The potential role of sediment organic phosphorus in algal growth in a low nutrient lake

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Abstract: The role of sediment–bound organic phosphorus (P_o) as an additional nutrient source is a component of internal P budgets in lake system that is usually neglected. Here we examined the relative importance of sediment P_o to internal P load and the role of bioavailable P_o in algal growth in Lake Erhai, China. Lake Erhai sediment extractable P_o accounted for 11−43% (27% average) of extractable total P, and bioavailable P_o accounted for 21−66% (40%) of P_o. The massive storage of bioavailable P_o represents an important form of available P, essential to internal loads. The bioavailable P_o includes mainnly labile monoester P and diester P was identified in the sequential extractions by H_2O, $NaHCO_3$, NaOH, and HCl. 40% of H_2O-P_o, 39% of $NaHCO_3-P_o$, 43% of $NaOH-P_o$, and 56% of $HCl-P_o$ can be hydrolyzed to labile monoester and diester P, suggesting that the bioavailability of P_o fractions was in decreasing order as follows: $HCl-P_o$ > $NaOH-P_o$ > H_2O-P_o > $NaHCO_3-P_o$. It is implied that traditional sequential fractionation of P_o might overestimate the availability of labile P_o in sediments. Furthermore, analysis of the environmental processes of bioavailable P_o showed that the stabler structure of dissloved organic matter (DOM) alleviated the degradation and release of diester P, abundant alkaline phosphatase due to higher algal biomass promoted the degradation of diester P. The stability of DOM structure and the degradation of diester P might responsible for the spatial differences of labile monoester P. The biogeochemical cycle of bioavailable P_o replenishs available P pools in overlying water and further facilitate algal growth during the algal blooms. Therefore, to control the algal blooms in Lake Erhai, an effective action is urgently required to reduce the accumulation of P_o in sediments and

interrupt the supply cycle of bioavailable P_o to algal growth.

Capsule abstract: Sediment bioavailable P_o represents an important internal P load, and the biogeochemical cycling of P_o can replenish additional nutrient source to support algal growth

Keywords: Organic phosphorus; Bioavailability; Algal growth; Sediment

1. Introduction

Eutrophication and consequent harmful algal blooms are the major environmental problems worldwide (Ngatia et al., 2017). In many aquatic ecosystems, algal blooms are controlled by the availability of phosphorus (P) in the water column (Ji et al., 2017). If a large amount of available P presented in the sediment, it can be released into overlying water, accelerating eutrophication and delaying damaged ecosystems recovery even after external inputs have been reduced. Numerous studies have focused on investigating the size of the internally−available P pool and evaluating the potential risk of eutrophication from sediment nutrient pools (Lei et al., 2018; Chen et al., 2018; Wang et al., 2019). Sediment organic P (P_o) constitutes the majority of P, and is critical to the biogeochemical cycling of P in many lakes (Ahlgrenet al., 2005; Ni et al., 2019; Baldwin, 2013; Ding et al., 2015). Although P_o is abundant in sediment in some aquatic ecosystems, many studies of internal P loads

64 are still focused on the flux of phosphate release (Shinohara et al., 2017; Mandal et al.,
65 2015). The contribution of bioavailable P_o is often neglected in estimations of internal
66 P load because of the complexly of P_o (Worsfold et al., 2008; Bai et al., 2009). As a
67 result, sediment P load is largely underestimated by current assessment systems. An
68 improved understanding of the contribution of P_o to internal P load from sediment is
69 essential to formulate effective control strategies for lake eutrophication.

70 P_o consists mainly of sugar phosphates, phospholipids, nucleic acids, inositol
71 phosphates, and residual P (Copetti et al., 2019). The mechanism of adsorption of P_o
72 to lake sediments is similar to that of inorganic P (P_i) in sediments (Worsfold et al.,
73 2008). Phytoplankton and other organisms can take up phosphate released from
74 sediment via the enzymatic hydrolysis of P_o, and some species of phytoplankton can
75 even use small P_o molecules directly (Huang et al., 2005; Yue et al., 2014). Evaluation
76 of the potential availability of P_o is usually dependent on geochemical fractionation
77 and enzymatic hydrolysis characteristics. Sequential extraction schemes have been
78 adopted by many scientists to determine the relative concentrations and fractions of P_o
79 (Ruban et al., 1999). Enzymatic hydrolysis has been used as a quantitative assessment
80 tool for hydrolysable P, since most P_o is hydrolyzed to orthophosphate before uptaken
81 by phytoplankton and other organisms; thus hydrolysable P is a good indicator of the
82 bioavailability of P_o in sediment (Bünemann, 2008). These analytical techniques are
83 the powerful tools to determine the bioavailability of P_o and can improve our
84 understanding on the behavior and fate of P_o.

85 Lake Erhai is the second largest freshwater lake on the Yun−Gui Plateau of

China, and important tourist attraction and drinking water source for surrounding cities such as Dali. At present, Lake Erhai is in the initial phase of eutrophication (Wang et al., 2015). Long−term monitoring has shown that Lake Erhai has a relatively high algal biomass, with average chlorophyll-*a* concentration of 14 μg L^{-1} between 2003 and 2017 (Wang et al., 2015), exceeding the threshold level (or limit) set by the Organisation for Economic Cooperation and Development (OECD) (1982) for eutrophication. There have been frequent outbreaks of cyanobacterial blooms observed in some part of the lake each year, especially in 2013 when almost 80% of the lake area was affected. However, Lake Erhai contains relatively clean water, with mean concentrations of total P, total dissolved P (TDP), and soluble reactive P (SRP) of 20, 9.5, and 3.8 μg L^{-1} from 2003 to 2017, respectively (Wang et al., 2015). Lake Erhai is characterized by a high sediment P content, with total P contents ranging from 419 to 1108 mg kg^{-1} (904 mg kg^{-1} average) (Zhao et al., 2013; Ni and Wang, 2015). As external P loads have significantly decreased in recent years, the release of internal P accumulated in sediments has become an important source of P. Thus, whether the release of P from sediments is sufficient to support high algal biomass and the formation of algal blooms is a key research area that needs to be addressed.

Lake Erhai is a potentially P−limited lake (Yu et al., 2014). Previous studies have focus mostly on the role of flux of inorganic P (P_i) release on lake eutrophication (Liu et al 2015). However, little research has reported contribution of P_o to internal P load, especially the impact mechanism of bioavailable P_o and the major classes of organic molecules on algal growth. Assessing the contribution of P_o to internal P load and

potential role of bioavailable P_o on algal growth are therefore essential to understand the fate of P_o. Accordingly, the main objectives of this study are to (1) investigate the relative importance of P_o to internal P loads by sequential extraction, (2) characterize the bioavailable P_o and the major classes of organic molecules using phosphatase hydrolysis, and (3) examine the environmental processes of bioavailable P_o and its potential role in algal growth.

2. Materials and methods

2.1. Study Area

Lake Erhai is adjacent to the suburbs of Dali City in Yunnan Province. It has an area of 256 km^2, a mean depth of 10.5 m, and a volume of 27×10^8 m^3. It supplies drinking water and supports the socioeconomic development of Dali City. The lake is at a high altitude (1972 m), located in an area with a subtropical monsoon climate, and has an annual temperature range of 5 ^oC–15 ^oC. The average water residence time is approximately 2.75 years. Topographically, the bottom of Lake Erhai can be divided into three parts, namely the northern (sampling sites N1, N2, N3 and N4), middle (sampling sites M1, M2, M3, M4 and M5), and southern (sampling sites S1, S2, S3, S4 and S5) areas (Fig. 1). The northern part receives pollutants from agricultural sources via three major rivers (Yongan, Miju, and Luoshi), with a mean depth of 9 m. The middle part has no aquatic plant life, and receives relatively low levels of pollutants, with a mean depth of 14 m. The southern part receives pollutants

mainly from domestic sources within the Dali administrative area, with a mean depth of 7 m. Aquatic plants have been disappeared from the southern area since 2003, with debris deposited onto the surface sediments.

2.2. Sample collection and procedure

Fourteen surface sediment samples (0−5 cm) were collected from the fourteen sampling sites listed above using a columnar sampler (HL−CN, Xihuayi Technology, Beijing, China) in September 2013. The sampling time represents a high−risk period for the decline in water quality and the presence of algal blooms in Lake Erhai. Overlying water samples were also collected from each sediment sampling site.

Sediment samples were stored in airtight plastic bags, and transported to the laboratory in the dark at 4 ^{o}C. The samples were freeze−dried, ground to a powder, and then passed through a 100 mesh sieve to ensure homogeneity prior to analysis.

2.3. Analytical methods

2.3.1. Physicochemical analysis

The organic matter (OM) content of the sediment samples was determined using the K_2CrO_4 external heating method using 0.3 g of dried sediment (Nanjing Institute of Soil, Chinese Academic of Science, 1978). Chlorophyll−*a* concentrations were determined using a hot–ethanol extraction method (Lorenzen, 1967).

150 *2.3.2. Sequential fractionation of sediment P*

151 Sediment total P and its fractions (P_o, P_i) were extracted sequentially using the
152 modified method of He et al., (2004) and Zhu et al (2013), which can rapidly detect P_i
153 and P_o fractions, and produces P_o fractions that are suitable for subsequent enzymatic
154 hydrolysis. This method classifies P into four different categories: H_2O−P, $NaHCO_3$−P,
155 NaOH−P and HCl−P. Briefly, 1.0 g of dry sediment was extracted with 25 mL of
156 deionized water at 25 ^oC for 2 h. Then centrifuged at 10,000 g for 15 min and the
157 supernatant were passed through a 0.45 μm glass fibrefilter (Whatman, UK). Using
158 the same procedure, residues were then sequentially extracted with 0.5 mol L^{-1}
159 $NaHCO_3$ (pH 8.5), 0.1 mol L^{-1} NaOH, and 1 mol L^{-1} HCl for 16 h each to separate
160 the $NaHCO_3$, NaOH, and HCl fractions. In order to reduce contamination of
161 subsequent extracts by residual extractant and P in the pellet, after $NaHCO_3$ and
162 NaOH extraction, residues were washed with 5 mL of deionized water, and the
163 supernatant was discarded after centrifugation. Total P and P_i of each fraction were
164 determined, respectively. P_o in each fraction was obtained using total P in each
165 fraction minus the corresponding P_i.

166 The pH of $NaHCO_3$, NaOH and HCl extractions was adjusted so as to avoid
167 effect of pH on the enzymatic activity. The pH of the $NaHCO_3$ and NaOH fractions
168 was adjusted to 7.0. The pH was adjusted to 5.15 in order to prevent HCl fractions
169 from precipitating after pH adjustment.

170

171 *2.3.3. Enzymes, buffers, and assay procedures*

Alkaline phosphatase (APase) and phosphodiesterase (PDEase) are widespread enzymes in the water and sediment and play a key role in the biogeochemical cycling of P (Hakulinen et al., 2005; Zhou et al., 2008 and 2002). APase and PDEase were selected to characterize the bioavailability of P_o in the sediment, namely labile monoester P and diester P.

For the hydrolase selection, preparation and combination, we used APase that originated from bovine intestinal mucosa (EC 3.1.3.1, Type I−S, solid activity: 28 U mg^{-1}), and PDEase that was derived from *Crotalus atrox* (EC 3.1.4.1, type I, solid activity 0.02 U mg^{-1}). Both were purchased from Sigma−Aldrich Chemicals (St. Louis, MO, USA). APase and PDEase were dissolved in Tris (hydroxymethyl) aminomethane−HCl buffer (0.1 mol L^{-1}, pH 9) at concentrations of 1 and 0.02 U mL^{-1}, respectively. The solutions were stored in the dark for up to a week at 4 °C. To characterize P_o more effectively, APase was used alone and PDEase was used in combination with APase to achieve the complete hydrolysis of diester P. These analyses were performed at pH 9 and at 37 °C.

For the assay procedure, we added $MgCl_2$ (0.002 mol L^{-1}) to the buffers as enzyme activators. Trisodium citrate ($Na_3C_6H_5O_7$ $2H_2O$) was added to prevent the adsorption of released P_i by metal hydroxides during the assay. Prior to each assay, we prepared mixtures of 5 mL of the H_2O fraction or pH adjusted extracts ($NaHCO_3$, NaOH, and HCl fraction), 0.44 mL of APase (pH 9) or combination of APase and PDEase (pH 9) in an appropriate buffer, and 0.05 mL of 0.68 mol L^{-1} trisodium citrate. The mixtures were incubated for 16 h at 37 °C in a colorimetric tube (Monbet et al.,

2007).

The concentration of hydrolyzed P_o by each enzyme preparation was calculated as the difference in P_i concentrations before and after incubation. Duplicate samples and enzyme−free buffers were incubated simultaneously to identify and correct for any non−enzymatic hydrolysis and/or matrix blank. Concentrations of phosphate were measured using the molybdenum blue−ascorbic acid method. In order to avoid enzyme precipitation, 1 mL of sodium dodecyl sulfate (2%) was added before analysis.

2.3.4. UV–visible absorbance spectroscopy

The extracted solution was analyzed using a 1 cm quartz cuvette on a Hach DR−5000 spectrophotometer at wavelengths ranging from 200−700 nm. E_2/E_3 and A_{253}/A_{203} are the ratios absorbance at 250 nm to that at 365 nm, and at 253 nm to that at 203 nm, respectively. Specific ultraviolet absorbances at 260 nm ($SUVA_{260}$) and 254 nm ($SUVA_{254}$) were calculated as 100 times the ratio of UV absorbance at 260 and 254 nm to the corresponding concentration of dissolved organic carbon. Dissolved organic carbon was determined using a TOC analyzer (Shimadzu TOC−500, Japan).

2.3.5. Microorganism number analysis

The total number of microorganisms was measured using the plate dilution method (Zhang et al., 2015).

2.4. Data analysis and quality control

Data were analyzed and presented using SPSS 21 (IBM, Armonk, New York, USA) and Origin 8.5 (OriginLab, USA). Duplicate field samples, spiked samples, and blanks were used to control data quality. Triplicate measurements were conducted on each sample and reported as arithmetic means. For each sample analysis, the variation among replicates was less than 10%, and precision was almost 10% with a confidence level of 95%.

3. Results and Discussion

3.1. Risk of P_i and P_o releasing

Extractable total P concentration in the surface sediments of Lake Erhai ranged from 169 to 641 mg·kg^{-1}, with an average of 289 mg kg^{-1}. The concentrations of extractable P_i and P_o fractions in the sediment are presented in Figure 2. The concentration of extractable P_i ranged from 109 to 563 mg kg^{-1} (218 mg·kg^{-1} average), which accounted for 57−88% of the extractable total P in the sediment. The P_i fractions of the Lake Erhai sediments decreased in the following order: HCl−P_i > NaOH−P_i > $NaHCO_3$−P_i > H_2O−P_i (Fig. 2a). The H_2O−P_i fraction was found in the pore water of the sediments or loosely adsorbed onto the sediment particles (Zhou et al., 2001), and accounted for 21−51% (39% average) of the total H_2O−P in the sediment. The $NaHCO_3$−P_i contains slightly more labile adsorbed P, accounting for

36−87% (59% average) of the total $NaHCO_3$−P. The NaOH−P_i (Fe and Al oxide−bound P) accounted for 48−92% (78% average) of the total NaOH−P. HCl−P_i (calcium−bound P) accounted for 51−98% (86% average) of the total HCl−P. The result of P_i fractions suggested that P_i in Lake Erhai sediments exists mainly in the forms of HCl−P and NaOH−P. HCl−P is considered to be nonbioavailable and difficult to release from sediment unless the environment is acidic (pH < 6) (Jin et al., 2006; Kim et al., 2003). NaOH−P is a potential source of labile P that could be released into overlying water when anoxic conditions prevail at the sediment−water interface (Ting and Appan, 1996). Lake Erhai exhibited relatively stable pH and DO concentrations in the bottom water from 1992 to 2014, with mean values that ranged from 8.7−9.7 mg L^{-1} and 5.2−7.3 mg L^{-1}, respectively (Wang et al., 2015). Therefore, the risk of P_i releasing from Lake Erhai sediments could be alleviated because of the persistent aerobic state and stable pH value. This largely explains the reason why the content of total P is high in Lake Erhai sediments (Zhao et al., 2013), but the flux of SRP release is relatively low compared with the China's five largest freshwater lakes (Fig. 3).

The concentration of extractable P_o ranged from 38 to 91 mg·kg^{-1}, which accounted for 11−43% (27% average) of the extractable total P in the sediment. The concentration of P_o fractions in the sediments decreased in the following order: $NaHCO_3$−P_o > NaOH−P_o > HCl−P_o > H_2O−P_o (Fig. 2b). H_2O−P_o and $NaHCO_3$−P_o accounted for 49−79% (61% average) and 13−64% (41% average) of the total H_2O−P and $NaHCO_3$−P, respectively. Of the fulvic and humic acid−associated P as NaOH−P_o,

the fulvic acid−combined P is considered to be comprised of moderately labile P_o, whereas humic acid−associated P is considered to be more resistant to biodegradation (Zhang and Shan, 2008), and accounted for 8−52% (22% average) of total NaOH−P. The HCl−P_o, representing moderately labile P_o in the sediment (Lü et al., 2016), accounted for 2−49% (14% average) of the total HCl−P. These finding suggest that there was more P_o presented as higher labile P species, which could easily release and enter the overlying water to support algal growth. Compared with China's five largest freshwater lakes, Lake Erhai have stronger UV−radiation and higher microbial activity because of higher altitude and warm and humid climatic conditions (Wang et al., 2015). UV−radiation and microorganisms are important drivers in the degradation of P_o release dissolved P_i through photochemical transformation and biomineralization (Li et al., 2019). Therefore, the higher content of P_o in Lake Erhai sediments than China's five largest freshwater lakes (Fig. 3), indicating that Lake Erhai sediment P_o might has greater potential to contribute internal P load.

3.2. Bioavailability of P_o

To further understand the bioavailability of P_o in the sediment, two kinds of active P_o species (labile monoester and diester P) in the H_2O, $NaHCO_3$, NaOH, and HCl fractions were hydrolyzed by APase and PDEase (Fig. 4). The concentration of labile monoester P in H_2O−P_o ranged from 0.1 to 2.2 mg kg^{-1}, and accounted for 4.9−61.3% of H_2O−P_o, with an average concentration of 0.9 mg kg^{-1} (24.7%). Concentrations of labile monoester P in $NaHCO_3$−P_o were 2.8−9.7 mg kg^{-1} (6.5mg kg^{-1} average), accounting for 9.7−38.6% (20.8% average) of $NaHCO_3$−P_o. Labile

monoester P, i.e.0.3−4.9 mg kg^{-1} (1.6 mg kg^{-1} average) and accounted for 1.7−24.2% (11.1% average) of all NaOH−P_o. For HCl−P_o, concentrations of labile monoester P were 0.7−3.6 mg kg^{-1} (1.8mg kg^{-1} average), accounting for 5.9−62.5% (28.4% average) of all HCl−P_o (Fig.4a).

Diester P consisting of DNA (DNA−P), ribonucleic acids (RNA−P) and phospholipids, is degraded more rapidly than monoester P (Makarov et al., 2002). Amounts of diester P were 0.1−1.1 mg kg^{-1} (average 0.6 mg kg^{-1}), and accounted for 4.3−29.4% (14.5% average) of H_2O-P_o. Corresponding values for the $NaHCO_3$−P_o fraction were 1.9−10.6 mg kg^{-1} (5.8−44.0%), with an average value of 5.5 mg kg^{-1} (18.8%), and the values for the NaOH−P_o fraction were 1.8−9.6 mg kg^{-1} (11.8−88.4% average), with an average of 4.5 mg kg^{-1} (32.9%). The amount of diester P in the HCl−P_o fraction was 0.3−5.6 mg kg^{-1} (5.2−62.4%), with average values of 1.7 mg kg^{-1} (27.0%) in (Fig.4b).

Contents of total P_o hydrolyzed by Apase and PDEase were in the decreasing order of size, $NaHCO_3$−P_o > NaOH−P_o > HCl−P_o > H_2O−P_o. However, percentages of hydrolyzed P_o in the P_o fractions were in decreasing order of size, HCl−P_o > NaOH−P_o > H_2O−P_o > $NaHCO_3$−P_o, with average values of 56%, 43%, 40%, and 39%, respectively (Fig. 4c). Therefore, P_o fractions characterized by sequential extraction and enzymatic hydrolysis in sediments from Lake Erhai could be classifed accordance with their decreasing bioavailability in order HCl−P_o > NaOH−P_o > H_2O−P_o > $NaHCO_3$−P_o. In the traditional sequential P_o fractionation, H_2O−P_o and $NaHCO_3$−P_o are considered to be labile P_o, while HCl−P_o and partly NaOH−P_o (fulvic

303 acid associated P_o) are moderately labile P_o (Ivanoff et al., 1998). However, this study
304 found that the percentages of hydrolyzed P_o in the HCl−P_o and NaOH−P_o were higher
305 than that of H_2O−P_o and $NaHCO_3$−P_o fractions in sediments. This suggested that
306 traditional sequential fractionation of P_o might overestimate the availability of labile
307 P_o in sediments. Overall, 21−66% (40% average) of the extractable P_o could be
308 hydrolyzed by APase and PDEase in Lake Erhai. The increase in alkaline phosphatase
309 during the period of algal growth could promote the degradation of bioavailable P_o,
310 replenish available P pools in the overlying water and further facilitate algal growth.
311
312 *3.3. Environmental process of bioavailable P_o*
313 The pools of P_o in sediments are intrinsically linked with the amount and
314 structure of the deposited OM (Lü et al., 2016). This reflects that, the amount and
315 structure of OM might be key drivers that responsible for the biogeochemical cycle of
316 bioavailable P_o, thereby influence the distribution pattern of P_o in the sediments.
317 UV–visible has been used as an effective tool to characterise the structure of
318 dissolved organic matter (DOM) (Zhou et al., 2015). The spatial distribution of
319 UV–visible parameters such as the A_{253}/A_{203}, $SUVA_{260}$, $SUVA_{254}$, and E_2/E_3 of DOM
320 solutions within Lake Erhai are presented in Fig. 5a. $SUVA_{260}$ values can indicate the
321 concentration of hydrophilic substances, with higher values corresponding to more
322 hydrophobic substances (Jaffrain et al., 2007). $SUVA_{254}$ values are proxies for
323 estimating the concentration of aromatic in DOM, with higher $SUVA_{254}$ values
324 corresponding to higher degree of aromaticity and humification (Yeh et al., 2014;

325 Weishaar et al., 2003). Li et al (2014) found that higher A_{253}/A_{203} ratios corresponded
326 to higher concentrations of substitution groups (hydroxyl, carbonyl, carboxyl, and
327 ester groups). In this study, $SUVA_{260}$, $SUVA_{254}$ values, and A_{253}/A_{203} ratios increased
328 steadily from the northern to the southern sampling areas, indicating increasing
329 hydrophilic substances, substitution groups, aromaticity and humification in the DOM.
330 Variations in the E_2/E_3 ratios were related to differences in the humification level and
331 molecular weight of DOM, with lower E_2/E_3 ratios reflecting higher molecular weight
332 (He et al., 2008; Peuravouri and Pihlaja, 1997). In Lake Erhai sediments, the E_2/E_3
333 ratios decreased steadily from the northern to southern areas, indicating that sediment
334 DOM has a higher molecular weight. The structure of the DOM in the sediments
335 became to more complex as you move from the northern to the southern part of Lake
336 Erhai. This fact can be inferred by two phenomena: (1) the deposition of OM in the
337 south experienced a longer period of degradation by UV−radiation due to the flow of
338 water from north to south (Cory et al., 2015), and (2) the relatively higher
339 humification of OM because of the somewhat higher microbial populations in the
340 southern areas due to domestic pollution (Fig. 5b).

341 The distribution of bioavailable P_o (as total hydrolyzable P_o) displayed a
342 decreasing content gradient from southern to northern sampling sites, with the mean
343 values of 26.1, 22.9, and 19.9 mg kg^{-1} in the southern, middle and northern areas,
344 respectively (Fig. 6a). The correlation between bioavailable P_o and UV−radiation
345 parameters was not strong except E_2/E_3 ratio (Table 1). However, the more
346 substitution groups of aromatic rings, and higher molecular weights and humification

347 degree that DOM contains in the sediment, the higher content of bioavailable P_o will
348 be in the sediment (Fig. 6a). From the northern to the southern sampling areas,
349 sediment DOM contains more substitution groups and has higher degree of
350 humification and higher molecular weights ($SUVA_{254}$, A_{253}/A_{203} and E_2/E_3). The
351 substitution groups could potentially form stable compounds via adsorption and
352 complexation with nutrient, redox metal ions and organic pollutants (Li et al., 2014).
353 High humification degree could better sustain the release and conversion of the DOM
354 in sediment (Li et al., 2015). Furthermore, microorganisms have difficulty degrading
355 and utilizing organic molecules with high molecular weights and high degree of
356 aromaticity (He et al., 2011). Therefore, the degradation and release of bioavailable P_o
357 in sediments can be alleviated due to the stabler structure of DOM from the northern
358 to the southern in Lake Erhai.

359 Besides the structure and composition of DOM, phosphatase hydrolysis is
360 another factor that significantly affects the bioavailable P_o content (Hakulinen et al.,
361 2005). Large amounts of phytoplankton are believed to enhance the activation of
362 alkaline phosphatase (Sabine et al., 2005; Zhou et al., 2008). As a result, the sediment
363 bioavailable P_o would degradate and enter into the overlying water, because there is
364 abundant alkaline phosphatase during the algal growth period. In Lake Erhai, higher
365 chlorophyll *a* concentrations appeared in the northern region (Fig. 5b), which would
366 signal the greater production of alkaline phosphatase, thereby enhance the degradation
367 of bioavailable P_o in this region. The relatively low content of bioavailable P_o
368 (especially diester P) in the northern sites demonstrated that phytoplankton biomass is

369 also an important factor in determining the accumulation of bioavailable P_o in
370 sediments.

371 The bioavailable P_o components also displayed variability in their spatial
372 distribution. The diester P showed a similar trend as bioavailable P_o in the sediment,
373 with mean values of 14.2, 13.7, and 8.3 mg kg^{-1} in the southern, middle, and northern
374 areas, respectively (Fig. 6b). Diester P was positively correlated with A_{253}/A_{203} and
375 $SUVA_{254}$, with correlation coefficients of 0.567 ($p < 0.05$) and 0.587 ($p < 0.02$),
376 respectively. Diester P also had negative relationship with the E_2/E_3 ratio ($r = 0.772$, p
377 < 0.01) (Table 1), indicating that the more complex structure of DOM is in the
378 sediments, the higher content of diester P will be in the sediment. Furthermore, diester
379 P was negatively correlated with chlorophyll *a* concentration ($r = 0.489$, $p < 0.05$),
380 which could be attributed to the great degradation of diester P in the sediment because
381 of somewhat richer alkaline phosphatase in higher phytoplankton biomass. Sediment
382 labile monoester P was greatest in the southern (11.9 mg kg^{-1}) and northern (11.6 mg
383 kg^{-1}) areas and lowest in the middle region (9.1 mg kg^{-1}) (Fig. 6c). The correlation
384 was not strong between the content of labile monoester P and OM (Table 1), which
385 possibly showed that labile monoester P was active P_o species in the sediments. The
386 conversion of diester P is an important factor affecting labile monoester P distribution.
387 Ahlgren et al (2006) found that diester P that originated from microorganisms will be
388 degraded to phosphate or labile monoester P rapidly after microorganisms die. In the
389 northern of Lake Erhai, the diester P was degradated more easily than that of in
390 middle and soutnern areas, which might enhance labile monoester P accumulation in

391 the sediments. The higher content of labile monoester P in southern are likely related
392 to the stabler structure of DOM.
393
394 *3.4. Potential role of bioavailable P_o in algal growth*
395 The contribution of sediment P_o to algal growth was associated with
396 bioavailability and environmental conditions (Ni et al., 2016, 2019). In Lake Erhai,
397 bioavailable P_o accounted for 21−66% of extractable P_o and 4−17% of extractable
398 total P in the sediments. Enzymatic hydrolysis is the most important driver of P_o
399 release from sediments (Hakulinen et al., 2005), and APase and PDEase are
400 widespread in Lake Erhai (Wang et al., 2015). During the periods of algal growth
401 (August to October), as phytoplankton biomass increases, activities of APase and
402 PDEase would also increase in the sediment, which especilly obvious during the
403 period of potential algal blooms (Zhou et al., 2002; Xu, 2005). Therefore, the large
404 accumulation of bioavailable P_o allows for possible degradation that would replenish
405 the available P at the sediment−water interface, and further support algal growth. This
406 could be the reason why Lake Erhai nutrients are very low, but its phytoplankton
407 biomass always maintains a high level, and outbreak blooms occur frequently. In turn,
408 Death and sedimentation of phytoplankton enhanced the accumulation of P_o and OM
409 in the sediments. Sediments OM was primarily derived from autochthonous sources
410 based on the results of TOC /TN ratio (5.6−11.8, 9.2 averages) (Fig. 5b). Meyers and
411 Ishiwatari, (1993) reported that the TOC /TN ration for vascular land plants is usually
412 greater than 20, whereas plankton and bacteria have a TOC / TN ratio of between 4

and 10. Therefore, the high phytoplankton biomass and blooming phenomena in Lake Erhai would likely persist indefinitely without an intervention to interrupt the supply cycle of P_o from the sediments (Fig. 7).

Taken with the results presented in section 3.3, these results indicate that the accumulation and release of bioavailable P_o are related to the structural of DOM. The overlying water of Lake Erhai is constantly exposed to strong UV−B radiation because of its high altitude (1974 m), which leads to a high degree of DOM humification in the sediment (Ni et al., 2019). As a result, bioavailable P_o released from the sediment could be inhibited due to the shielding action of the high degree of DOM humification. Compared with the southern and middle regions of Lake Erhai, the humification degree of DOM in northern region is lower (Fig. 5), indicating the greater potential risk of bioavailable P_o release in this region. Furthermore, gradual increases in the number of microorganisms and phytoplankton (Zhang et al., 2015; Wang et al., 2015) might increase the degradation rate of bioavailable P_o from sediment via the enzymatic hydrolysis. Therefore, reducing internal sediment P_o accumulation, controlling sediment P_o degradation and release, strengthening algal removal, improving microbial habitats, optimizing key environnmental conditions, and human intervention in the biogeochemical cycle of bioavailable P_o are important aspects to consider in the management and control of the algal blooms in Lake Erhai.

4. Conclusions

We investigated the importance of P_o to internal P loads in Lake Erhai. Our

results showed that exractable P_o accounted for 11%−43% (27% average) of extractable total P, and bioavailable P_o accounted for 21−66% (40%) of extractable P_o in the sediments. The massive storage of bioavailable P_o represents an important form of available P, essential to internal loads. The bioavailable P_o includes mainnly labile monoester P and diester P was identified in the sequential extractions by H_2O, $NaHCO_3$, NaOH, and HCl. 40% of H_2O-P_o, 39% of $NaHCO_3-P_o$, 43% of $NaOH-P_o$, and 56% of $HCl-P_o$ can be hydrolyzed to labile monoester and diester P, suggesting that the bioavailability of P_o fractions was in decreasing order as follows: $HCl-P_o$ > $NaOH-P_o$ > H_2O-P_o > $NaHCO_3-P_o$. It is implied that traditional sequential fractionation of P_o might overestimate the availability of labile P_o in sediments.

We also examined the environmental processes of bioavailable P_o and its potential role in algal growth. The results showed that the stabler structure of DOM alleviated the degradation and release of diester P, abundant alkaline phosphatase due to higher algal biomass promoted the degradation of diester P. The stability of DOM structure and the degradation of diester P might responsible for the spatial differences of labile monoester P. The biogeochemical cycle of bioavailable P_o could replenish available P pools in overlying water and facilitate algal growth.

Conflict of interest

The authors declare that they have no conflict of interests.

Acknowledgements

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Figures:

Fig. 1. Location of Lake Erhai in China showing sampling sites in the northern (N1, N2, N3 and N4), middle (M1, M2, M3, M4 and M5), and southern (S1, S2, S3, S4 and S5) parts of the lake.

Fig. 2. Amount and percentage of P_i (a) and P_o (b) in H_2O, $NaHCO_3$, NaOH, and HCl fractions of total phosphorus, respectively.

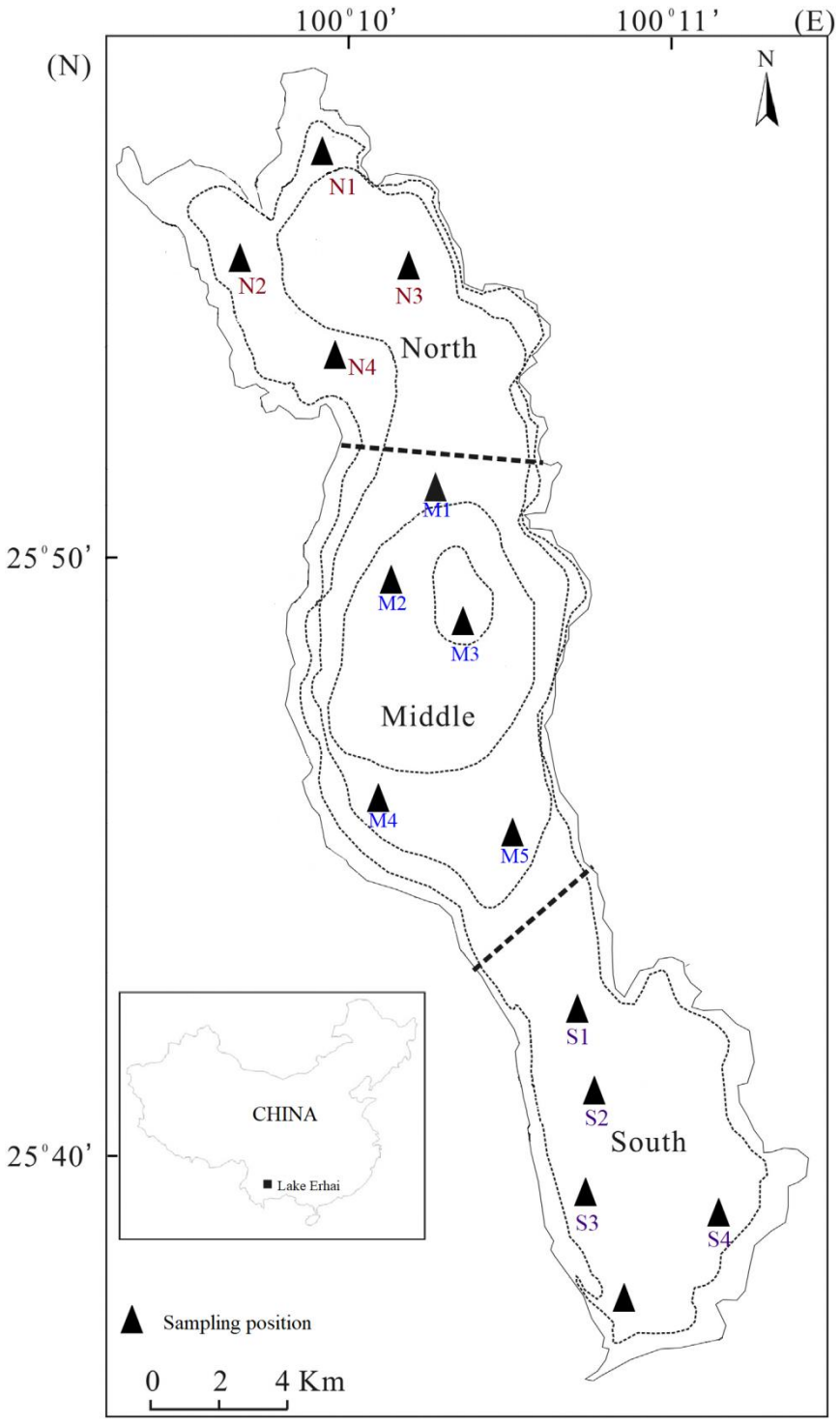
Fig. 3. Comparison of release flux of soluble reactive phosphorus (SRP) and P_o contents of Lake Erhai and China's five largest freshwater lakes.

Fig.4. Content (a) and relative abundance (b) of labile monoester P and diester P characterized by phosphatase in the H_2O, $NaHCO_3$, and NaOH fractions, respectively.

Fig. 5. Spatial distributions of UV–visible parameters (A_{253}/A_{203}, $SUVA_{260}$, $SUVA_{254}$, and E_2/E_3) (a), OM, TOC / TN ratio, total microbial population, and Chlorophyll *a* (b) in Lake Erhai.

Fig. 6. Spatial distribution of total hydrolyzable P_o (a), diester P (b), and labile monoester P (c) hydrolyzed by enzymes (APase and PDEase) in the H_2O, $NaHCO_3$, NaOH, and HCl fractions in Lake Erhai sediments.

Fig. 7. Biogeochemical cycle of sediment P_o and the supply model of bioavailable P_o to algal growth in Lake Erhai.



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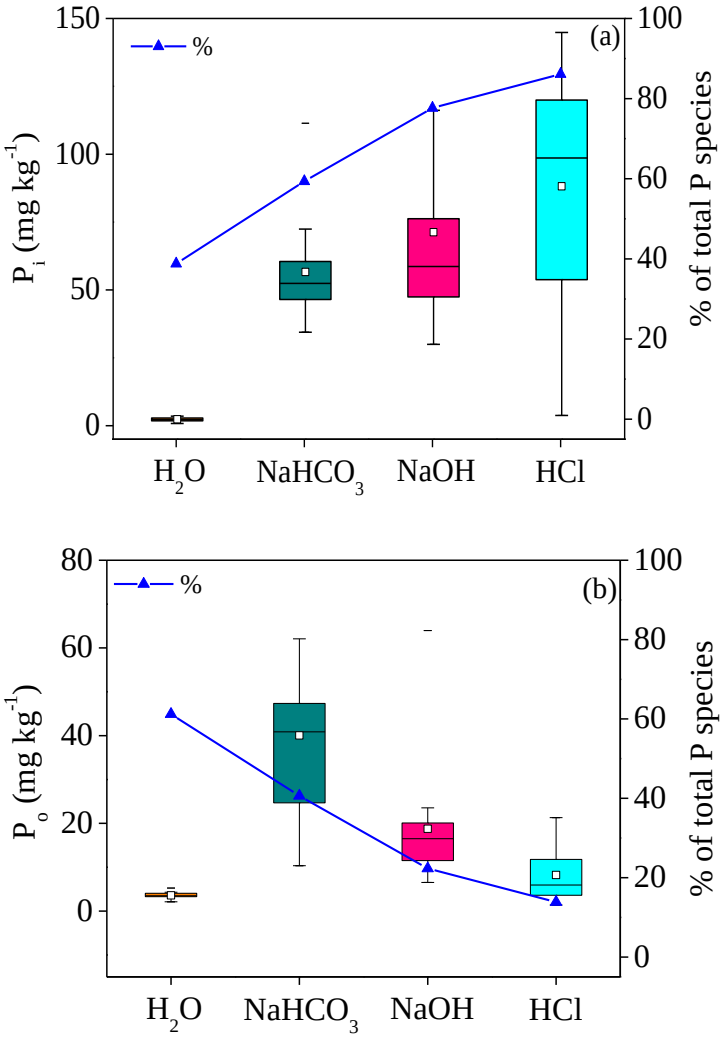

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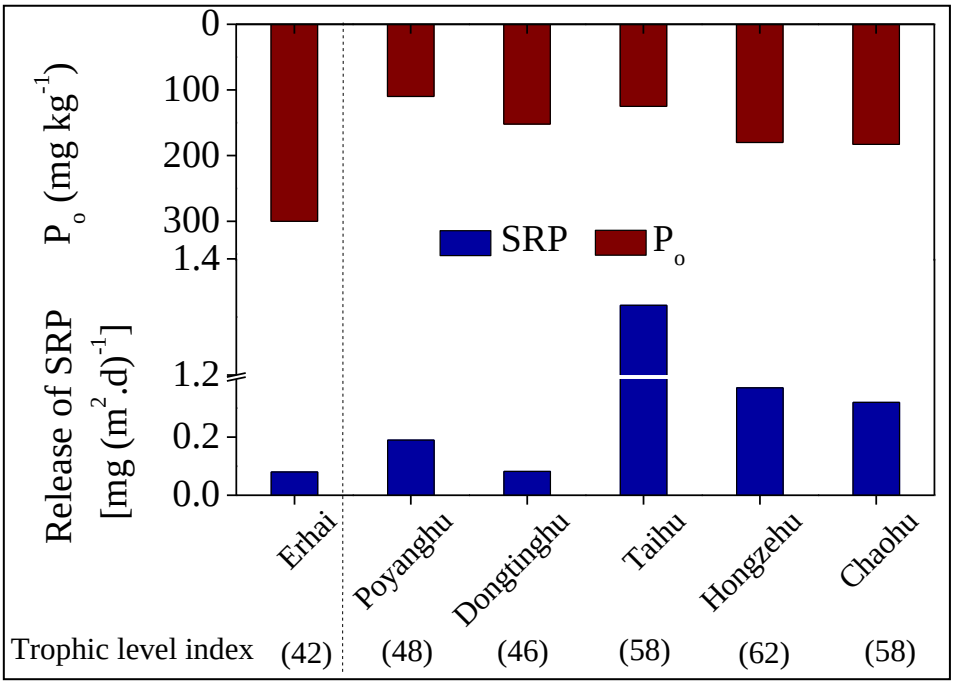

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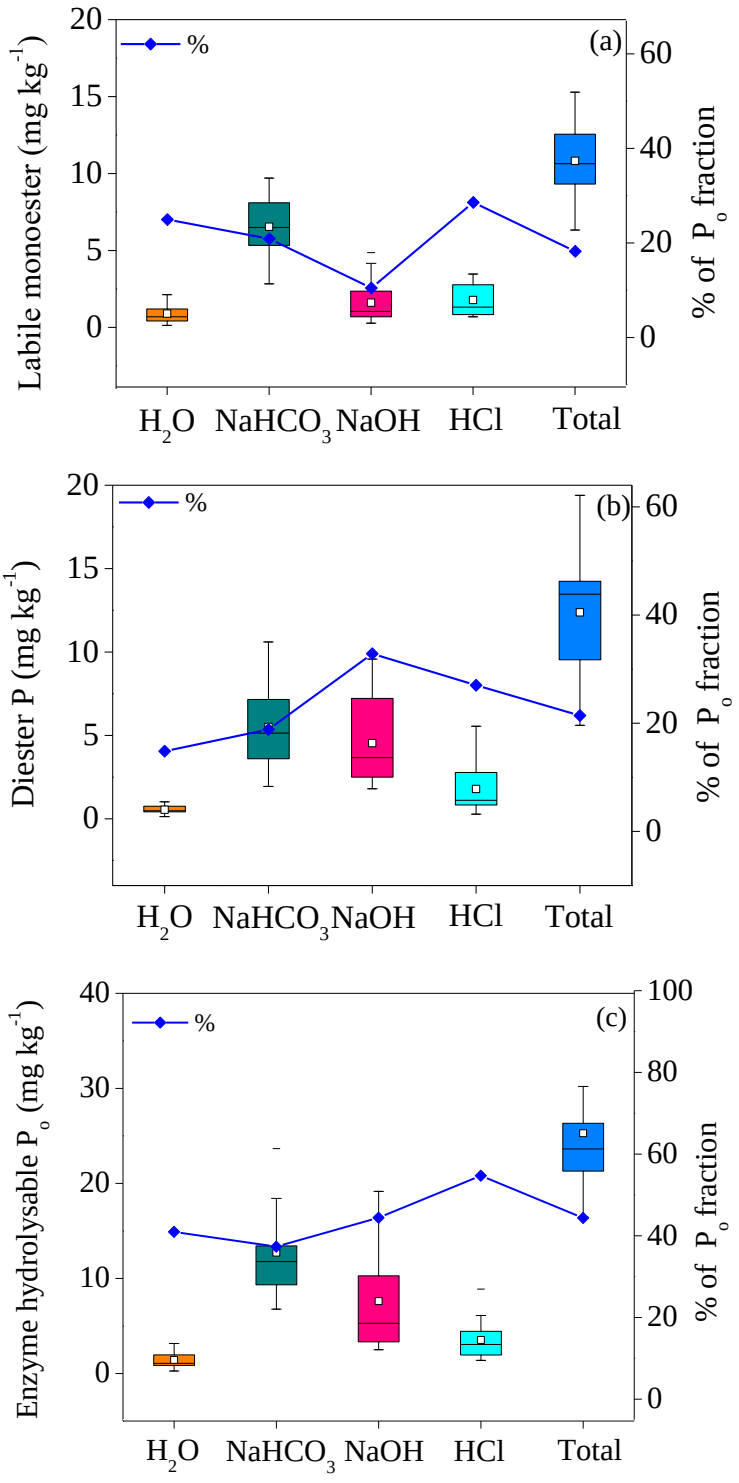

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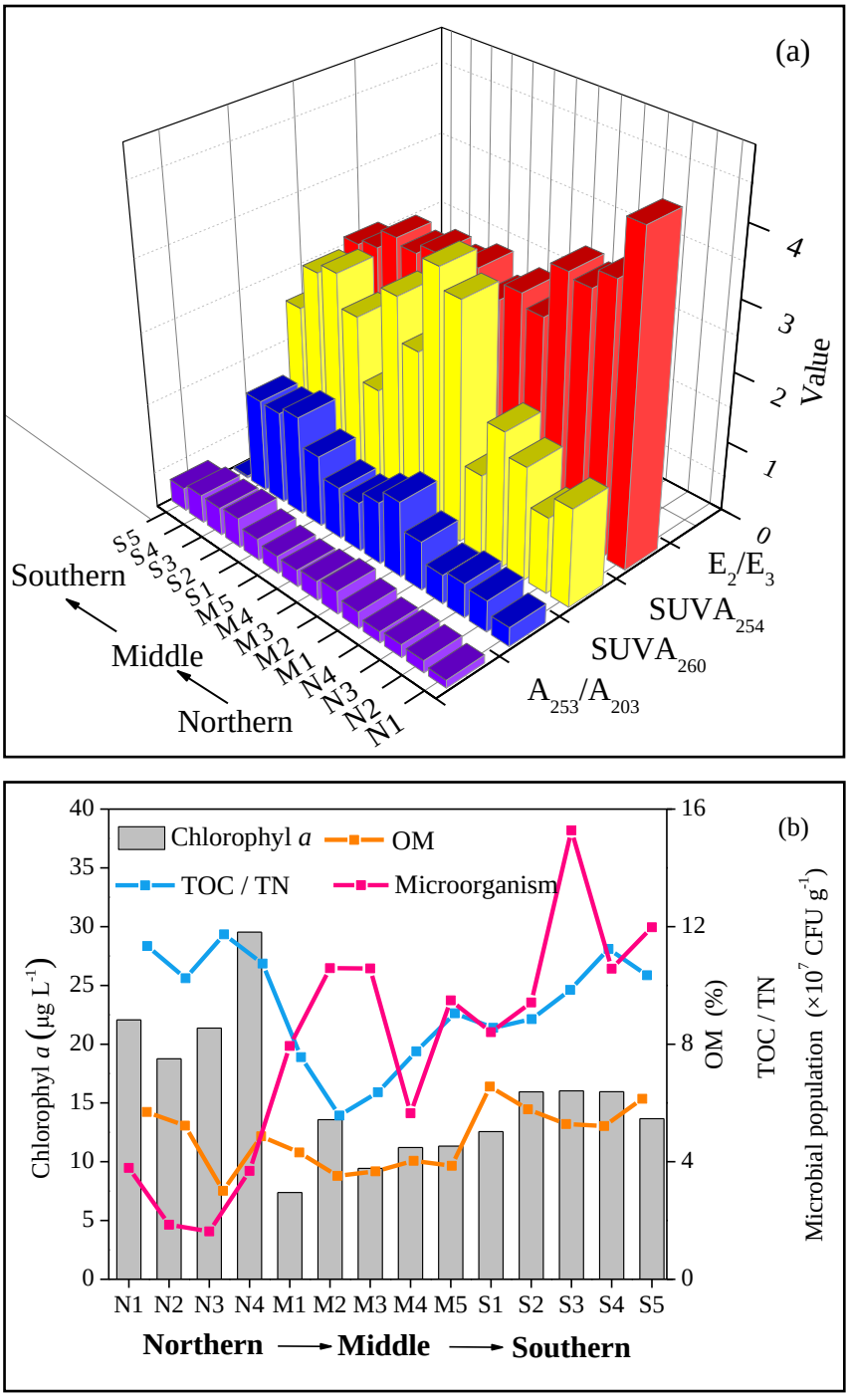

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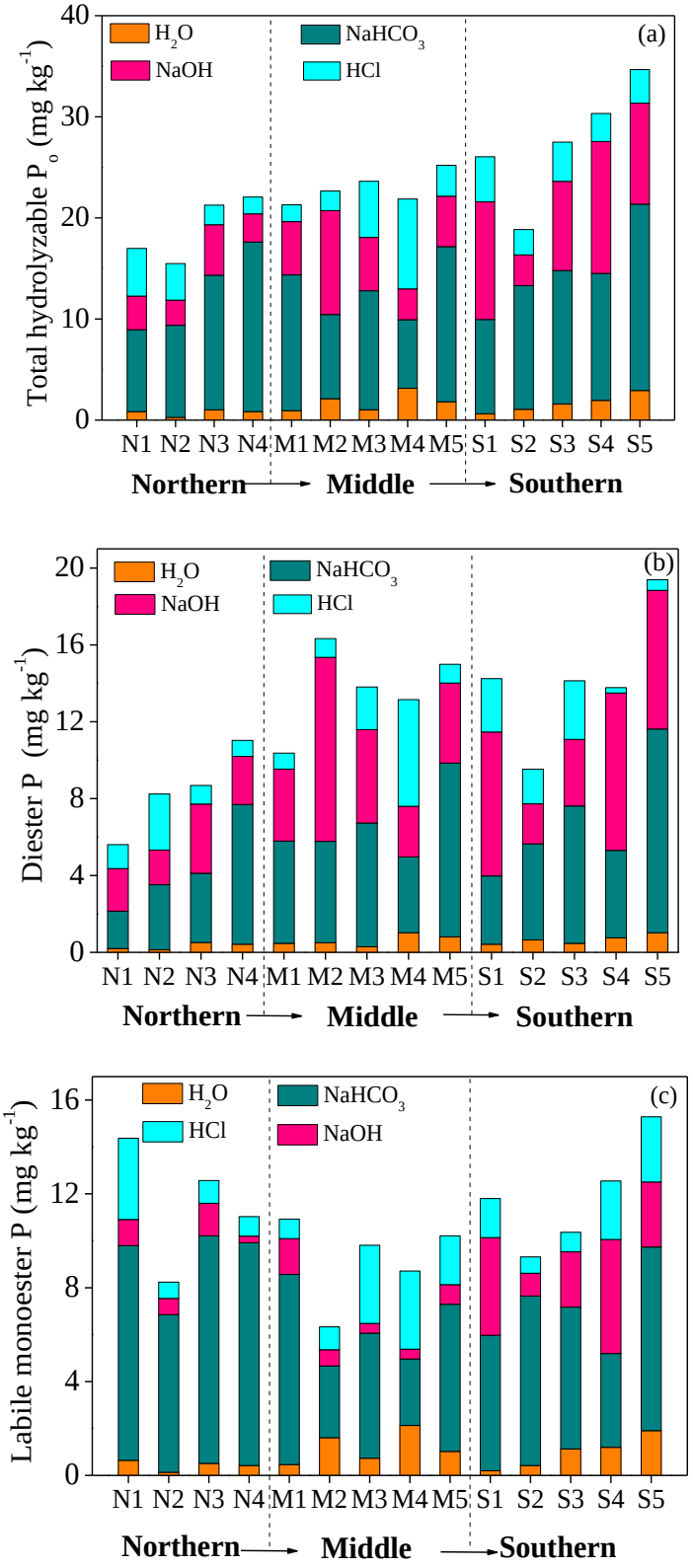

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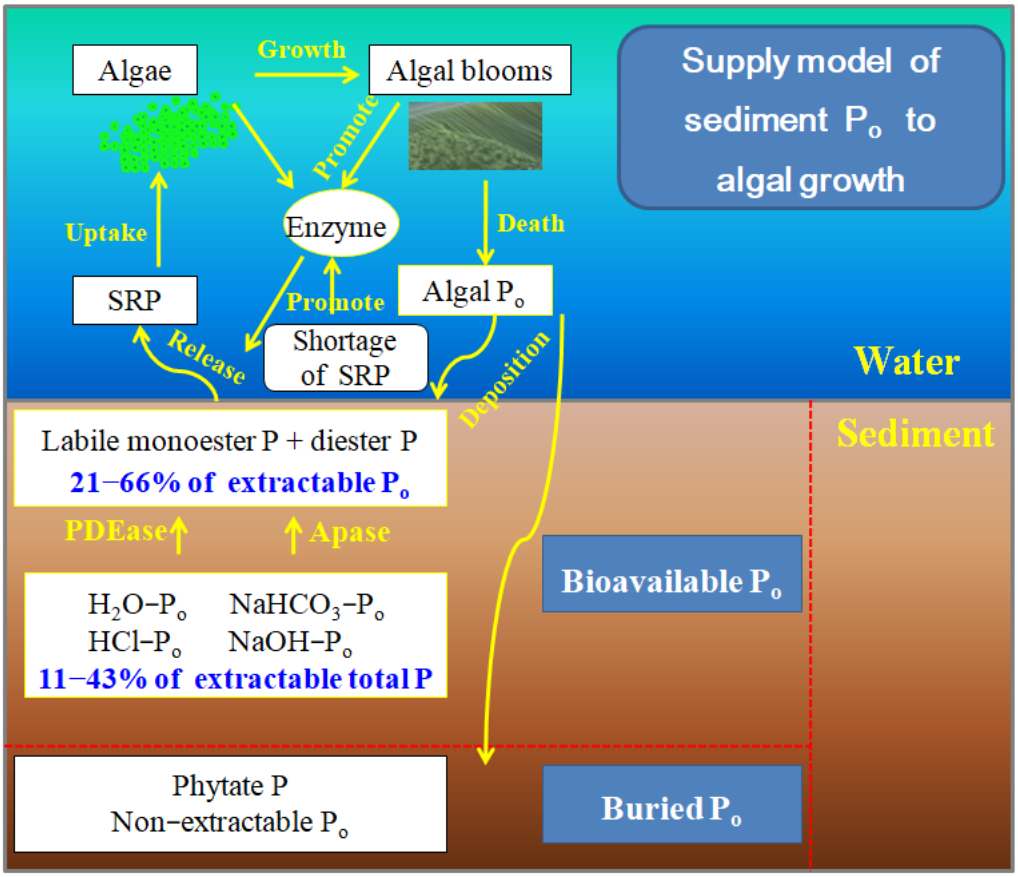

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