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Microbial Biomass and Community Composition Involved in Cycling of Organic Phosphorus in Sediments of Lake Dianchi, Southwest China

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ABSTRACT

Organic phosphorus (P_o) was a major fraction of phosphorus (P) in sediments of lakes, and microbes were involved in most of its relevant biogeochemical cycling. Forms and quantification of P_o were investigated by sequential fractionation in 18 sediments of Lake Dianchi, Southwest China. Microbial biomass and community structure in these sediments were determined by phospholipid fatty acids (PLFAs). Distribution of P_o fractions were in the rank order that humic P_o > nucleic acid and polyphosphate > residual P > Ca-Al-P_o > Fe-P_o > sugar P_o > acid soluble P_o > H_2O-P_o. The recoveries of P_o and P_i in these detailed sequential fractions including residual P shows that the total contents of P_o in sediments of lakes were overestimated by the Standards, Measurements and Testing (SMT) protocol (ignition method). Microbial biomass including Gram-positive bacteria (14.4–20.0%), Gram-negative bacteria (32.7–38.4%), microeukaryotes (14.9–24.4%), aerobic bacteria (43.6–55.8%), anaerobic bacteria (0–2.9%) and type *y* methanotrophs (17.6–24.4%) were assigned. Microbial mass and their composition were strongly correlated with H_2O-P_o, Fe-P_o, nucleic acid and polyphosphate, and humic P_o, though residual P was likely inert for microbes in sediments. The formation and degradation of P_o was closely related with microbial activities in sediments. These findings have implications for understanding the role of microbes on cycling of P_o and organic matter in sediments of lakes.

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Correlation analysis; eutrophication; fatty acid analysis; microbe; organic phosphorus

Introduction

Phosphorus (P) is widely recognized as a key element resulting in eutrophication of lakes (Schindler et al. 2008), and microbes drive most of its relevant biogeochemical cycling (Søndergaard et al. 2003; McMahon and Read, 2013). The sediment plays an important role in the P dynamics and microbial activities of lakes (Qian et al. 2011). Internal P loading in the sediments could be a significant source for bioavailable P of algae and other organisms in overlying water, especially after reducing external loading in a eutrophic lake (Søndergaard et al. 2003; Zhu et al. 2013a). Organic P (P_o) can constitute 12–80% of this internal P load in sediments of lakes (Ding et al. 2010; Torres et al. 2014). Especially, biogeochemical cycling of P_o in sediments played an important role in the eutrophic status of lakes, such as Lake Dianchi and Lake Taihu in China (Zhu et al. 2013a; Zhu et al. 2015a).

Forms and labilities of P_o (generally including condensed phosphate) in sediments of lakes have been investigated by sequential extraction (Golterman, 1996; Zhang et al. 2008), ^{31}P-NMR (Zhang et al. 2013; Torres et al. 2014) and enzymatic hydrolysis (Zhu et al. 2013a; Giles et al. 2015). These P_o compounds and condensed P include phosphate monoester (e.g., AMP, inositol phosphates, α-glycerophosphate, β-glycerophosphate and glucose phosphate); diester phosphate (e.g., phospholipids, RNA and DNA); phosphonates; pyrophosphate; and polyphosphate (Ding et al. 2010; Jørgensen et al. 2011; Zhu et al. 2015b). The biogeochemical cycling of these P_o and condensed P including generation, degradation and transformation is closely related with activities of microbes in sediments of lakes (Søndergaard et al. 2003; Hupfer et al. 2004; McMahon and Read, 2013). However, there were still significant gaps in knowledge of relationships between internal cycling of P_o and microbial activities in sediments of lakes.

Phospholipid fatty acid (PLFA), as a biomarker for microbial biomass and community structure of microorganisms, has been applied to investigate the microbial biomass and community composition in sediments of lakes (White et al. 1979; Findlay et al. 1989; Steger et al. 2011; Zhao et al. 2011). Extraction of PLFA allows quantification of viable microbial biomass in sediments without cultivation, which could avoid biases of the true community composition (White et al. 1979; Zhao et al. 2011). Thus, the relationships between microbial biomass and other quantified parameters such as dissolved organic carbon, total nitrogen (TN) and total P (TP) in the water and sediments of lakes could be analyzed further (Steger et al. 2011; Zhao et al. 2011). Although there are many methods for quantification

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of P_o forms in the sediments, relationships between these P_o forms with microbial biomass characterized by PLFA have not, to the knowledge of the authors, been discussed previously. Sequential extraction can be used to fractionate P forms and evaluate their bioavailabilities in sediments (Golterman, 1996; Golterman et al. 1998; Zhang et al. 2008). A sequential extraction procedure had been developed by Golterman and coworkers (1996; 1998), which has been tested and calibrated for specific chemical P forms such as sugar bound phosphates and Ca-associated P (McDowell et al. 2005). Thus, P_o forms were characterized by the sequential extraction procedure developed by Golterman and coworkers (1996; 1998) for sediments of Dianchi Lake, a eutrophic lake, Southwest China. Microbial biomass and community composition in those sediments were analyzed by PLFA. Relationships between P_o forms and microbial biomass in sediments were analyzed further.

Materials and methods

Study area and sampling

Lake Dianchi (24°40′–25°02′ N, 102°36′–102°47′ E) is the sixth largest freshwater lake in China (Figure 1). It has an area of approximately 306 km^2 and is located in the southwest of Kunming, Yunnan Province, China. The average depth is approximately 5 m, and the maximum depth is 9.7 m. Lake Dianchi is very important as a source of drinking water supply, for industrial production, climate regulation and ecological protection. However, in recent decades Lake Dianchi has become eutrophic with frequent and massive blooms of algae (Wang et al. 2009). Phosphorus is the nutrient that currently limits productivity in Lake Dianchi (Gao et al. 2005). Lake Dianchi is a relatively closed system with a residence period of 3–8 years, which might exacerbate retention and accumulation of nutrients in the lake. Now that external inputs of nutrients

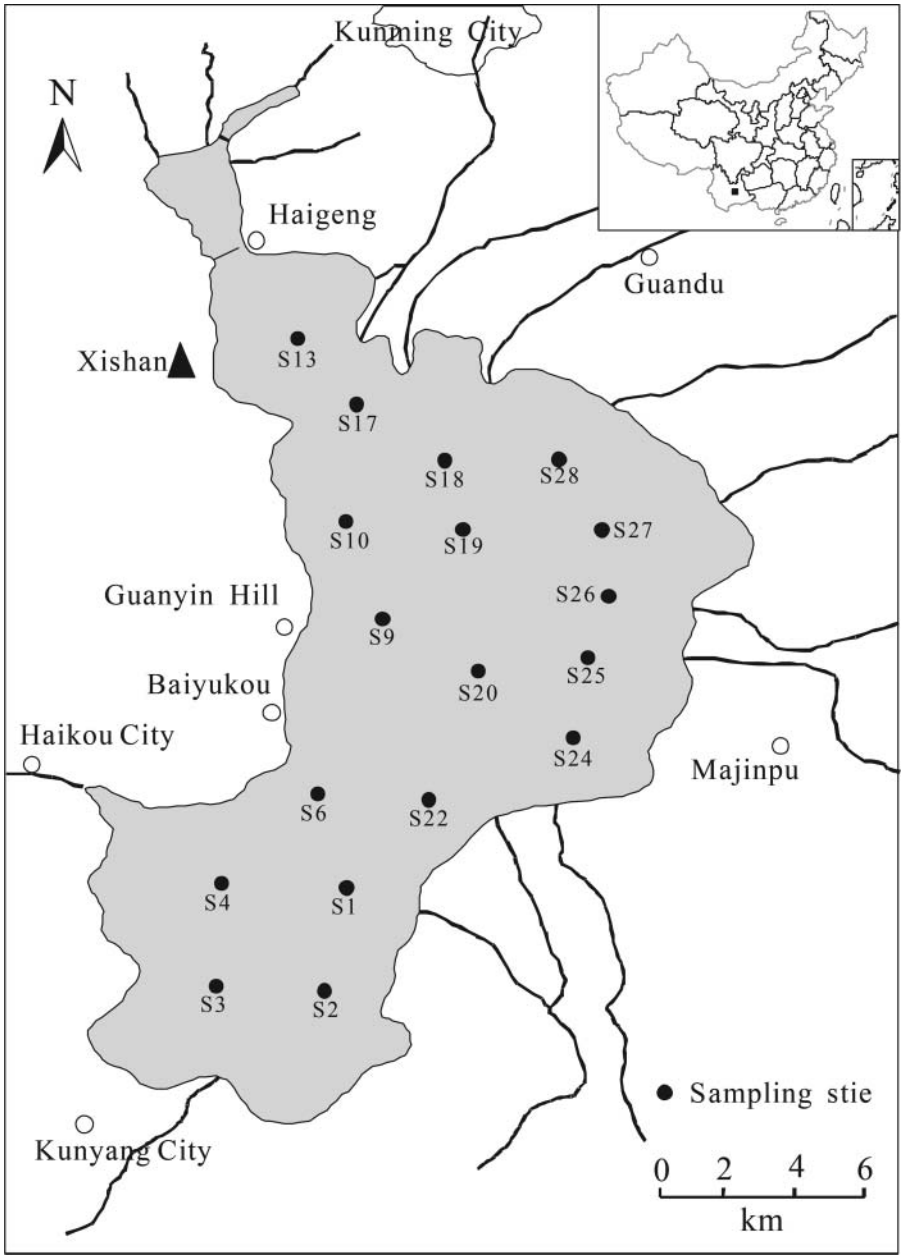

Figure 1. Map of Lake Dianchi showing sampling sites.

have been controlled to a certain extent (Lu et al. 2012), large loads of internal nutrients are a major factor that continue to influence the trophic status of Lake Dianchi (Gao et al. 2005; Zhu et al. 2013a). In May 2010, surface sediments were collected from 18 locations (identified as S1–S24; Figure 1) by use of a Peterson grab sampler. Sediments were transported to the laboratory in air-sealed plastic bags and cold storage with ice. Sediment samples were lyophilized and ground to powder and stored at −20°C before analysis.

Analysis of sediment properties

Total concentrations of Al, Ca, Fe and Mn were measured using inductively coupled plasma optical emission spectrometry after microwave acid (HNO_3–HCl–HF) wet digestion of sediment samples. General characteristics of P forms including contents of TP, inorganic P (P_i) and P_o in sediments were determined by the method that had been harmonized and validated by use of the SMT program of the European Commission (Ruban et al. 1999; Ruban et al. 2001). Concentrations of P were determined by molybdenum blue method (Murphy and Riley, 1962). Sediments were pretreated by an excess of 1 mol L^{-1} HCl to remove carbonates, and then analyzed for total organic carbon (TOC) and TN using an elemental analyzer (Vario EL III, Elementar, Germany).

Sequential fractionation of sediment phosphorus

Phosphorus in sediments was extracted using the sequential fraction scheme developed by Golterman et al. (1996; 1998) (Figure 2). Briefly, 0.5 g of each lyophilized sediment was sequentially extracted via shaking with 15 mL of deionized water (2 h), 0.05 M Ca-EDTA (+1% Na-dithionite, pH 7.5), 0.1 M Na-EDTA (pH 4.5), 0.5 M H_2SO_4, cold 0.5 M trichloroacetic acid (TCA; 0°C, 4 h), hot 0.5 M TCA (95°C, 30 min), and finally 2 M NaOH (90°C, 1 h) before the remaining P was released by digestion with $K_2S_2O_8$. These fractions represent, in sequential order, water soluble or sediment interstitial water P (H_2O-P), Fe associated P (Fe-P), Ca and Al associated P (Ca-Al-P), acid soluble inorganic and organic P (ASIP or ASOP), sugar bound P (Sugar P) after digestion by $K_2S_2O_8$ (cold TCA), nucleic P and polyphosphate (NP and PP) after digestion by $K_2S_2O_8$ (hot TCA), humic bound P and phytate (NaOH P_i and humic P_o), and residual P. Following extraction, each suspension of sediment was centrifuged (8000 × g) for 10 min, decanted, and an aliquot was taken for quantification of P. To determine the P_i from the chelating reagents extracts, a 25–50 time dilution was used to prevent interference of chelating reagents with the Mo-P colorimetric reaction. In addition, the P_o fraction was defined as the difference between detectable molybdate reactive P before and after digestion with $K_2S_2O_8$ at 121°C for 30 min.

Fatty acid analysis

For the PLFA extraction, 1.5 g freeze-dried sediment samples were analyzed by a modified one-phase Bligh–Dyer method (Frostegård et al. 1991). Total microbial biomass was determined as total phospholipids phosphate (PLP) by digestion of $K_2S_2O_8$ followed by quantification using phosphomolybdate–malachite green analyzed at 610 nm (Findlay et al. 1989). Structure of microbial community was determined by quantifying fatty acid methyl esters (FAMEs) from phospholipids. Total lipids were fractionated into neutral, glyco-, and polar lipids using solid phase extraction with silicic acid columns (Supelclean ™ LC-Si SPE Tubes, Supelco, Inc.). The polar fraction containing the phospholipids was subjected to a mild alkaline methanolysis for conversion to FAMEs. Organic fractions were dried by a stream of nitrogen and stored at −20°C prior to gas chromatography (GC) analysis.

FAMEs were dissolved in hexane for GC analysis. The fatty acid 19:0 (nonadecanoic acid methyl ester) was added to the samples as an internal standard. The FAMEs were identified by GC (Agilent 6890N) with an autosampler, an Agilent 7683B injector and a flame ionization detector. The following temperature program was used in the column oven: 170°C at the starting point, followed by an increase of 5°C/min to 260°C, and then increase of temperature at 40°C/min to 310°C, where temperature was maintained for 90 s. Vaporization chamber and detector temperature were 250°C and 300°C respectively. Volume injected was 1 μl, and the split ratio was 20:1. The FAMEs were identified by use of Sherlock MIS software (v. 4.5), and qualified relative to the internal standard.

Standard nomenclature for molecular formula as "X: YωZ(c/t)," was used for FAMEs (Table S1): "X" is the total number of carbon atoms and "Y" is the number of unsaturated double bonds. The position of the first double bond is indicated by "ω" and the number of carbon atoms from the aliphatic end is indicated by "Z." Furthermore, the suffixes "c" and "t" specify the cis and trans conformations of the double bond respectively. Methyl branching at the iso and anteiso positions was designated by the prefixes "i" and "a" respectively. The prefix "cy" denotes cyclopropane fatty acids. Additionally, the suffixes "G" and "Alcohol" specify the uncertain double bond and fatty alcohol. Certain PLFAs were assigned to microbial groups based on previous studies (Vestal and White, 1989; White et al. 1996; Liu et al. 2009; Steger et al. 2011). Thus, all iso- and anteiso-branched fatty acids with 14–19 carbons plus 15:0 were considered as representing Gram-positive bacteria (G^+); all monounsaturates containing 14–19 carbons (except 16:1ω9 and 18:1ω9) plus cyclopropane fatty acids were considered to be markers for Gram-negative bacteria (G^-). The sum of 14:0; 16:1ω9, 17:0, 18:0, 18:2, 18:3, 18:1ω9, 20:4, 20:5 and 24:0 were considered as representing microeukaryotes; all monounsaturates containing 14–19 carbons were regarded as aerobic bacteria; cyclopropane fatty acids (cy 17:0 and cy 19:0) were considered as representing anaerobic bacteria. Furthermore, monounsaturated 16 carbon fatty acids indicate type *y* methanotrophs, a type of methane-oxidizing bacteria. Moreover, Gram-positive bacteria would concert some monounsaturated carbon fatty acids to cyclopropane fatty acids under the stress of hunger. Therefore, contents of cyclopropane fatty acids could be stress biomarker for nutrients (Liu et al. 2009).

Statistical analysis

Data were checked for deviations from normality of variance before analysis. To check whether there were significant

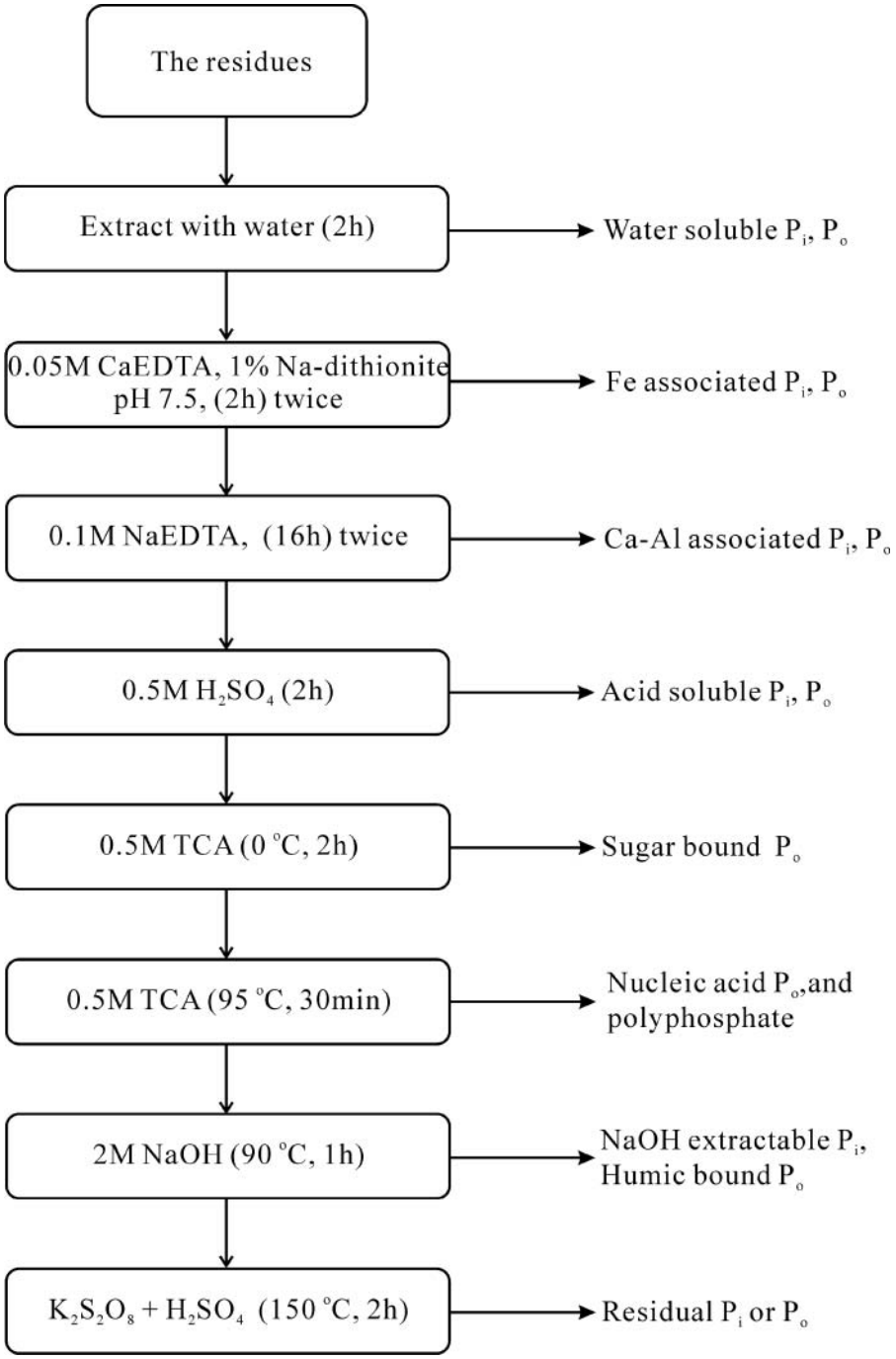

Figure 2. Procedure for sequential extraction of forms of organic phosphorus based on Golterman et al. (1996; 1998).

correlations between sequential P_o forms and microbial community structure in the sediments, Pearson correlation coefficients (r values, two-tailed) at $P < 0.01$ and $P < 0.05$ were determined using SPSS 11.5 for windows. Factor analysis including principal component analysis (PCA) was further carried out by SPSS 11.5.

Results and discussion

Sediment characteristics

Sediment characteristics of Lake Dianchi including TP, P_i, P_o, TOC, TN and TOC/TN (Table 1) were also discussed in a the previous study (Zhu et al. 2013a). Concentrations of TP in sediments of Lake Dianchi ranged from 1574 to 2623 mg kg^{-1}. Inorganic P was the main component of TP, concentrations of which ranged from 860 to 1847 mg kg^{-1} and accounted for 51.7–70.6% of TP. However, the HCl extractable P_i was the main composition of P_i in these sediments, which was P primarily bound to calcium that was released from sediments with difficulty (Zhu et al. 2013a; Zhu et al. 2013b). Concentrations of P_o ranged from 393 to 630 mg kg^{-1}, which accounted for 20.6% to 29.8% in sediments of Lake Dianchi. Contents of TOC and TN ranged from 3.31% to 8.29% and 0.42% to 1.00% respectively. Molar ratios of TOC/TN ranged from 8.2 to 9.7, which

Table 1. Characteristics of sediments from Lake Dianchi.

	mg kg^{-1}			%			g kg^{-1}			
Site	TP[a]	P_i[a]	P_o[a]	TOC[a]	TN[a]	TOC/TN[a,b]	Al	Ca	Fe	Mn
S1	2227	1323	570	6.63	0.95	8.2	70.4	48.9	58.3	0.8
S2	2215	1516	607	6.18	0.83	8.7	75.1	48.9	61.3	0.9
S3	2307	1441	630	5.81	0.81	8.4	74.2	49.7	62.0	0.8
S4	2278	1466	584	6.72	0.89	8.8	69.8	52.2	59.1	0.8
S6	1933	1159	551	5.69	0.75	8.8	69.9	58.2	64.2	0.9
S9	2015	1408	531	7.44	0.95	9.1	63.5	73.1	60.8	0.9
S10	1901	1254	567	6.76	0.89	8.9	67.5	73.9	63.8	0.9
S13	1743	1153	506	5.52	0.71	9.1	69.8	78.2	66.7	1.0
S17	1678	1058	433	4.90	0.70	8.2	67.8	72.0	67.4	1.0
S18	1632	860	450	3.31	0.42	9.1	80.2	36.0	77.0	0.9
S19	2264	1557	602	7.50	0.94	9.3	67.3	68.7	58.3	0.8
S20	2623	1847	540	8.29	1.00	9.7	66.9	51.1	59.6	0.8
S22	1773	916	471	5.91	0.74	9.3	72.2	30.5	69.8	0.8
S24	2023	1367	555	6.76	0.86	9.2	66.7	51.1	63.0	0.8
S25	1915	1193	523	4.90	0.66	8.7	71.7	41.1	71.2	0.9
S26	1631	974	470	3.44	0.45	9.0	66.0	36.8	75.1	1.0
S27	1772	1051	416	3.57	0.47	8.9	66.8	63.6	72.0	1.2
S28	1574	975	393	4.00	0.56	8.3	64.3	84.5	66.2	1.2

[a]These data also published in the previous paper; details could be found in the Supporting Information of Zhu et al. (2013a).
[b]Molar ratios between TOC and TN.

indicated that the organic matter derived mainly from the autochthonous sources such as algae, bacteria and aquatic macrophytes (Meyers and Ishiwatari, 1993). This was also supported by the molecular compositions of organic matter, $\delta^{13}C$ and $\delta^{15}N$ in sediments of Lake Dianchi from the previous studies (Wang et al. 2009; Xiong et al. 2010). Contents of Al ranged from 63.5 to 80.2 g kg^{-1}. Contents of Ca ranged from 30.5 to 84.5 g kg^{-1}. Contents of Fe were varied from 58.3 to 77.0 g kg^{-1}. Contents of Mn ranged from 0.8 to 1.2 g kg^{-1}. Contents of Al, Ca and Fe, especially Ca, was greater than that of other lakes in China, such as Lake Taihu and Lake Poyang (Ding et al. 2010). This is likely due to the fact that sediments from Lake Dianchi are calcareous sediments.

Distribution and recoveries of P_o and P_i in sequentially extracted fractions

Contents of H_2O-P_o ranged from 1.4 to 5.1 mg kg^{-1}, which accounted for 0.1–0.3% of TP in the sediments (Figure 3). H_2O-P_o was loosely adsorbed to sediment particles or in interstitial water of sediments, which was transferred easily across

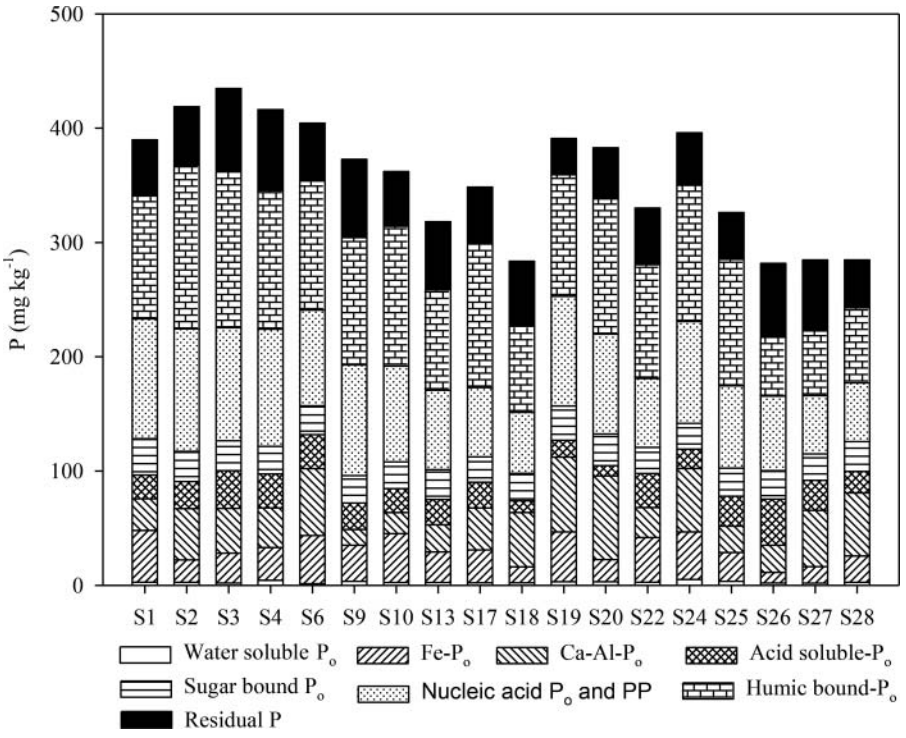

Figure 3. Distributions of P_o including condensed P (PP, polyphosphate) in the sequential extraction fractions.

the sediment–water interface (Zhu et al. 2013a; Zhu et al. 2015b). Though there was only a small proportion of H_2O-P_o in TP, large proportions of H_2O-P_o extracted from the sediments of Lake Dianchi could be hydrolyzed by phosphatase (e.g., alkaline phosphatase, phosphodiesterase and phytase), thus bioavailable for algal blooming (Zhu et al. 2013a). Contents of Fe-P_o ranged from 9.2 to 45.3 mg kg^{-1} and accounted for 0.6–2.3% of TP in sediments. Fe associated P was an important internal source of P in sediments that could be released to support blooms of cyanobacteria in Lake Dianchi (Hu et al. 2007; Zhu et al. 2015a). Fe-P_o in sediments could be released into the overlying water under anoxic conditions, thus some dissolved P_o (e.g., phytate) could be hydrolyzed by phosphatase (Golterman et al. 1998; Zhu et al. 2013a). Some P_o (e.g., glucose phosphate) associated with Fe oxides such as goethite could be hydrolyzed by phosphatase on the surface of mineral directly without desorption (Olsson et al. 2011), and thus could be as a proportion of Fe-P_i (Figure 4). Contents of Ca-Al-P_o ranged from 13.8 to 73.2 mg kg^{-1}, and accounted for 0.7–3.5% of TP in sediments. Aluminum hydroxide has been widely used to precipitate P from overlying water of lakes and to immobilize P in sediments (Jensen et al. 2015). Ca-Al-P_o in sediments was thought to be released with difficulty and thus hardly bioavailable to support algal blooming (Zhu et al. 2015a). Contents of ASOP ranged from 9.0 to 40.3 mg kg^{-1}, and accounted for 0.3–2.5% of TP in sediments. ASOP was thought to be a component of moderately labile P_o (Zhang et al. 2008). Contents of sugar P_o ranged from 22.2 to 31.8 mg kg^{-1} with relative contributions to TP in sediments of 1.1–1.7%. Sugar P_o, such as glucose phosphate, which would be hydrolyzed by phosphatase and release bioavailable phosphate (Zhu et al. 2013a), has also been detected by ^{31}P-NMR (Giles et al. 2015). Humic P_o, NP and PP defined in this procedure of sequential fractionation were the main constituents of P_o in sediments from Lake Dianchi (Figure 3). Contents of NP and PP ranged from 51.0 to 107.3 mg kg^{-1}, and accounted for 2.9–4.9% of TP in sediments. Nucleic acid P_o (e.g., DNA, RNA) and PP (e.g., pyrophosphate) could be hydrolyzed by phosphatase, thus was bioavailable for algal blooming (Zhu et al. 2013a; Zhu et al. 2015b). Contents of humic P_o ranged from 51.5 to 143.0 mg kg^{-1}, and accounted for 3.2–7.5% of TP in sediments. Humic P_o, characterized by ^{31}P-NMR and hydrolysis by phytase, included orthophosphate, phosphonates, monoester P (e.g., glucose phosphate, inositol phosphates, α- and β-glycerophosphate) and diester P (Golterman et al. 1998; He et al. 2015). When evaluated by ultraviolet irradiation and enzymatic hydrolysis, only 10–63% humic bond P was considered to be labile (He et al. 2015). Bioavailability of humic P_o would be reduced by interaction between P_o and humic substances in sediments of lakes (Zhu et al. 2015b). Additionally, contents of residual P ranged from 31.5 to 72.4 mg kg^{-1} and accounted for 1.4–4.0% of TP in sediments. Residual P after sequential fractionation would be a component of P_o or P_i in sediments from Lake Dianchi, which could be refractory P_o or P_i.

Concentrations and distributions of P_i fractions extracted simultaneously with the P_o fractionation are shown (Figure 4). Ca-Al-P_i was the dominant fraction in sediments from Lake Dianchi, ranging from 732.6 to 1297.7 mg kg^{-1}, with contributions of 41.4–50.5% of TP in sediments. This is likely due to abundant phosphate rock in the area of Lake Dianchi. For example, concentrations of Ca-Al-P_i were greater in sediments from south part of Lake Dianchi (S1, S2, S3 and S4), which was the result of phosphate rock mining in this area in the past. H_2O-P_i and Fe-P_i were the important internal source of bioavailable P in sediments of Lake Dianchi (Hu et al. 2007; Zhu et al. 2015a). Contents of H_2O-P_i ranged from 0 to 1.4 mg kg^{-1} and accounted for only 0–0.1% of TP in sediments; contents of Fe-P_i ranged from 188.1 to 524.1 mg kg^{-1} and accounted for

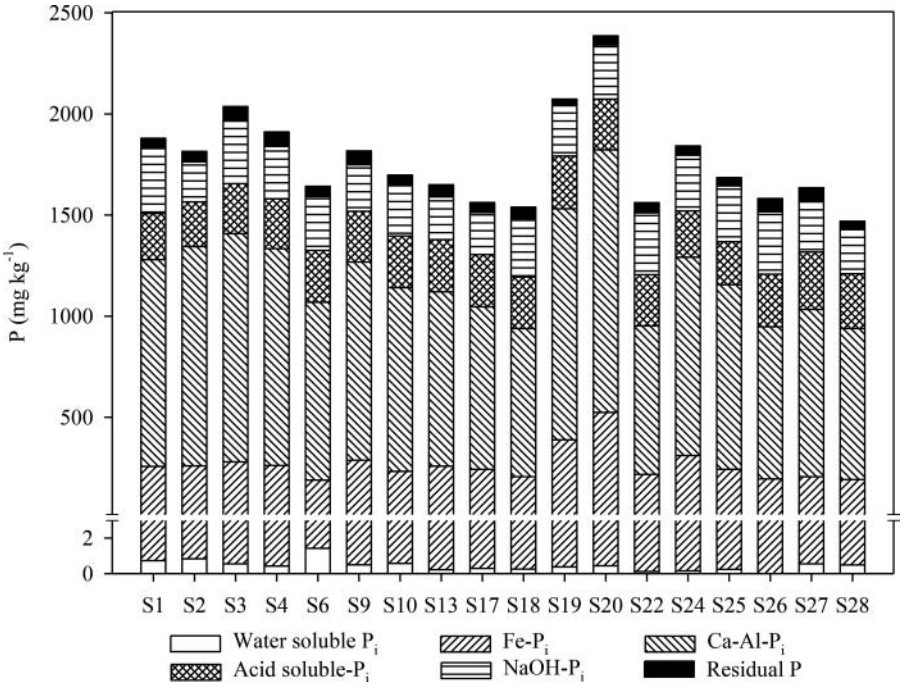

Figure 4. Distributions of P_i in the sequential extraction fractions.

Table 2. Recoveries (%) of P including TP, P_i and P_o by sequential extraction in sediments of Lake Dianchi.

Recoveries	S1	S2	S3	S4	S6	S9	S10	S13	S17	S18	S19	S20	S22	S24	S25	S26	S27	S28
TP[a]	99.7	98.5	104.0	99.0	103.3	105.3	105.8	109.5	110.9	108.3	107.4	103.9	103.9	108.3	102.9	110.3	104.8	108.8
P_o[b]	68.4	69.0	69.0	71.2	73.5	70.2	63.8	62.9	80.5	63.1	65.0	70.9	70.1	71.3	62.3	60.0	68.4	72.4
P_i[c]	138.4	116.3	136.3	125.5	137.3	124.2	131.6	138.0	143.0	172.3	131.1	126.8	165.0	131.4	137.8	155.8	149.7	146.4

[a]TP in the sediments were determined by the SMT procedure, and recovery of TP including sequential extraction TP and residual P.
[b]Contents of total P_o were determined by the SMT procedure, and recovery of P_o including sequential extraction P_o and residual P.
[c]Contents of total P_i were determined by the SMT procedure, and recovery of P_i including sequential extraction P_i, except for residual P.

9.7–20.0% of TP in sediments. Contents of ASIP ranged from 212.1 to 283.7 mg kg^{-1} and accounted for 9.6–17.2% of TP in sediments. ASIP is likely the proportion of Ca-P not extracted by Na_2EDTA in the previous step. Contents of NaOH-P_i ranged from 198.6 to 323.0 mg kg^{-1} and accounted for 9.0–19.0% of TP in sediments. NaOH-P_i is likely the proportion of Fe-P_i or Al-Ca-P_i not extracted in previous steps. Residual P was also likely a component of P_i fractions, with relatively low proportions in P_i fractions (Figure 4).

Recoveries of TP, P_o and P_i from sediments of Lake Dianchi by this sequential extraction procedure are given in Table 2. Recoveries of TP were close to 100%, which ranged from 98.5% to 110.9% in these sediments. However, recoveries of P_o varied from 60.0% to 80.5% in these sediments. Recoveries of P_i were 116.3–172.3% in these sediment samples. The reason for this might have been due to labile P_o being hydrolyzed during extraction and measurement. For example, solutions including H_2SO_4 and NaOH could result in hydrolysis of P_o (Turner et al. 2005). Second, total contents of P_o in these sediments were overestimated by use of the SMT protocol. Total contents of P_i in these sediments were underestimated by use of the SMT protocol. Overestimation of P_o in sediments by use of the SMT protocol was also supported by results of sequential extraction and ^{31}P-NMR analysis (Zhu et al. 2016). It is likely that some P_i is immobilized strongly by sediments—due to this the solution of HCl in the SMT protocol could not recover all of the P_i from sediments in the first step. Thus, the remaining P_i in sediments would be determined as a proportion of P_o after ignition and extraction by HCl solution again (Zhu et al. 2016). Also, the ignition method tends to overestimate contents of P_o in soils by increasing solubility of P_i after ignition (Condron et al. 1990; Turner et al. 2005). Thus, caution is needed in quantification of P_o in sediments by use of the ignition method.

Microbial biomass and community structure characterized by PLFA

Microbial biomass in sediments from Lake Dianchi, as measured by the PLP, ranged from 420.0 nmol PLP g^{-1} to 1247.9 nmol PLP g^{-1} dm (dry mass) (Figure 5). Total amounts of PLP in sediments of Lake Dianchi were compared with contents of PLP in the sediments of eight lakes from Sweden (Steger et al. 2011) and an eutrophic Lake Acton (Smoot and Findlay, 2001). This result indicated that the microbial biomass in sediments from eutrophic Lake Dianchi was similar to those in sediments of other lakes described previously. Microbial community structure in Lake Dianchi was further characterized by use of the compositions of FAMEs from PLFA (Table 3 and Table S1). In sediments from Lake Dianchi, the proportion of G^+ ranged from 14.4% to 20.0%, while the proportion of G^- ranged from 32.7% to 38.4%. The proportion of microeukaryotes ranged from 14.9% to 24.4%. The proportion of aerobic bacteria accounted for 43.6–55.8% of the microbial biomass in sediments of Lake Dianchi. However, based on relative proportions of FAMEs, the proportion of anaerobic bacteria accounted for only 0–2.9% of the microbial biomass in sediments of Lake Dianchi. Additionally, the proportion of type *y* methanotrophs accounted for 17.6–24.4% of microbial biomass in sediments. Proportions of G^+, G^- and microeukaryotes in sediments of Lake Dianchi were similar with those in sediments

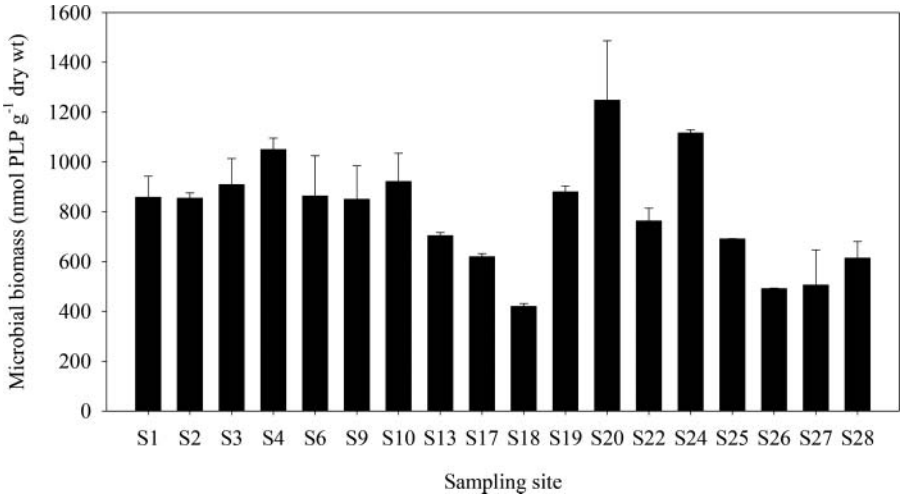

Figure 5. Distribution of microbial biomass (characterized by PLP) in sediments from Lake Dianchi. Data presented as the average value with standard deviation ($n = 2$).

Table 3. Microbial community structure in sediments from Lake Dianchi.

Sites	Percentage of different microbial groups in total microbial biomass (%)						G^+/G^-	nmol/g Stress
	G^+	G^-	Microeukaryotes	Aerobic bacteria	Anaerobic bacteria	Type I methanotrophs		
S1	14.4	34.4	22.5	50.8	0.5	21.2	0.42	1.90
S2	14.8	36.9	22.3	53.2	0.5	23.3	0.40	1.78
S3	16.2	38.4	22.4	51.4	2.9	23.2	0.42	8.68
S4	17.9	33.3	22.6	49.5	0.5	21.9	0.54	1.79
S6	17.1	36.5	24.4	55.4	0.6	24.1	0.47	1.92
S9	17.6	36.1	22.8	53.4	0.5	23.7	0.49	2.02
S10	18.0	37.7	23.8	55.8	0.5	24.2	0.48	1.73
S13	17.2	37.1	23.6	55.4	0.0	24.4	0.46	0.00
S17	17.6	35.0	23.9	52.9	0.0	22.7	0.50	0.00
S18	18.3	35.2	18.3	47.9	0.0	17.6	0.52	0.00
S19	18.9	36.6	16.7	47.2	0.6	21.7	0.52	1.49
S20	20.0	34.5	14.9	43.8	0.6	21.4	0.58	2.36
S22	16.5	35.3	16.4	43.6	2.1	20.8	0.47	6.01
S24	18.7	34.4	22.6	49.5	1.1	21.9	0.54	5.45
S25	17.0	36.9	23.3	51.3	2.9	21.1	0.46	7.60
S26	16.3	35.1	18.4	46.7	1.8	21.3	0.46	4.53
S27	18.6	32.7	18.4	46.2	0.0	19.8	0.57	0.00
S28	19.3	35.9	21.1	49.2	2.8	22.2	0.54	7.45

from boreal lakes of Sweden (Steger et al. 2011). Ratios of G^+ to G^- (G^+/G^-) ranged from 0.4 to 0.6 (Table 3), which suggested that G^- was the predominant composition of microbial biomass in sediments from Lake Dianchi. These results further showed that Gram-negative bacteria were the most important contributors to community composition in sediments of lakes (Steger et al. 2011).

Proportions of aerobic bacteria were relatively great (Table 3), though relatively large amounts of organic matter have been accumulated in sediments due to frequent blooms of algae. Values of DO in the overlying water of Lake Dianchi now range from 5 to 10 mg L^{-1}; hypoxia (DO $\leq$2 mg L^{-1}) occurred rarely in the overlying water (Liu et al. 2014). Based on the composition of FAMEs, proportions of anaerobic bacteria were small in sediments of Lake Dianchi. Branched fatty acids containing 14–16 carbons also likely represented some anaerobic bacteria (Zaady et al. 2010). However, only cyclopropane fatty acids (cy 17:0 and cy 19:0) were selected as representing anaerobic bacteria based on previous studies (Vestal and White, 1989). Thus, the proportion of anaerobic bacteria might underestimate in this study. Type *y* methanotrophs are physiologically and phylogenetically distinct and fall into the group of Gammaproteobacteria. Type *y* methanotrophs are an important methane-oxidizing bacteria in sediments of Lake Dianchi (Table 3), which also support by the abundance of Gammaproteobacteria (Bai et al. 2012) and quantification of type *y* methanotrophs (Yang et al. 2016) in sediments of Lake Dianchi. Type *y* methanotrophs play an important role in cycling of carbon, nitrogen and oxygen in lakes, and are also widespread in other lakes such as Lake Washington (Auman et al. 2000; Costello et al. 2002). Lake Dianchi, is a hypereutrophic lake, where debris derived from cyanobacteria and aquatic plants accumulated in sediments (Dong et al. 2006; Qu et al. 2013). Under suitable conditions in Lake Dianchi, methane would be produced from accumulated organic matter (Dong et al. 2006; Yang et al. 2016). The flux of methane was detected at the water–air interface (Chen et al. 2007). Type *y* methanotrophs would play a key role in oxidizing and mitigating the methane emission in sediments of Lake Dianchi (Yang et al. 2016). Although the methane was the only source of carbon for methanotrophs, they could degrade other organic matter in sediments, which would also play an important role on cycling of organic matter in the sediments of Lake Dianchi. Additionally, the "stress" of nutrients for microbes was low, which indicated that the organic matter accumulated in the sediments of Lake Dianchi received sufficient nutrients.

Relationships between P fractions and microbial community structure

Bulk compositions of organic matter including TOC, TN and TP; some P_o fractions including H_2O-P_o, Fe-P_o, NP and PP; and humic P_o were significantly correlated with microbial biomass and other microbial composition except for anaerobic bacteria (Table 4). Greater concentrations of TOC in sediments would result in greater abundances of microbial mass in

Table 4. Relationships between microbial community and TOC, TN and P_o forms in the sediments from Lake Dianchi.

Microbial community	TOC	TN	TP	H_2O-P_o	Fe-P_o	Ca-Al-P_o	ASOP	Sugar-P_o	NP and PP	Humic P_o	Residual P
Microbial biomass	0.904**	0.884**	0.837**	0.586*	0.494*	0.283	−0.245	0.237	0.744**	0.707**	−0.134
G^+	0.751**	0.700**	0.623**	0.725**	0.347	0.243	−0.255	0.070	0.571*	0.399	−0.057
G^-	0.788**	0.785**	0.669**	0.619**	0.442	0.052	−0.091	0.196	0.761**	0.552*	0.006
Microeukaryotes	0.609**	0.661**	0.458	0.585*	0.493*	−0.135	0.038	0.054	0.720**	0.552*	0.127
Aerobic bacteria	0.736**	0.757**	0.586*	0.588*	0.475*	−0.035	−0.055	0.147	0.767**	0.560*	0.068
Anaerobic bacteria	−0.065	−0.040	0.025	0.207	0.019	−0.056	0.334	−0.037	0.000	0.060	−0.070
Type I methanotrophs	0.780**	0.783**	0.628**	0.591**	0.448	0.025	−0.059	0.152	0.752**	0.553*	0.053

**$P < 0.01$; *$P < 0.05$; $n = 18$.

sediments of Lake Dianchi. This indicated that microbial activities would be important for decomposition of organic matter in sediments. Decomposition of organic matter with microbial activities would result in degradation or release of nutrients such as P_o and N complexed with organic matter (Wu et al. 2001; Wu et al. 2010). Microbes have been shown previously to be the key factor for cycling of N in sediments of lakes (Keeney, 1973). Contents of TN were significantly correlated with microbial mass in sediments from Lake Dianchi. Contents of TP were also significantly correlated with microbial mass in sediments, which indicated that microbes also play an important role on biogeochemical cycling of P in Lake Dianchi. Bacterial communities and their roles in mineralization of P in sediments of Lake Dianchi has also been studied previously (Xia et al. 2004). Results of this study suggested that numbers of P-accumulating bacteria were greater in areas where P was accumulating, and that bacteria can absorb soluble P and transport it into the form of polyphosphate after self-synthesis in the sediments of Lake Dianchi (Xia et al. 2004). After death of P-accumulating bacteria, the P would be accumulated in sediments as various minerals. Additionally, P-decomposing bacteria were much greater (approached 490 times) in the P_o culture medium rather than that in the P_i culture medium (Xia et al. 2004). This result indicated that accumulating P_o in sediment during eutrophication of Lake Dianchi resulted in increased growth of bacteria in sediments.

H_2O-P_o was significantly correlated with microbial mass, G^+, G^-, microeukaryotes, aerobic bacteria and type *y* methanotrophs (Table 4), which was likely that the decomposition of organic matter by bacteria results in increasing labile P_o in sediments of lakes. Additionally, bacteria play an important role on bioavailability of H_2O-P_o that dissolved into overlying water, which was readily available for algal blooming (Zhao et al. 2012). Bacteria such as *Gordonia sp.* and *Burkholderia sp.* can degrade constituents of labile H_2O-P_o such as glucose phosphate and mononucleotide phosphate and release bioavailable orthophosphate to support blooming of algae in eutrophic lakes (Zhao et al. 2012). Fe-P_o was also closely related with microbes such as microeukaryotes and aerobic bacteria (Table 4). These microorganisms would consume O^2, NO_3- and so on during organic matter decomposition, thus providing necessary conditions for reduction of Fe(III) and subsequent release of Fe-P_o (Gächter et al. 1988). There were no significant relationships between microbial biomass and Ca-Al-P_o, ASOP and sugar P_o (Table 4). The distribution of microbial biomass was significantly correlated with NP and PP in the sediments of Lake Dianchi. Contents of NP and PP characterized by the same sequential fractions in this study were significantly correlated with contents of diester such as DNA and RNA characterized by ^{31}P-NMR in the soils ($P < 0.01$) (McDowell et al. 2005). DNA or RNA also could represent the microbial mass. Thus, NP and PP could be directly related to microbial mass; it is likely that NP and PP were derived from microbial mass in the sediments of Lake Dianchi. The NP and PP attributed by microbial mass would play an important role on cycling of P in lakes (Hupfer et al. 2004; Shinohara et al. 2012). Humic P_o was also closely related with microbial biomass (Table 4). Humic P_o includes monoester P, diester P and pyrophosphate complexed with humic substances (He et al. 2006). Some labile P_o in humic substance could be hydrolyzed by activities of microbial biomass, such as enzymatic hydrolysis (Lovley et al. 1996). Some P_o derived from debris of microbial biomass would be complexed with humic substances in sediments (He et al. 2015; Zhu et al. 2015b). Further investigation was needed for understanding the role of microbial biomass on the formation of humic P_o in sediments. Additionally, there were no relationships between residual P and microbial biomass, which further indicated that residual P would be refractory and could not be recycled by the activities of microbes in sediments of Lake Dianchi.

Relationships between the P_i fractions and microbial biomass were also analyzed (Table 5). There were no relationships between H_2O-P_i and microbial biomass. However, Fe-P_i was significantly correlated with microbial mass, G^+, G^-, which indicated that cycling of Fe-P_i was closely related with microbes in sediments of Lake Dianchi. Ca-Al P_i was also significantly correlated with microbial biomass. Fe-P_i and Ca-Al P_i were the main component of P_i in the sediments of Lake Dianchi (Figure 4). It has been reported that microbes were the key factor in formation of Fe-P_i and Ca-Al P_i in sediments of Lake Dianchi (Xia et al. 2004). Phosphorus-concentrating bacteria assimilated soluble P into P_o or condensed P_i, which would form precipitates with Fe, Ca or Al hydrated P_i after death of microbes and diagenesis in sediments of Lake Dianchi (Xia et al. 2004). The negative correlation between ASIP and G^-, microeukaryotes and aerobic bacteria indicated that carbonic acid, nitric acid or organic acids would be important for dissolution of ASIP in sediments (Xia et al. 2004). Additionally, there was no significant correlation between NaOH P_i and residual P with microbial biomass (Table 5).

In order to present an in-depth analysis of correlations between P_o fractions and microbial biomass in sediments, a PCA was carried out (Table 6). Results of the PCA showed that the proportion of total variance explained by PC1–PC6 was 91.549%, which explained the majority of relationships

Table 5. Relationships between microbial community and P_i forms in the sediments from Lake Dianchi.

R	Water soluble P_i	Fe-P_i	Ca-Al-P_i	Acid soluble P_i	NaOH-P_i	Residual P
TPLFA	0.233	0.710**	0.814**	−0.380	0.035	−0.134
G^+	0.112	0.598**	0.617**	−0.270	−0.007	−0.057
G^-	0.258	0.477*	0.646**	−0.474*	0.036	0.006
Microeukaryotes	0.337	0.161	0.443	−0.520*	−0.053	0.127
Aerobic bacteria	0.335	0.347	0.571*	−0.479*	−0.044	0.068
Anaerobic bacteria	−0.196	−0.068	−0.001	−0.294	0.379	−0.070
Type I methanotrophs	0.295	0.435	0.614**	−0.419	−0.047	0.053

**$P < 0.01$; *$P < 0.05$; $n = 18$.

Table 6. Principal component analysis for TOC, TN, P_o fractions and microbial community from sediments of Lake Dianchi.

	Component					
	PC1	PC2	PC3	PC4	PC5	PC6
TOC	**0.918**	−0.196	0.132	−0.063	−0.103	0.089
TN	**0.927**	−0.151	0.227	−0.129	−0.079	0.075
TP	**0.800**	−0.248	0.277	0.338	0.191	0.166
Water soluble P_o	**0.630**	0.098	−0.542	−0.025	−0.043	0.039
Fe-P_o	**0.571**	−0.137	0.110	**−0.704**	−0.106	−0.099
Ca-Al-P_o	0.128	**−0.698**	−0.290	0.384	0.211	0.123
Acid soluble P_o	−0.215	**0.758**	0.323	−0.061	0.331	0.008
Sugar P_o	0.302	**−0.518**	**0.474**	0.094	0.346	**−0.501**
Nucleic acid P_o and PP	**0.856**	0.055	**0.418**	0.096	−0.068	−0.022
Humic P_o	**0.717**	−0.009	0.297	−0.171	−0.039	**0.540**
Residual P	−0.104	**0.736**	0.276	**0.492**	−0.158	0.061
TPLFA	**0.943**	−0.100	−0.058	0.081	0.061	0.184
G^+	**0.875**	0.069	−0.399	0.184	−0.025	−0.081
G^-	**0.944**	0.197	−0.141	0.070	0.055	−0.140
Microeukaryotes	**0.845**	0.389	−0.106	−0.062	−0.089	−0.179
Aerobic bacteria	**0.920**	0.272	−0.098	0.029	−0.072	−0.187
Anaerobic bacteria	0.086	0.295	−0.277	−0.226	**0.846**	0.134
Type I methanotrophs	**0.935**	0.238	−0.122	0.072	−0.024	−0.145
% of variance	52.565	13.607	8.327	6.550	6.162	4.338
Cumulative % of variance	52.565	66.172	74.499	81.049	87.211	91.549

between P_o fraction and microbial biomass. PC1, which accounted for 52.565% of the total variance, was primarily positively correlated with nutrients and P_o fractions (TOC, TN, TP, H_2O-P_o, Fe-P_o, NP and PP, and humic P_o) and microbial composition (microbial biomass, G^+, G^-, microeukaryotes, aerobic bacteria and type *y* methanotrophs). This result showed that P_o fractions including H_2O-P_o, Fe-P_o, NP and PP, and humic P_o were closely related with decomposition or accumulation of organic matter and activities of microbes in sediments of Lake Dianchi. PC2, which accounted for 13.607% of the total variance, was negatively loaded by Ca-Al-P_o and sugar P_o, and was positively loaded by ASOP and residual P. These P_o fractions were likely more influenced by other factors rather than microbes in sediments of Lake Dianchi. PC3, which accounted for 8.327% of the total variance, was primary correlated with sugar P_o and NP and PP. This indicated that the origin of sugar P_o and NP and PP was likely similar, such as from microbe or plankton. Origin and decomposition of sugar P_o were also related with microbe in the sediments. PC4, accounted for 6.550% of the total variance, was negatively loaded by Fe-P_o and positively loaded by residual P. This further indicated that biogeochemical cycling of Fe-P_o and residual P was different; an anaerobic condition would result in release of Fe-P_o. However, residual P would be preserved in sediments. PC5, which accounted for 6.162% of the total variance, was correlated with anaerobic bacteria. This is likely that the anaerobic bacteria could not be characterized well by the PLFA in the sediments in this study. Thus, the role of anaerobic bacteria in sediments of Lake Dianchi needs further investigation. PC6 accounted for 4.338% of the total variance and was negatively loaded by sugar P_o and positively loaded by humic P_o. This likely indicated that sugar P_o as operationally defined here was labile and could be hydrolyzed or assimilated by microbes in lakes, whereas large humic P_o was likely formed under the activities of microbe in sediments of Lake Dianchi.

Conclusions

Based on a sequential fractionation of P, the concentrations and proportions of P_o fractions to TP (average value) in sediments from Lake Dianchi, Southwestern China were in the rank order that humic P_o (104.0 mg kg^{-1}, 5.3%) > nucleic acid and polyphosphate (79.7 mg kg^{-1}, 4.0%) > residual P (53.2 mg kg^{-1}, 2.8%) > Ca-Al-P_o (39.8 mg kg^{-1}, 2.0%) > Fe-P_o (29.0 mg kg^{-1}, 1.5%) > sugar P_o (25.3 mg kg^{-1}, 1.3%) > acid soluble P_o (23.2 mg kg^{-1}, 1.2%) > H_2O-P_o (2.8 mg kg^{-1}, 0.1%). The recoveries of P_o and P_i in these detailed sequential fractions including residual P show that the total contents of P_o in sediments of lakes were overestimated by the SMT protocol (ignition method).

There was abundant microbial biomass (measured by PLP) in sediments from Lake Dianchi. With this microbial biomass, G^+ (14.4–20.0%), G^- (32.7–38.4%), microeukaryotes (14.9–24.4%), aerobic bacteria (43.6–55.8%), anaerobic bacteria (0–2.9%) and type *y* methanotrophs (17.6–24.4%) were assigned. Gram-negative bacteria were the most important contributors to community composition in sediments of Lake Dianchi. Large amount of organic matter accumulated in the sediments of Lake Dianchi received enough nutrients for these microbes.

The microbial mass and their composition were closely related with TOC, TN and TP in sediments of Lake Dianchi. The P_o fractions including H_2O-P_o, Fe-P_o, nucleic acid and polyphosphate, and humic P_o were closely related with the activities of microbial mass in the sediments of Lake Dianchi. Residual P in sediments was not being recycled by the activities of microbes. Accumulation of organic matter and nutrients in sediments of eutrophic lakes would result in more activities of microbes, thus biogeochemical cycling of P_o in those lakes. However, microbes involved in cycling of P_o in sediments of lakes, such as molecular mechanisms controlling metabolism of P_o should be further investigated (McMahon and Read, 2013).

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Table S1 Individual fatty acid concentrations (nmol g^{-1}) in the sediments from Lake Dianchi, Southwest China (FAME: fatty acid methyl ester)

FAME	S1	S2	S3	S4	S6	S9	S10	S13	S17	S18	S19	S20	S22	S24	S25	S26	S27	S28
C 10:0 2OH	0.000	1.599	1.663	1.640	0.000	1.531	0.000	0.000	0.000	0.000	0.000	1.591	0.000	0.000	0.000	3.209	0.000	1.767
C 12:0	14.358	15.954	0.000	15.166	0.000	15.942	0.000	0.000	0.000	0.000	0.000	13.694	14.778	0.000	0.000	0.000	0.000	0.000
C 13:0 ANTEISO	13.477	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.353	0.000	0.000	0.000	0.000	0.000	0.000
C 14:0 ISO	8.032	7.441	7.085	9.445	7.380	10.336	8.266	6.622	5.326	5.432	8.017	12.043	7.131	13.814	6.876	9.012	9.030	7.690
C 14:0	8.793	8.802	7.497	10.280	7.499	10.606	8.287	6.819	4.711	4.423	6.946	10.463	7.203	13.145	6.261	6.700	5.743	6.195
C 15:1 ISO G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.448	0.000	0.000	0.000	0.000	0.000	0.000
15:0 ISO	12.008	13.553	12.061	13.549	13.197	14.847	12.415	10.008	7.403	7.609	10.943	16.957	11.052	17.347	10.426	10.539	9.975	10.936
15:0 ANTEISO	19.509	17.464	15.676	26.547	18.483	28.631	21.866	15.670	11.692	9.134	18.651	33.463	16.201	33.858	15.089	13.281	16.763	19.037
C 15:0	3.492	3.604	3.229	3.923	3.473	3.553	2.950	2.411	1.849	1.559	2.561	3.308	2.762	4.930	2.724	2.416	0.000	2.325
C16N Alcohol	7.908	7.453	6.857	7.312	7.378	7.062	7.000	6.948	5.996	7.304	6.928	7.020	6.922	13.855	7.394	13.226	12.753	7.718
16:0 ISO	5.197	4.641	4.055	6.644	5.035	6.753	5.166	4.011	3.032	2.450	4.827	7.818	4.188	8.618	3.908	3.176	4.434	4.844
16:1 ω7c	72.915	74.835	62.260	75.177	68.448	86.855	68.283	55.482	37.850	24.018	51.461	80.304	52.492	93.696	49.646	48.622	41.649	53.266
16:1ω5c	8.204	7.884	6.073	9.306	8.075	11.056	8.866	6.601	4.961	2.817	6.884	8.853	6.014	11.426	5.422	4.781	4.804	5.326
C 16:0	49.639	49.703	44.062	57.300	44.647	52.042	41.846	33.850	25.635	21.195	35.369	50.001	38.037	71.914	36.932	34.612	31.280	36.241
ISO 17:1 G	9.336	10.280	8.090	11.652	10.122	8.533	7.805	6.517	4.804	4.392	5.223	7.231	6.820	13.649	7.597	5.718	7.207	6.861
17:1 ANTEISO B/i I	9.194	9.108	8.141	9.043	9.085	8.995	8.538	8.255	7.139	8.714	7.539	9.166	8.129	11.875	8.638	9.873	10.623	9.310
17:0 ISO	2.829	2.619	2.334	3.171	2.659	3.086	2.587	2.009	1.654	0.000	2.212	2.948	2.201	4.115	2.298	0.000	0.000	2.173
17:0 ANTEISO	4.076	3.272	3.246	5.849	3.961	5.690	4.169	3.062	2.144	1.661	3.681	6.591	2.992	7.012	3.167	2.399	3.352	3.875
17:0 CYCLO	0.000	0.000	7.070	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.275	5.453	6.011	4.526	0.000	5.889
17:1ω8c	1.882	1.423	0.000	1.490	1.733	1.743	1.463	0.000	0.000	0.000	0.000	1.714	0.000	0.000	0.000	0.000	0.000	0.000
17:1ω6c	5.890	4.646	0.000	0.000	0.000	5.777	4.445	3.045	0.000	0.000	3.249	5.623	0.000	0.000	0.000	0.000	0.000	0.000
C 17:0	3.835	2.914	3.437	4.352	3.652	2.810	2.538	1.881	1.552	0.000	1.982	2.470	2.475	4.264	2.731	0.000	0.000	2.308
18:3 ω3c/18:1 ω8c	3.680	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	15.328	21.080	14.757	0.000	0.000	0.000	0.000	0.000
18:1ω9c	0.000	0.000	13.990	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	13.393	0.000	0.000	11.771
18:1ω9t	63.045	58.170	32.758	63.087	59.011	70.327	57.395	45.092	33.844	19.308	28.718	38.327	29.323	77.853	31.634	33.549	31.784	29.449

18:1ω5c	22.069	20.744	19.969	19.970	16.757	24.293	18.955	14.601	11.038	13.729	22.532	26.932	20.101	28.964	17.490	14.456	12.334	12.763
C 18:0	8.746	7.924	7.077	9.397	7.442	9.448	7.614	6.177	4.819	4.146	7.246	9.686	7.221	13.284	6.856	5.732	5.677	6.079
19:0 cyclo c 11-12	1.903	1.780	1.611	1.791	1.918	2.018	1.725	0.000	0.000	0.000	1.494	2.360	1.738	0.000	1.589	0.000	0.000	1.557
19:1 (w 8?) Alcohol	0.000	0.000	0.000	1.695	0.000	1.628	0.000	0.000	0.000	0.000	1.468	2.216	0.000	0.000	0.000	0.000	0.000	0.000
C 19:0	9.936	8.280	8.280	8.280	8.280	8.280	8.280	8.280	8.280	8.280	8.280	8.280	8.280	15.385	8.280	15.385	15.385	8.280
C 20:0	2.036	1.893	1.718	1.979	1.548	2.069	1.648	1.305	0.000	0.000	1.640	1.954	1.815	3.318	1.614	0.000	0.000	0.000
21:0 ISO	5.892	4.523	4.083	5.204	5.209	4.408	4.497	4.488	4.448	6.199	4.380	4.875	3.883	12.298	5.071	9.171	11.660	7.359
21:1ω3c	1.507	1.446	0.000	1.338	1.659	1.982	1.711	1.480	0.000	0.000	1.394	1.603	0.000	0.000	0.000	0.000	0.000	1.244
22:1ω5c	0.000	0.000	0.000	0.000	1.101	1.270	0.000	0.000	0.000	0.000	0.000	1.044	0.000	0.000	0.000	0.000	0.000	0.000
C 22:0	1.267	1.122	1.082	1.073	0.000	1.197	0.000	0.000	0.000	0.000	0.000	11.534	1.066	0.000	0.000	0.000	0.000	0.000
C 24:0	1.387	1.290	1.132	0.000	0.000	1.075	0.000	0.000	0.000	0.000	0.000	1.167	0.000	0.000	0.000	0.000	0.000	0.000