mu, Y., … Tang, Z. (2015). Influence of natural organic matter on the bioavailability and preservation of organic phosphorus in lake sediments. *Chemical Geology*, *397*, 51–60. https://doi.org/10.1016/j.chemgeo.2015.01.006

Zhu, Y., Wu, F., He, Z., Guo, J., Qu, X., Xie, F., … Guo, F. (2013). Characterization of organic phosphorus in lake sediments by sequential fractionation and enzymatic hydrolysis. *Environmental Science & Technology*, *47*, 7679–7687. https://doi.org/10.1021/es305277g

How to cite this article: Zhang C, Feng W, Chen H, et al. Characterization and sources of dissolved and particulate phosphorus in 10 freshwater lakes with different trophic statuses in China by solution ^{31}P nuclear magnetic resonance spectroscopy. *Ecol. Res*. 2019;34:106–118. https://doi.org/10.1111/1440-1703.1006