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Composition characterization and biotransformation of dissolved, particulate and algae organic phosphorus in eutrophic lakes

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ABSTRACT

Characteristics and transformation of organic phosphorus in water are vital to biogeochemical cycling of phosphorus and support of blooms of phytoplankton and cyanobacteria. Using solution ^{31}P nuclear magnetic resonance (NMR), combined with field surveys and lab analyses, composition and structural characteristics of dissolved phosphorus (DP), particulate phosphorus (PP) and organic P in algae were studied in two eutrophic lakes in China, Tai Lake and Chao Lake. Factors influencing migration and transformation of these constituents in lake ecosystems were also investigated. A method was developed to extract, flocculate and concentrate DP and PP from lake water samples. Results showed that orthophosphate (Ortho-P) constituted 32.4%–81.3% of DP and 43.7%–54.9% of PP, respectively; while monoester phosphorus (Mono-P) was 13.2%–54.0% of DP and 32.9%–43.7% of PP, respectively. Phosphorus in algae was mostly organic P, especially Mono-P, which was $\geq$50% of TP. Environmental factors and water quality parameters such as temperature (T), electrical conductivity (EC), pH, secchi depth (SD), dissolved oxygen (DO), chemical oxygen demand (COD_{cr}), chlorophyll-a (Chl-a), affected the absolute and relative concentrations of various P components in the two lakes. Increased temperature promoted bioavailable P (Ortho-P and Mono-P) release to the lake waters. The results can provide an important theoretical basis for the mutual conversion process of organic P components between various media in the lake water environment.

1. Introduction

In most countries, where dense humans populate, eutrophication is a challenging problem (Zhang et al., 2014; Yu et al., 2020). Phosphorus (P) is generally the nutrient limiting primary production in freshwater lakes, while excessive P causes greater primary production, which results in hyper-eutrophication and subsequent water quality deterioration (Ahlgren et al., 2005). Once lakes become eutrophic, it is hard to reverse the circumstances, even if external loadings are reduced or controlled (Sas, 1990). In recent decades, exogenous P has been effectively controlled in several lakes of China, thus internal recycling of P among environmental media, such as water, sediment and phytoplankton, has become the dominant research area in eutrophic lakes (Chen et al., 2018; Ding et al., 2018b). Previous studies have focused mostly on P recycling from sediment to water column, or cycling between phytoplankton and water. Little was known about the transformation of P, especially organic matter dissolved in water, particulate matter and algae. Thus, chemical identification of P in those matrices was necessary to understand internal recycling of P in eutrophic lakes.

Dissolved phosphorus (DP) can be directly accumulated in phytoplankton, with dissolved inorganic orthophosphate (PO_4^{3-}; Ortho-P) being the most bioavailable form to support primary

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production (Giles et al., 2015). Concentrations of DP in lake waters decreased due to the rapid absorption by algae, during the algae blooming periods (Hudson et al., 2000). Characterizations of DP would be restricted and the forms of P could not be identified by the solution ^{31}P NMR if the concentrations of DP were too low. Particulate phosphorus (PP), which represents a portion of total P in lake water, might enter lake water and subsequently either settle to the bottom or release DP to the water bodies (Bai et al., 2017). Particulate phosphorus serves as a source of P to maintain the blooms of phytoplankton (Read et al., 2014). Therefore, in order to gain insight into circulation and control of P in eutrophic lakes, it has been deemed important to characterize DP and PP as a function of various conditions.

At present, characterization of P in environmental matrices, such as soil (He et al., 2003), sediment (Zhu et al., 2015; Zhang et al., 2013), algae, phytoplankton (Feng et al., 2016a, b) and manure (He et al., 2003), has mostly been done by ^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy. To characterize chemical forms of DP in lakes, Cade-Menun et al. (2006) and Read et al. (2014) employed ^{31}P NMR after concentration of DP by lyophilization. Enriched P could also be obtained by flocculation of 20 L of lake water followed by rotary evaporation (Reitzel et al., 2009; Bai et al., 2017). Volume reduction by rotary evaporation was limited by temperature, which could denature and alter the forms of DP, and the process was also time-consuming and tedious. Hence, while ^{31}P NMR could be used to characterize P in various matrices, it was not adequate for characterizing DP and PP in water without any pretreatment, such as enrichment, concentration, flocculation and extraction.

In the present study, we investigated two representative tropical lakes in China, Tai Lake and Chao Lake. Both lakes are shallow, hyper-eutrophic lakes, but the P sources of the two lakes are different (Bai et al., 2017; Feng et al., 2018). Phosphorus in DP, PP and algae was characterized by solution ^{31}P NMR spectroscopy with dominant environmental factors being investigated, so that appropriate remediation could be applied to effectively control the blooming of algae and cyanobacteria in these two culturally eutrophied lakes.

2. Methods and materials

2.1. Research areas and sample collection

Tai Lake (N 30°55′40"~31°32′58″, E 119°52′32"~120°36′10″), located in the south basin of Yangtze River, is the third largest freshwater lake in China and the main source of drinking water for the city of Wuxi (Feng et al., 2016a). The surface area of Tai Lake is approximately 2427.8 km^2, with mean and maximum depths of 2.1 m and 4.8 m, respectively (Li et al., 2019). Chao Lake (N 30°56’~32°02’, E 117°00’~118°29’) is the fifth largest, freshwater lake in the Yangtze River basin, with a surface area of 770 km^2, and a mean depth of 2.7 m (Feng et al., 2019). Chao Lake is the one of most important drinking water sources for Hefei and other surrounding cities. Both of the two lakes have been affected by urban inputs, industrial runoff, waste water effluents and agricultural runoff, all of which have contributed P causing water eutrophication with consequent algae blooms and resultant water quality deterioration (Liu et al., 2012). In the present study, Tai Lake was divided into seven regions on the basis of the geographical location and spatial distribution of the lake district: Meiliang Bay (T1), Zhushan Bay (T2), Gonghu Bay (T3 and T4), Center region (T5 and T6), south-west coast (T7), south coast (T8), east coastal (T9), and east region (T10). Among those seven regions, Meiliang Bay and Zhushan Bay were the most eutrophic (Zhou et al., 2008; Ding et al., 2013). Similarly, Chao Lake was divided into five regions, T1, T2, T3, T4 and T5. Samples were collected from the above regions in Tai Lake and Chao Lake (Fig. 1).

Water samples were collected from 0.5 m below the surface and 0.5 m above the sediments at the same sampling site. And the two water samples, 20 L in total from the same site, were homogenized and transferred into an acid-cleaned 25-L tank. Samples were prepared for DP extraction by vacuum through GF/C filters (Whatman, UK), and then transferred to a flocculation and precipitation chamber (Bai et al., 2017; Zhang et al., 2019). Particulates retained on the filters were air dried and translocated to the laboratory in air-sealed plastic bags and stored until extraction of PP.

Samples of phytoplankton were obtained by a phytoplankton collector (200 mesh, pore diameter 0.064 mm), sealed in sampling bottles and immediately transported to the laboratory. All algae samples were freeze-dried. Then the lyophilized powder was pass through 2-mm sieve and stored at −20 °C until further processed within 24 h (Feng et al., 2016b).

2.2. Chemical properties and trophic state of the eutrophic lakes

Tai Lake and Chao Lake exhibited typical characteristics of trophic lakes, and their trophic status was assessed in several regions of both lakes. Water quality parameters, including total P (TP), total nitrogen (TN), ammonia nitrogen (NH_3—N), nitrate nitrogen (NO_3—N), chlorophyll-a (Chl-a), chemical oxygen demand (COD) and secchi depth (SD). TP was determined by the harmonized and validated SMT method developed by European Committee for Standardization. TN was determined by potassium persulfate oxidation method. NH_3—N was obtained by the method of Nash’s reagent. NO_3—N and Chl-a were gained with ultraviolet spectrophotometry. COD was evaluated by potassium dichromate method. Secchi depth (SD) was determined by Secchi disc method. Details of analytical methods were described by previous publications (Huo et al., 2013; Ding et al., 2018a). Salinity (SAL), temperature (T), electrical conductivity (EC), pH, dissolved oxygen (DO), turbidity (NTU), and total dissolved solids (TDS) were determined by fully automatic portable chemical analyzer (Yang et al., 2016).

The trophic status index (TSI) was used to classify lakes and to compare their trophic status with other lakes (Li et al., 2019a). Chl-a was a key estimator for phytoplankton biomass, which could measure abundances of algae in water. TSI was calculated based on concentrations of Chl-a, TP, TN, SD and COD. Trophic status of lakes can be classified as: Oligotrophic ($0 < TSIc \leq 30$), oligo-mesotrophic ($30 < TSIc \leq 40$), mesotrophic ($40 < TSIc \leq 50$), light eutrophic ($50 < TSIc \leq 60$), middle eutrophic ($60 < TSIc \leq 70$), and highly eutrophic ($70 < TSIc \leq 100$) (Huo et al., 2013; Li et al., 2019a). The computational formula for TSI is as follows:

$$\text{TLI}\ (\text{Chl"TT5843c571""ADa}) = 10(2.5 + 1.086\ln(\text{Chl"TT5843c571""ADa}))$$
$$\text{TLI}\ (\text{TP}) = 10(9.436 + 1.624\ln(\text{TP}))$$
$$\text{TLI}\ (\text{TN}) = 10(5.453 + 1.694\ln(\text{TN}))$$
$$\text{TLI}\ (\text{SD}) = 10(5.118 - 1.94\ln(\text{SD}))$$
$$\text{TLI}\ (\text{COD}) = 10(0.109 + 2.661\ln(\text{COD}))$$

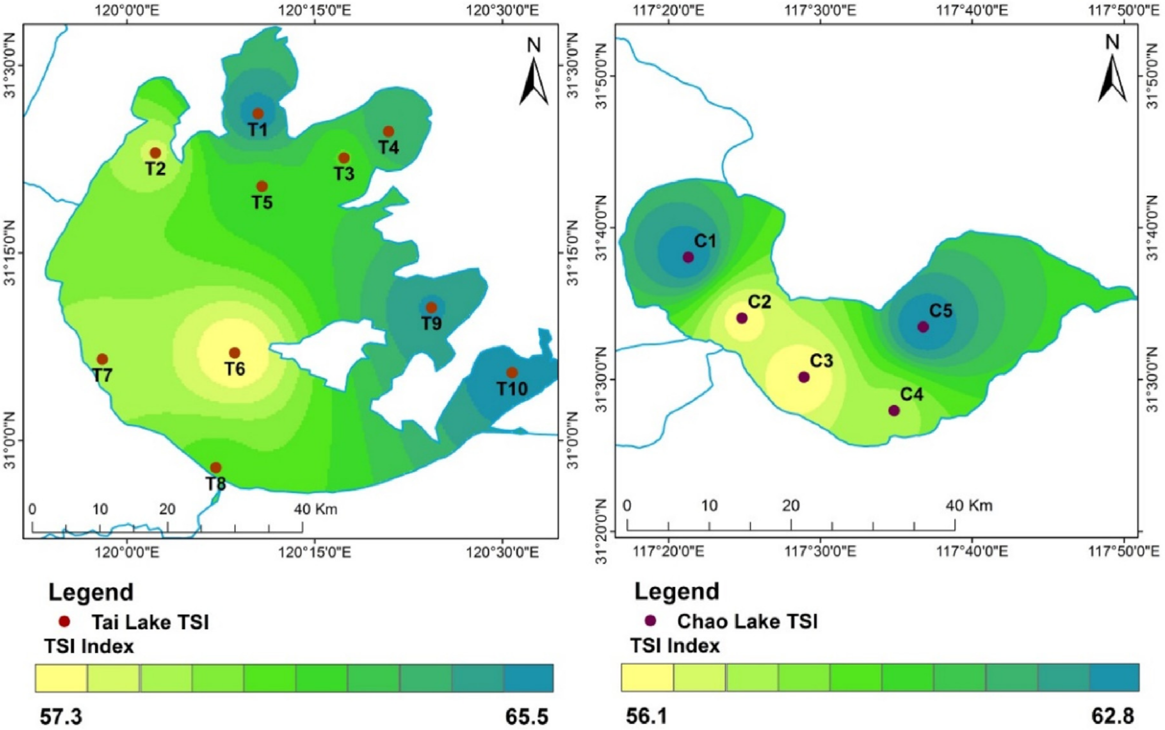


Fig. 1. Distribution of trophic state index (TSI) of Tai Lake and Chao Lake.

Table 1
Properties and trophic states of Tai Lake and Chao Lake.

Lake	No.	Samples	Chl-a ($\mu g\ L^{-1}$)	TP ($mg\ L^{-1}$)	TN ($mg\ L^{-1}$)	SD[a] (cm)	COD_{cr}	Standard scale	Trophic state
Tai Lake	T1	Meiliang Bay	56.04	0.16	2.14	20	128.8	IV[b]	Mid-eutrophic
	T2	Zhushan Bay	56.05	0.33	3.51	65	79.3	III[c]	Light-eutrophic
	T3	Gonghu Bay 1	55.84	0.09	1.90	30	92.5	IV	Mid-eutrophic
	T4	Gonghu Bay 2	56.05	0.09	2.13	30	126.4	IV	Mid-eutrophic
	T5	Center region 1	22.00	0.05	2.04	15	96.7	IV	Mid-eutrophic
	T6	Center region 2	5.10	0.09	2.72	15	98.1	III	Light-eutrophic
	T7	South-west coast	9.90	0.12	2.20	13	97.2	III	Light-eutrophic
	T8	South coast	11.50	0.12	2.25	13	109.3	IV	Mid-eutrophic
	T9	East coast	55.96	0.05	1.78	14	102.5	IV	Mid-eutrophic
	T10	East region	56.04	0.03	2.07	15	111.4	IV	Mid-eutrophic
Chao Lake	C1	West Region	42.50	0.40	2.82	10	50.2	IV	Mid-eutrophic
	C2	South-west Region	25.30	0.29	2.14	13	34.7	III	Light-eutrophic
	C3	South Region	34.70	0.28	2.76	13	23.2	III	Light-eutrophic
	C4	North-east Region	30.80	0.24	3.44	14	30.8	III	Light-eutrophic
	C5	East Region	41.50	0.28	2.88	17	75.3	IV	Mid-eutrophic

[a] SD: Secchi depth; COD_{cr}: Chemical oxygen demand ($mg\ L^{-1}$).

[b] The second-grade protection zone of centralized drinking water source; fish and prawn wintering grounds, migration channel, aquaculture etc.

[c] Industrial water and human indirect contact recreation water.

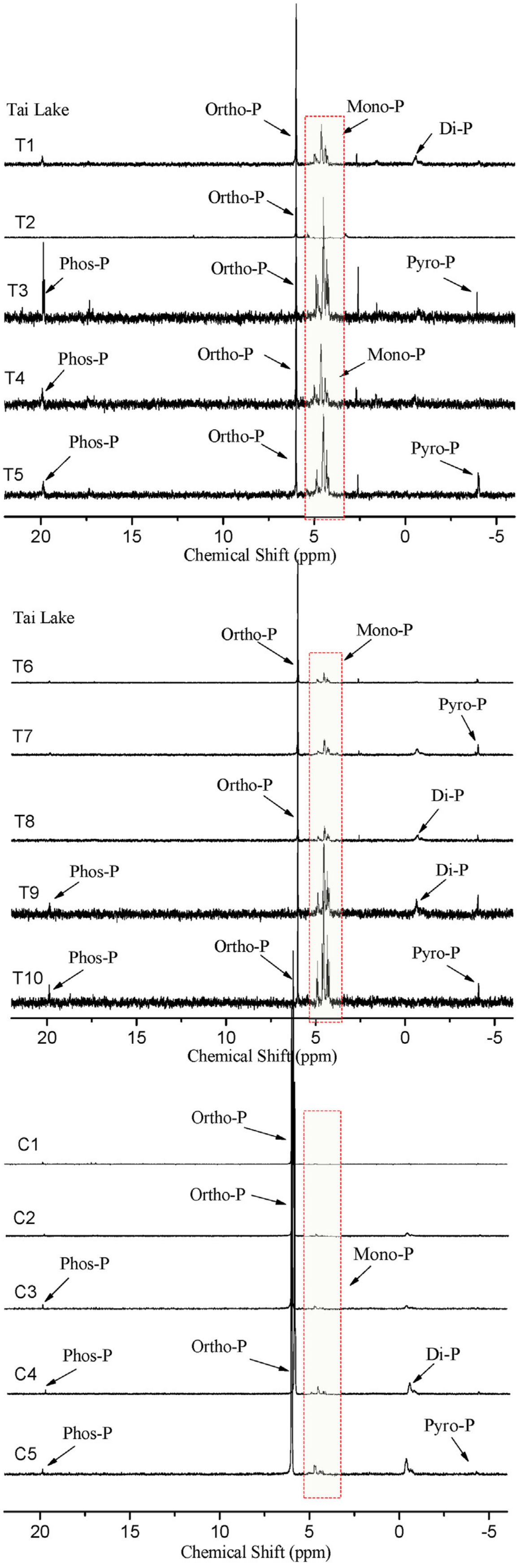


Fig. 2. ^{31}P NMR spectra of dissolved phosphorus (DP) in water from Tai Lake and Chao Lake.

where, Chl-a is in 10^{-3} mg L^{-1}, SD is in m, and others parameters are in mg L^{-1}.

The computational formula for the comprehensive TSI is as follows:

$$TLI(\Sigma) = \Sigma Wj \cdot TLI(j)$$

where, $TLI(\sum)$ is the comprehensive TSI, Wj is the weight of the nutritional status index of the parameter, and TLI(j) represents the nutritional status index of the parameter.

2.3. Enrichment of DP and PP in Tai Lake and Chao Lake

DP was enriched by precipitation combined with freeze-drying of the water samples. Flocculating agent (4.0 g $AlCl_3$) was added to filtrated water to precipitate and thus enrich DP (Reitzel et al., 2009; Bai et al., 2017; Zhang et al., 2019). Quick flocculation was performed with stirring and the precipitate was collected by centrifugation at 8000*g* for 20 min. The DP was then extracted from the precipitate by use of 20 mL of 1 M NaOH +0.05 M EDTA at 25 °C for 16 h using the vibrating incubator. Extracts were freeze-dried for use in ^{31}P-NMR analysis (Cade-Menun, 2015). Particulate P in particulate matter collected by filters in Section 2.1 was extracted by use of 1 M NaOH +0.05 M EDTA (20 mL) at 25 °C for 16 h using the vibrating incubator and then centrifuged at 8000 g for 15 min. Supernatants containing PP were collected. Extracts were also freeze-dried for use in subsequent ^{31}P-NMR analysis.

2.4. ^{31}P NMR spectroscopy

Each of all the lyophilized samples was re-dissolved in 1 mL of 1 M NaOH+0.01 M EDTA solution with ultrasonic vibration for 30 min. After dissolution, the solution was centrifuged at 8000 g for 30 min. The clear supernatant (0.5 mL) was transferred to a 5-mm NMR tube and 0.1 mL of D_2O was added to the tube to lock the signal for ^{31}P NMR. ^{31}P NMR spectra were obtained by use of a Bruker AV 600 MHz spectrometer equipped with a 5-mm BBO probe. NMR parameters were set as: 1.35 s acquisition time, 2.0 s relaxation delay, 25 °C, and a resonance frequency of 242.96 MHz without decoupling. The scan time was about 18 h for each sample. Phosphorus compounds included inorganic P [orthophosphate (Ortho-P), pyrophosphate (Pyro-P), polyphosphate (Poly-P)] and organic P [monoester P (Mono-P), diester P (Di-P), phosphonates (Phos-P)]. Phosphorus compounds were identified by shifts of solution ^{31} P NMR spectra, and peaks were identified by comparing to values published previously (Cade-Menun and Paytan, 2010; Cade-Menun, 2015; Giles et al., 2015; Feng et al., 2016a).

2.5. Data analysis

Data were checked for deviations from normality of variance before analysis.^{31}P NMR spectra and integrations were analyzed by MestReNova10.0 software. Heatmap chart analysis of the correlation between DP and PP of lake water bodies and environmental factors was performed using Matlab2012a software, and the map with sampling points and P composition distribution were obtained with Arcgis10.1.

3. Results

3.1. Lake TSI and trophic status analysis

The TSI, which is a comprehensive eutrophication indicator of lakes (Li et al., 2019a; Zhang et al., 2019), was relatively high in Tai

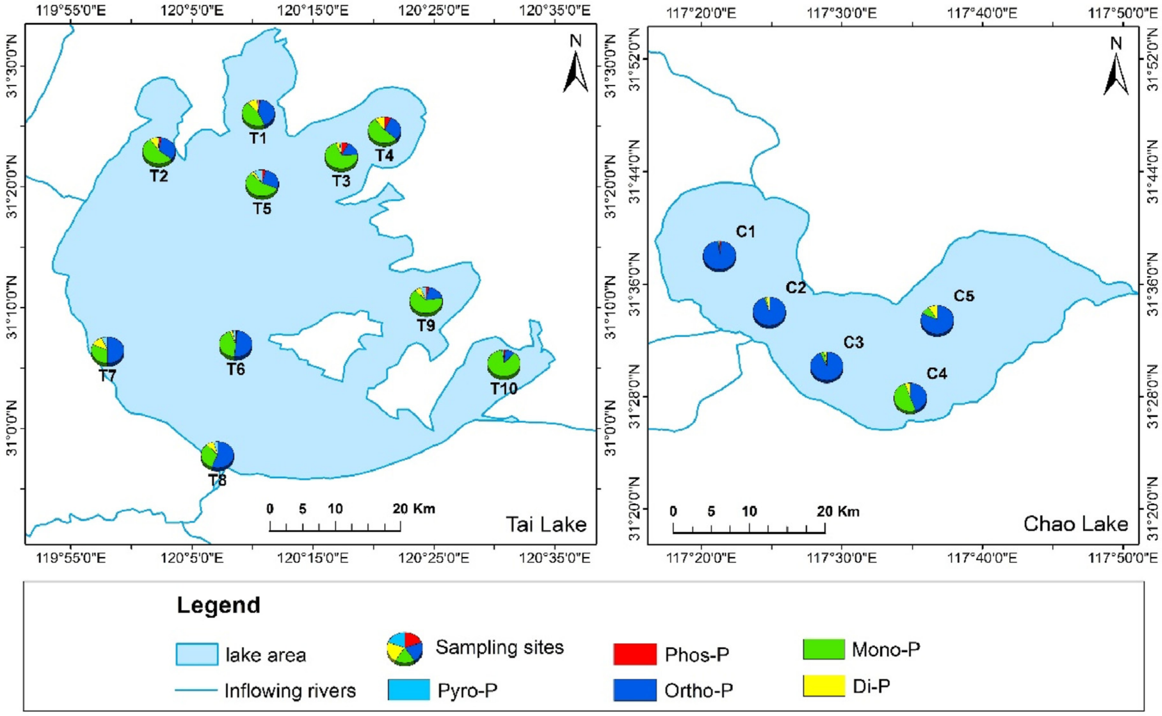


Fig. 3. Distributions of proportions of P in dissolved phosphorus (DP) in water from Tai Lake and Chao Lake.

Lake and Chao Lake. In Tai Lake, the TSI was greater at T1, T9 and T10, because cyanobacterial blooms were occurred frequently in Meiliang Bay (T1) (Fig. 1 and Table 1). The result was consistent with previous report for Tai Lake (Zhou et al., 2008; Ding et al., 2013). T9 and T10 were characterized to have large numbers of aquatic macrophytes, and the TSI values in the two regions were greater due to nutrients produced from decay and decomposition of dead aquatic plants. The center region of Tai Lake (T6) exhibited the least TSI (Fig. 1), and the water quality at that location was better than that at other sampling locations. In Chao Lake, C1 and C5 were the most polluted areas by P (Fig. 1), which was consistent with previous research (Feng et al., 2018).

Results of the previous research showed that there was a positive relationship between eutrophication status and mass of cyanobacteria. For instance in Erie lake, serious eutrophication was directly correlated with increasing concentrations of Chl-a during the summer (Rinta-Kanto et al., 2009). Some studies have reported that the risk of blooms was also greater when Chl-a concentration was higher than 10 μg/L (Nikolay, 2017). When Chl-a content exceeds 40 μg/L, risk of cyanobacterial outbreak increases sharply (Li et al., 2019a). Concentrations of Chl-*a* exceeding 40 μg/L were observed at T1, T2, T3, T4, T9 and T10 at Tai Lake, and C1 and C5 at Chao Lake. Risks of blooms in these regions were also greater than other parts of the lakes (Table 1).

3.2. *Characterization of dissolved phosphorus (DP) in eutrophic lakes*

Dissolved P components were different in Tai Lake and Chao Lake. In nature, DP is the main form available by plants and plays an important role in biogeochemical cycling processes of eutrophic lakes (Liu et al., 2016). Monoester phosphorus (Mono-P) was the main organic P component with a mean concentration of 30.3 μg/L, and accounted for 30.5%–85.0% (54.0% in average) of the DP in Tai Lake. Ortho-P was the second most abundant organic P component with a mean concentration of 17.8 μg/L, and composed of 10.5%–56.6% (32.4% in average) of the DP in Tai Lake. The other constituents were diester P (Di-P), pyrophosphates (Pyro-P) and phosphonates (Phos-P), which accounted for 6.9%, 3.6%, and 3.2%, respectively (Figs. 2 and 3, SI. Table 1). Relative concentrations of the DP constituents in Chao Lake were different from those in Tai Lake. In Chao Lake, Ortho-P, which is a bioavailable inorganic form, was the main constituent of DP, accounting for 43.7%–96.5% of the DP in the lake, with a mean proportion of 81.3% and a mean concentration of 170.7 μg/L. Mono-P was the next most abundant component, which accounted for 1.2%–49.7% of the DP in Chao Lake, with a mean contribution of 13.2% and a mean concentration of 22.1 μg/L. Other constituents of DP were Di-P, Phos-P and Pyro-P, which accounted for 4.5%, 0.7% and 0.4%, respectively.

3.3. *Characterization of particulate phosphorus in the eutrophic lakes*

Particulate matter was one of the important forms involved in P circulation in lake waters. Particulate phosphorus (PP) includes several components, which have different bioavailabilities for bioaccumulation (Boström et al., 1988; Li and Brett, 2013). Ortho-P was the main P component in the PP from Tai Lake, and accounted for 35.5%–69.7% of the PP, with a mean of 54.9% and a mean concentration of 27.5 μg/L. Mono-P was the second most abundant component accounting for 21.2%–52.4% of the PP, with a mean proportion of 32.9% and a mean concentration of 21.6 μg/L. The mean concentrations of Pyro-P and Di-P in PP were 9.6 μg/L and 2.2 μg/L, respectively, with mean proportions of 8.3% and 3.9% of PP, respectively (Fig. 4, Fig. 5, SI. Table 2).

Absolute and relative concentrations of constituents of the PP in Chao Lake were different from those in Tai Lake. In Chao Lake, there were two major forms of P, Ortho-P and Mono-P, which accounted for 43.69% and 43.72% of the PP, respectively. In Chao Lake, proportions of Pyro-P and the Di-P were 9.0% and 3.4% of the PP,

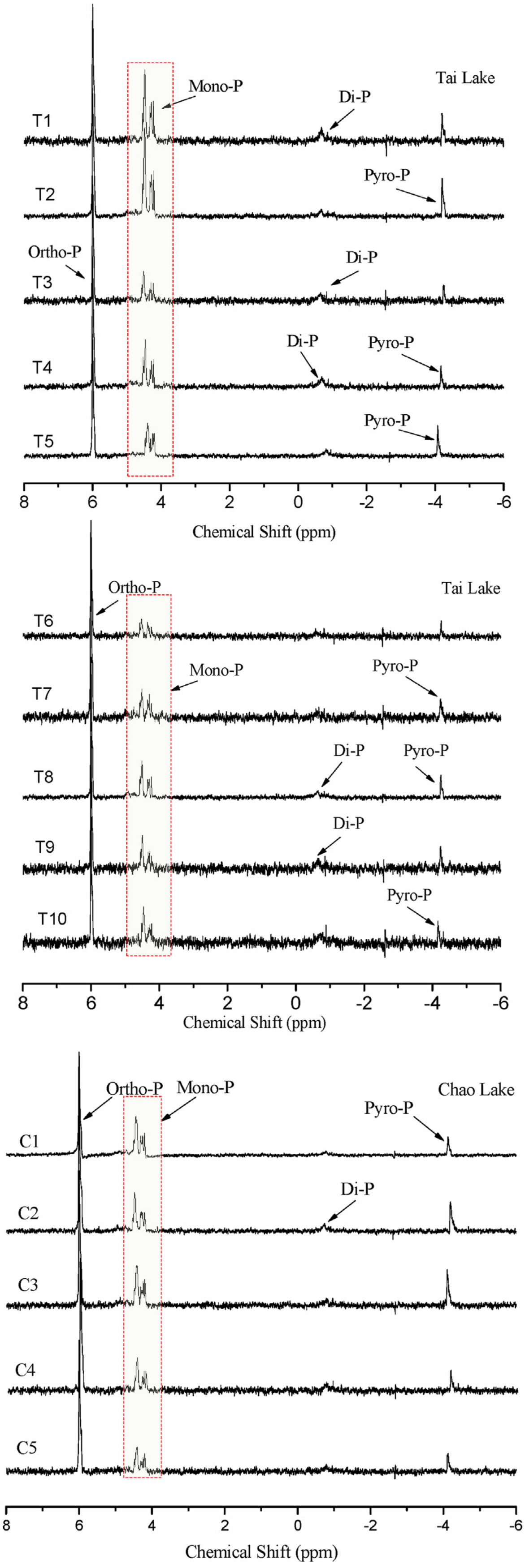


Fig. 4. ^{31}P NMR spectra of particulate phosphorus (PP) in water from Tai Lake and Chao Lake.

respectively.

3.4. Compositional characteristics of organic P in algae in the eutrophic lakes

Decomposition of algae debris promotes the biochemical cyclic processes of P, increasing the risk of algal blooms (Feng et al., 2018; Lehman et al., 2017). Therefore, the algae phosphorus components were detected by^{31}P NMR (Fig. 6, SI.Table 3) at representative sampling points in Lake Tai (T1, T2, T5, T7) and Chao Lake (C1 to C5). Mono-P was the main P component in algae in both Tai Lake and Chao Lake, and the mass concentrations were 1.3 mg g^{-1} and 0.7 mg g^{-1}, respectively, accounting for 50.7% and 50.0% of the TP in algae, respectively. The second largest P component in algae was Ortho-P, for which the proportion in Chao lake (47.2%) was slightly greater than that at Tai Lake (42.2%), and the proportion of Pyro-P of the TP in algae from Tai Lake (6.8%) was two-fold greater than that from Chao Lake (2.6%). The Di-P content in algae from Tai Lake and Chao Lake were both less than 0.3% of the TP in the algae. As in phytoplankton, which is related to the biological composition of algae itself, Mono-P generally accounted for approximately 50.0% of TP in both Lakes. The second most abundant component of algae was Ortho-P (42.2%–47.2%), which could be directly absorbed and utilized by algae.

4. Discussion

4.1. Correlation between DP, PP, algal P components and environmental factors in lakes

Environmental factors were related to characteristics of P in lakes and were positively or negatively associated with the eutrophication of lakes. These influencing factors could not be ignored in control of eutrophication and restoration of lakes. In the two eutrophic lakes, Tai Lake and Chao Lake, chemical and physical characteristics were closely related to concentrations of DP, PP and the amounts of algae (Kong and Gao, 2005; Li et al., 2011a). Environmental factors, such as pH, T, DO and EC, were fundamental conditions affecting the morphology, migration, and transformation of P components in biological and ecological systems (Li et al., 2019b). A heat map demonstrated the results of Pearson pairwise correlations between DP, PP, algal and environmental factors in Tai Lake and Chao Lake (Fig. 7). The EC, pH, SD and DP components were positively correlated in Tai Lake (Fig. 7 (a) Area I). Particularly, there was a significant positive correlation between SD and the main components of DP [Mono-P ($R^2 = 0.887$), Ortho-P ($R^2 = 0.673$)], because SD was the most important factor to predict photosynthesis of aquatic plants (He et al., 2008), which was likely due to shading effects of phytoplankton that attenuated light from reaching macrophytes. The T, DO and COD_{cr} were all negatively correlated with PP and DP, respectively [Fig. 7 (a) Area II]. In particular, DO and Mono-P of DP were significantly negatively correlated ($R^2 = 0.640$), because organic matter decomposition (such as Mono-P) consumed a large amount of oxygen in lake water.

There was also a significant difference between DP and environmental factors in Chao Lake [Fig. 7(b)]. Seven environmental factors, T, EC, pH, SD, DO, COD_{cr}, and Chl-a, were positively correlated with Phos-P in DP of Chao Lake [Fig. 7 (b) Area I], especially DO ($R^2 = 0.988$), EC ($R^2 = 0.839$), and COD_{cr} ($R^2 = 0.816$). These parameters were positively correlated with Phos-P because greater concentrations of DO promoted organic P release, including Phos-P and Mono-P. The seven environmental factors were negatively correlated with concentrations of Mono-P and Di-P in DP [Fig. 7 (b) Area II], especially pH was significantly negatively correlated with

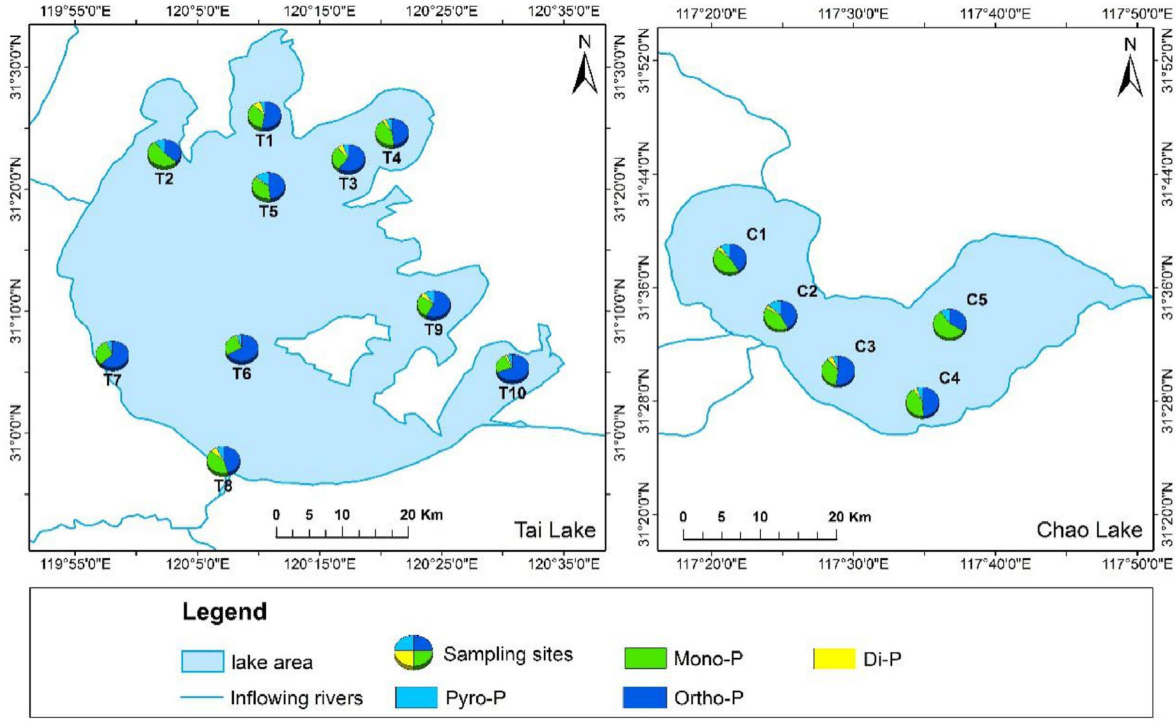


Fig. 5. Distributions of proportions of P in particulate phosphorus (PP) in waters of Tai Lake and Chao Lake.

Mono-P ($R^2 = 0.971$), because Mono-P and Di-P were easily decomposed under alkaline conditions (Feng et al., 2016b).

Constituents of P in algae were also related to environmental factors. Concentrations of P in algae of Tai lake were significantly correlated with T and concentrations of Chl-*a* (Fig. 7 (c)). Temperature and concentrations of Chl-a were both significantly negatively correlated with Ortho-P ($R^2_T = 0.881$, $R^2_{Chl} = 0.854$) and Mono-P ($R^2_T = 0.886$, $R^2_{Chl} = 0.858$), and positively correlated with Di-P ($R^2_T = 0.991$, $R^2_{Chl} = 0.999$). Previous studies have shown that T was an important factor in eutrophic lakes (Li et al., 2019). Surface water temperature of lakes significantly increases at a rate of 0.26–0.28 °C per decade (Ho et al., 2019; Li et al., 2019). Temperature increase can promote release of bioavailable P including Ortho-P, Mono-P, to lake waters.

Concentrations of Ortho-P and Mono-P in algae from Chao Lake were positively correlated with SD and Chl-a concentration (Fig. 7 (d)). Concentrations of Chl-a were significantly positively correlated with concentrations of Ortho-P ($R^2 = 0.681$), which further proved that Ortho-P was more easily absorbed and utilized by algae. However, T, pH, and COD_{cr} were all negatively correlated with concentrations of Di-P and Pyro-P; Particularly, T was significantly negatively correlated with concentration of Pyro-P ($R^2 = 0.876$). Phosphorus has a strong influence on the water quality in environment with high temperature (Li et al., 2011b). For example, algae grow more rapidly at higher temperatures. Pyro-P was consumed when Ortho-P was insufficient to support algae growth, which resulted in lower concentrations of Pyro-P(Feng et al., 2018).

4.2. Phosphorus accumulation and transformation by algae

PO_4^{3-} (Ortho-P) was easily absorbed by algae in the two eutrophic lakes (Feng et al., 2018). Other studies have found trophic status of lakes as mid-eutrophic, light-eutrophic, mesotrophic, and the status was significantly correlated with concentrations of Ortho-P (Zhang et al., 2019). Concentration of Mono-P was greater in mid-eutrophic and light-eutrophic lakes (Giles et al., 2015). Chao Lake could be classified as a light-eutrophic lake, since concentrations of Ortho-P accounted for 81.3% of DP (TSI: 56.1 to 62.8). When Ortho-P is insufficient to support primary production and phytoplankton growth in lakes, part of the unstable organic forms of Mono-P can be converted to Ortho-P under the action of alkaline phosphatase and then be used to support further growth and reproduction of algae (Zhu et al., 2016; Feng et al., 2018). Thus, Mono-P is a potential source of P to support algae blooming. Results of a previous study indicated that α-glycerophospholipids and β-glycerophospholipids of Mono-P were the main components of algae cell membranes (Liu et al., 2016). Mono-P, was the major P component in algae, which accounted for 50.0–50.7% of the TP (Fig. 6, SI.Table 3).

Previous studies have shown that PP was mainly made up of biological P, which was comprised of living or dead phytoplankton and bacterial cells. Biological P could be used as an alternative source of P for phytoplankton, and contributed significantly to the physiological nutritional status of lakes (Read et al., 2014; Shinohara et al., 2016). The composition of PP, with Ortho-P and Mono-P as the primary component, could indirectly affect trophic status of lakes (Bai et al., 2017). For example, relative proportions of Ortho-P and Mono-P in PP were 43.69% and 43.72% in Chao Lake, respectively, while in Tai Lake, Ortho-P and Mono-P in PP accounted for 54.9% and 32.9%, respectively (Fig. 5). However, there were also differences between PP and DP in the lakes. For example, the mean proportion of Pyro-P in PP, which was 8.3%, greater than that (3.6%) of DP in Tai lake (Figs. 3 and 5). This result demonstrated Pyro-P tended to be stored in PP. These results were consistent with previous research (Bai et al., 2017; Zhang et al., 2019).

Algae debris would sank to sediments of lakes, where Ortho-P was the primary component released by organically-bound P by microbes. In Tai Lake and Chao Lake, Ortho-P accounted for 54.9% and 43.7% of PP, respectively (Fig. 5). Phosphorus in sediments

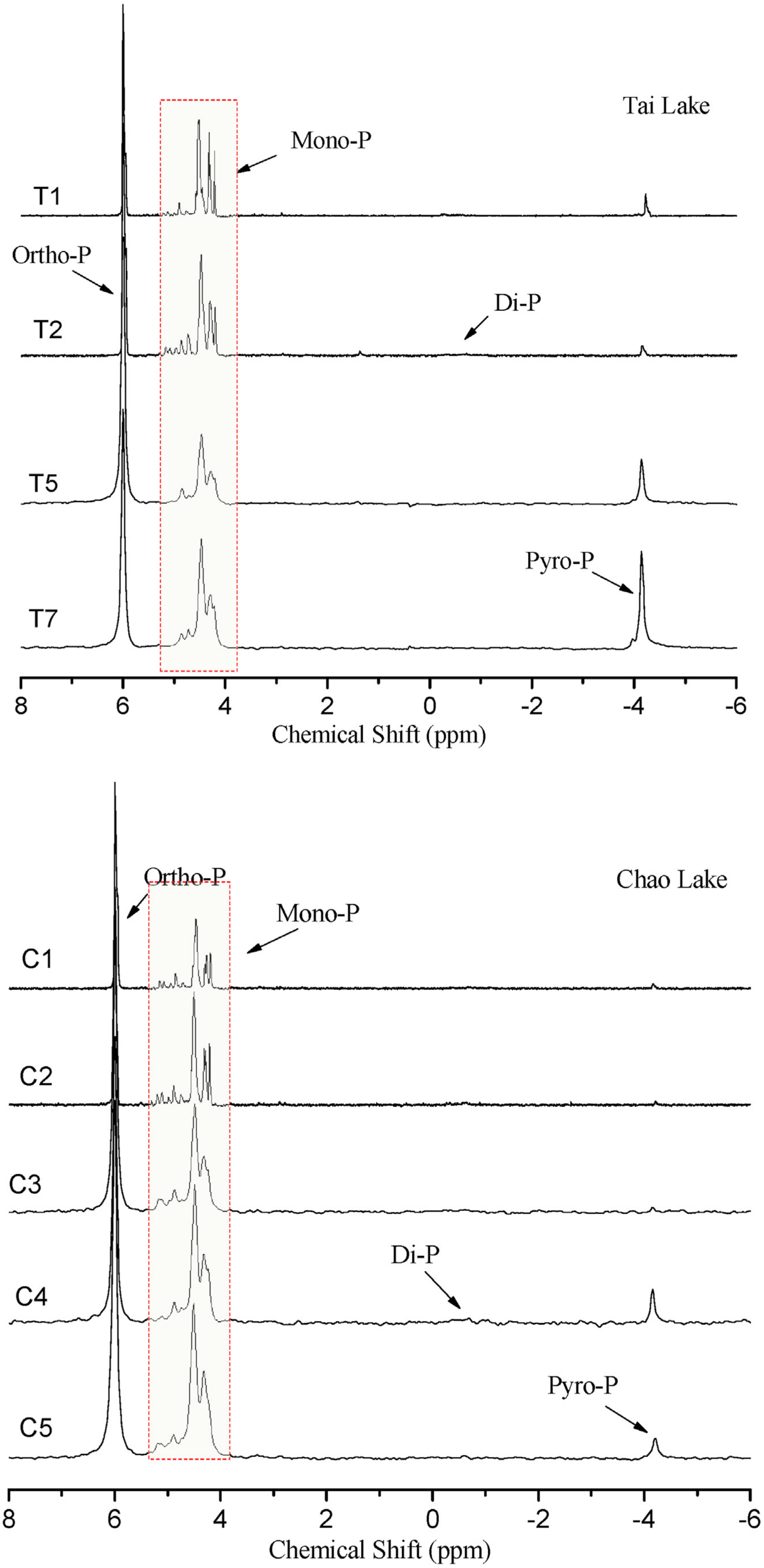
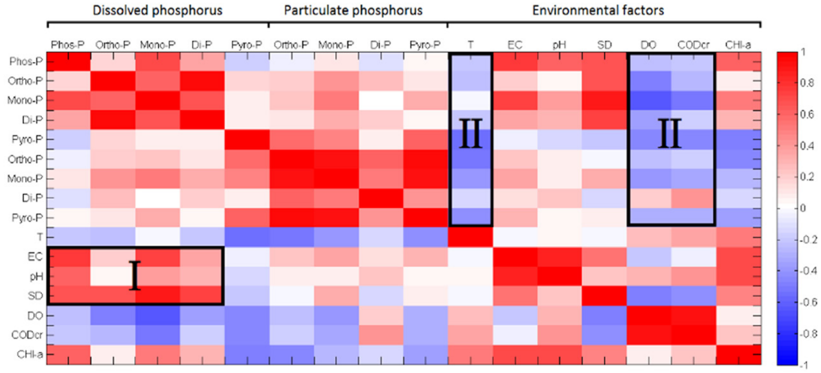
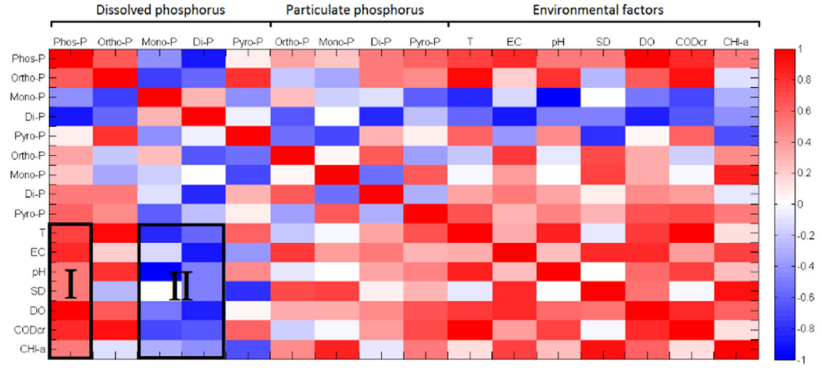


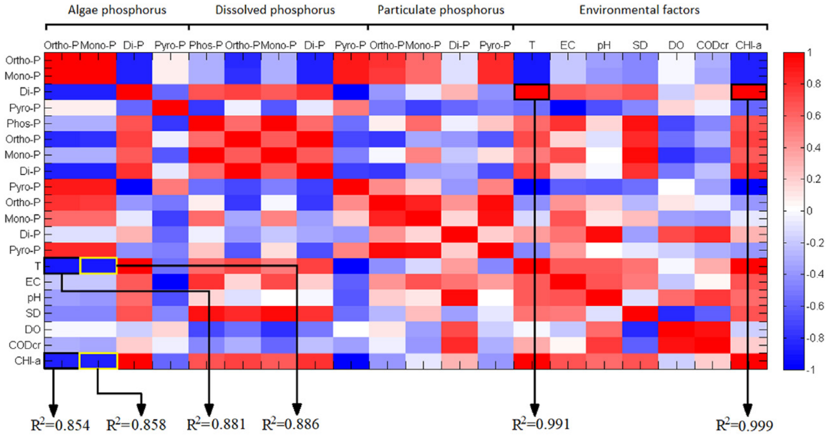
Fig. 6. ^{31}P NMR spectra of phosphorus (P) in algae from Tai Lake and Chao Lake.



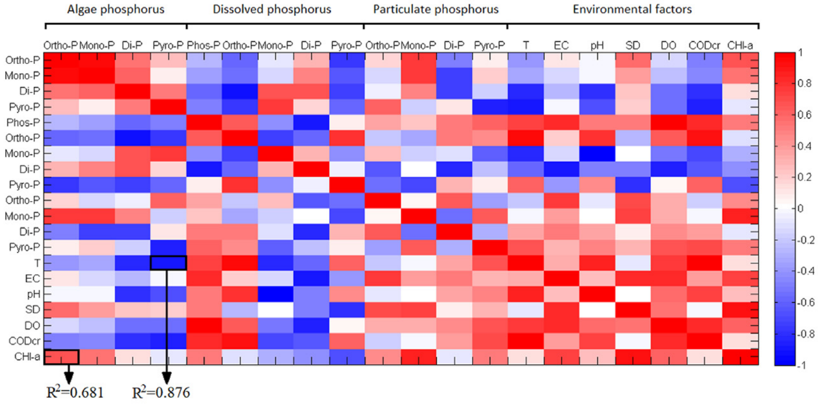
(a) Correlation analysis of DP and PP and environmental factors in Tai Lake



(b) Correlation analysis of DP and PP and environmental factors in Chao Lake



(c) Correlation analysis of algae P, DP and PP and environmental factors in Tai Lake



(d) Correlation analysis of algae P, DP and PP and environmental factors in Chao Lake

Fig. 7. Heat map showing results of a correlation analysis of DP, PP, algae P components with environmental factors in lakes.

came primarily from decomposition of detritus from algae and aquatic macrophytes, and the primary form of inorganic P and organic P were Ortho-P and Mono-P, respectively (Ahlgren et al., 2005; Reitzel et al., 2006; Jarosch et al., 2015; Defforey et al., 2017).

4.3. Mechanisms of endogenous P cycling in eutrophic lakes

Nutrients such as P (i.e. DP and PP) were released into lake water when algae died and were decomposed. Within each fraction (DP and PP), P compounds could be inorganic (Ortho-P, Pyro-P) and organic (Mono-P, Di-P). These forms of P undergo continuous migration and transformation (Paytan and McLaughlin, 2007). Mono-P and Pyro-P from DP together with Ortho-P and Mono-P from PP provided P sources for algae growth. It has been estimated that organic P could be of the same importance as inorganic P in the upper water column (Zhu et al., 2013). There was an interaction between DP and PP. Ortho-P and Mono-P from PP could migrate to DP under appropriate environmental factors (e.g., increasing temperature), when Pyro-P of DP was stored in PP as the nutrient matter. Part of PP was deposited into sediments due to gravity. Risk of algae re-outbreak may be effectively controlled if the accumulation of Ortho-P and Mono-P in water were slowed down. There is still a lot needed to be done before the P cycle of eutrophic lakes could be fully characterized.

5. Conclusions

1) Ortho-P and Mono-P, which respectively accounted for 32.4—81.3% and 13.2—54.0% of TP in the lakes, were the primary components of DP and PP in the two typical eutrophic lakes in China, Tai Lake and Chao Lake. The main form of P in algae was organic P, particularly the Mono-P, which accounted for up to 50% of the TP in algae.
2) Environmental factors have different effects on various forms of P in lakes. Secchi depth, was the most relevant factor for predicting concentrations of DP in Tai Lake, which also indicated that DP was increasing in lake water. In Chao Lake, DO, EC and COD_{cr} were associated with DP and related to releases of Phos-P. For example, in Tai Lake, DO was a key factor that limited degradation of Mono-P, whereas pH showed more significant effect in Chao Lake, which also indicated that Mono-P has potential bioavailability only in suitable environments.
3) Global warming, to some extent, could promote releases of bioavailable P in lakes and could slow the recovery of eutrophic lakes. For example, Ortho-P and Phos-P from algae decreased significantly with T in Tai Lake, and Pyro-P in algae increased as a function of T in Chao Lake. Results indicated that the influence of various factors on P components in different lakes was dependent on changing environmental conditions.

Author statement

The authors hereby certify that this paper consists of original, unpublished work which is not under consideration for publication elsewhere. All authors have reviewed the manuscript and approved it for submission.

Declaration of competing interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114838.

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Composition characterization and biotransformation of dissolved, particulate and algae organic phosphorus in eutrophic lakes

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Support Information (SI)

SI.Table 1 The concentrations (μg/L) and relative abundance (%) of dissolved phosphorus characterizations in Tai Lake and Chao Lake

	Phosphorus	Phos-P		Ortho-P		Mono-P		Di-P		Pyro-P	
		C[a]	R[b]	C	R	C	R	C	R	C	R
Tai Lake	Meiliang	1.73	2.29	30.88	40.68	33.54	44.18	8.64	11.39	1.12	1.47
	Zhushan	3.55	2.44	47.08	32.36	80.00	54.98	13.18	9.06	1.71	1.17
	Gonghu 1	5.23	8.18	10.20	15.96	45.07	70.54	2.80	4.39	1.36	2.13
	Gonghu 2	3.35	7.51	12.61	28.29	23.69	53.13	4.93	11.07	n.d.	0.00
	Center region 1	1.86	4.34	11.40	26.62	37.06	86.53	1.67	3.91	4.10	9.57
	Center region 2	0.27	1.26	10.60	50.45	8.68	41.29	0.74	3.53	0.73	3.47
	South-west coast	0.58	0.99	28.67	49.53	17.66	30.50	7.07	12.22	3.91	6.76
	South coast	n.d.	0.00	16.75	56.62	9.08	30.69	2.47	8.34	1.29	4.35
	East coast	1.27	3.47	7.16	19.50	23.51	64.05	2.28	6.22	2.48	6.75
	East region	0.64	2.18	3.09	10.51	24.97	85.02	n.d.	0.00	0.67	2.29
	Phosphorus	Phos-P		Ortho-P		Mono-P		Di-P		Pyro-P	
		C	R	C	R	C	R	C	R	C	R
Chao Lake	West Region	4.28	1.69	244.21	96.5	3.10	1.2	0.73	0.3	0.68	0.27
	South-west Region	1.15	0.43	250.02	93.6	4.49	1.7	10.22	3.8	1.12	0.42
	South Region	1.20	0.60	183.66	91.8	8.92	4.5	5.50	2.7	0.72	0.36
	North-east Region	0.60	0.36	73.05	43.7	83.02	49.7	9.80	5.9	0.53	0.32
	East Region	0.66	0.52	102.49	80.7	11.14	8.8	12.25	9.6	0.46	0.37

[a] concentrations of phosphorus; [b] relative abundance of phosphorus.

[c] Phos-P: phosphonates, Ortho-P:orthophosphate, Mono-P: monoester phosphorus, Di-P:diester phosphorus, Pyro-P:pyrophosphate

[d] n.d. not detected

SI.Table 2 The concentrations (μg/L) and relative abundance (%) of particulate phosphorus characterizations in Tai Lake and Chao Lake

	Phosphorus	Ortho-P		Mono-P		Di-P		Pyro-P	
		C[a]	R[b]	C	R	C	R	C	R
Tai Lake	Meiliang	63.96	42.52	40.88	45.64	9.61	4.41	6.21	6.24
	Zhushan	56.73	35.21	83.68	51.94	1.55	0.96	17.86	11.08
	Gonghu 1	11.57	60.56	4.87	25.51	1.24	6.47	1.24	6.49
	Gonghu 2	19.44	47.07	16.86	40.84	1.65	3.99	2.84	6.87
	Center region 1	165.13	46.99	122.17	34.77	6.21	1.77	52.48	14.93
	Center region 2	39.19	62.77	14.40	23.07	1.11	1.78	3.35	5.37
	South-west coast	36.32	60.53	16.36	27.27	0.60	1.01	4.11	6.85
	South coast	38.14	44.55	34.69	40.53	5.46	6.38	7.19	8.40
	East coast	7.80	54.86	3.39	23.87	0.69	4.85	1.42	9.97
	East region	0.23	66.55	0.07	19.11	0.01	3.37	0.02	6.72
	Phosphorus	Ortho-P		Mono-P		Di-P		Pyro-P	
		C	R	C	R	C	R	C	R
Chao Lake	West Region	54.8	40.27	60.8	44.69	5.6	4.15	14.5	10.68
	South-west Region	35.7	41.99	34.0	40.03	3.4	3.98	11.2	13.18
	South Region	57.2	52.46	38.5	35.36	6.5	6.01	6.3	5.74
	North-east Region	53.1	49.19	44.1	40.79	3.5	3.24	6.3	5.81
	East Region	43.8	33.19	74.5	56.41	0.2	0.17	12.7	9.62

[a] concentrations of phosphorus; [b] relative abundance of phosphorus.

[c] Ortho-P:orthophosphate, Mono-P: monoester phosphorus, Di-P:diester phosphorus, Pyro-P:pyrophosphate

SI. Table 3. Concentrations and relative abundance of phosphorus characterizations of algae in Tai and Chao Lakes.

Lake	No.	Samples	Ortho-P		Mono-P		Di-P		Pyro-P	
			C[a]	R[b]	C	R	C	R	C	R
Tai Lake	T1	Meiliang	0.7	39.4	1.0	55.6	0.0	0.5	0.1	4.5
	T2	Zhushan	0.9	42.0	1.1	55.1	0.0	0.7	0.0	2.2
	T5	Center region 1	1.8	49.5	1.8	49.0	0.0	0.0	0.1	1.5
	T7	South-west coast	1.2	38.0	1.4	43.0	0.0	0.0	0.6	19.0
Chao Lake	C1	West Region	0.6	50.9	0.6	47.8	0.0	0.0	0.0	1.3
	C2	South-west Region	0.3	37.1	0.5	62.9	0.0	0.0	0.0	0.0
	C3	South Region	0.8	52.1	0.7	43.8	0.0	0.0	0.1	4.0
	C4	North-east Region	0.6	46.7	0.6	45.9	0.0	0.7	0.1	6.7
	C5	East Region	1.3	49.0	1.3	49.5	0.0	0.3	0.0	1.2

[a] Concentrations of phosphorus (mg/g);

[b] Relative abundance of phosphorus (%).