

1 **Response of sediment organic phosphorus composition to lake**  
2 **trophic status in China**

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14 **Abstract:** Organic phosphorus ( $P_o$ ) constitutes the most important fraction of P in  
15 lake sediments, and the compositional properties of  $P_o$  affect its behavior in lake  
16 ecosystems. In this study,  $^{31}P$  NMR, FT-IR spectroscopy, and UV–visible absorbance  
17 spectroscopy were combined to identify the dynamic composition of sediment  $P_o$   
18 across two sets of lakes in China ranging from oligotrophic to eutrophic, and their  
19 possible effects on lake eutrophication were evaluated. The results showed that  
20 sediment  $P_o$  content (accounting for 24–75% of TP) was positively correlated with  
21 trophic status in both Eastern Plain and Yun-Gui Plateau lakes of China, and the linear  
22 relationship was more stable compared to total P (TP), implying that sediment  $P_o$  may  
23 be a superior indicator of trophic status than TP. The  $P_o$  component, phosphonate  
24 accounted for only 0.5% or less of  $P_o$ , while the monoester P and diester P, accounted  
25 for 2–24% and 0.5–5% of  $P_o$ , respectively, and were the main factors causing  $P_o$  to  
26 increase with the increasing trophic status. The factors were closely related to the  
27 enhanced organic sewage load and intensification of contemporary sedimentation of  
28 phytoplankton. As trophic status increased, sediment  $P_o$  might integrate into larger  
29 amounts of aromatic substances and functional groups, which could enhance the  
30 stability of  $P_o$  in sediments. Furthermore, sediments from lakes with higher trophic  
31 status exhibited a higher degree of humification and molecular weights, which impart  
32 resistance to biodegradation, and therefore, reduced the risk of sediment  $P_o$  release.  
33 However, the massive accumulation of bioavailable  $P_o$  (monoester and diester P)  
34 allows possible degradation, supporting algal growth and maintains eutrophic status  
35 because there is abundant alkaline phosphatase in eutrophic lakes. Thus, to control

36 lake eutrophication more effectively, targeted actions are urgently required to reduce  
37 the accumulation and degradation of  $P_o$  in lake sediment.

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39 **Keywords:** Organic phosphorus, Compositional characterization, Eutrophication,  
40 Sediment

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## 42 1. Introduction

43 Phosphorus (P) is the most limiting nutrient for productivity in the biosphere,  
44 and thus, excessive P loading is associated with increased risk of cyanobacterial  
45 bloom formation in many lakes (Schindler et al., 2016). As external P inputs have  
46 been gradually reduced, the release of sediment P has become a major source of P that  
47 continues to enter into water under certain environmental conditions (Shinohara et al.,  
48 2012; Søndergaard et al., 2003). Sediment organic P ( $P_o$ ), including sugar phosphates,  
49 inositol phosphate, nucleic acids, phospholipids, and condensed P, represents an  
50 important P source that is similar in magnitude to inorganic P (Turner et al., 2005;  
51 Worsfold et al., 2008). However, sediment  $P_o$  has long received much less attention  
52 than inorganic P because of the limitations of analytical techniques and its complexity  
53 of composition (Bai et al., 2009). Sediment  $P_o$  is currently recognized as a potential  
54 pool for bioavailable P, resulting in extensive studies investigating  $P_o$  fractionation,  
55 composition, bioavailability, decomposition, migration, and transformation (Zhu et al.,  
56 2013; Lu et al., 2016; Zhang et al., 2017; Feng et al., 2018). These studies have  
57 demonstrated that the biogeochemical cycle of  $P_o$  plays a key role as a source of P in

58 water columns and algal growth. Understanding the compositional characteristics of  
59  $P_o$  in sediment from different trophic status lakes is, thus, essential for better  
60 evaluation of  $P_o$  behavior and its effects on lake eutrophication processes. However,  
61 little is known about the relationship between the compositions and bioavailabilities  
62 of  $P_o$  in sediments and lake eutrophication processes.

63 The biogeochemical cycle of  $P_o$  is usually closely related to its composition and  
64 structural characteristics in the sediment. In recent years, various methods have been  
65 introduced to characterize  $P_o$ , mainly phosphorus-31 nuclear magnetic resonance ( $^{31}P$   
66 NMR), Fourier-transform infrared spectroscopy (FT-IR), soft X-ray fluorescence  
67 spectroscopy, near-infrared spectroscopy (NIR), high-performance liquid  
68 chromatography (HPLC), flow injection analysis (FIA), inductively coupled plasma  
69 emission spectrometry (ICP-AES) and traditional chemical extraction (Vestergren et  
70 al., 2012; Brandes et al., 2007; Cooper et al., 2005; Worsfold et al., 2008; Bünemann,  
71 2008). In comparison,  $^{31}P$  NMR can be utilized to characterize  $P_o$  species and provide  
72 considerable information to distinguish P compounds, including orthophosphate,  
73 polyphosphate, pyrophosphates, monoester P, diester P, and phosphonates (Ahlgren et  
74 al., 2006). Fourier transform infrared (FTIR) spectroscopy is widely used to  
75 characterize leachate-derived  $P_o$  and provide considerable information regarding  
76 functional groups containing P (Zhang et al., 2009). Ultraviolet visible light  
77 absorbance (UV-visible) can characterize the structure and stability of organic  
78 molecule (Matilainen et al., 2011), and thereby indirectly indicates the presence of  
79 unidentifiable  $P_o$  because some of it is incorporated into humus. Although the

80 compositional characteristics of  $P_o$  are important to understanding the behavior of  $P_o$ ,  
81 a single analytical technique is inadequate to characterize the  $P_o$  compositional  
82 characteristics from different angles. Thus, using multiple combined analytical  
83 techniques is beneficial to providing more detailed compositional information.

84 China has some of the most serious eutrophication in lakes worldwide because of  
85 excessive P loading. Indeed, the area of eutrophic lakes in China exceeds 8,700 km<sup>2</sup>,  
86 and almost 25% of all lakes in China are facing the threat of eutrophication (Ni and  
87 Wang, 2015). Confronted by the challenge of severe lake eutrophication problems, the  
88 state has issued a series of five-year plans and relevant measures to implement  
89 watershed load reduction. As a result, external P loading has been reduced to a certain  
90 extent in many eutrophic lakes in recent years (Tong et al., 2017). However, the  
91 decline of water quality and the frequent outbreak of algal blooms have not  
92 fundamentally improved, especially in the Eastern Plain (EP) and Yun-Gui Plateau  
93 (YGP) of China. Moreover, the release of sediment P has been found to be a major P  
94 source (Li et al., 2015), and  $P_o$  could account for 21%–60% of total sediment P (TP)  
95 in the EP and YGP lakes (Ding et al., 2010; Ni et al., 2016). This illustrates that  
96 sediment  $P_o$  might make a vital contribution to the P cycle in these lake ecosystems.  
97 Therefore, determination of the relationships and effects of  $P_o$  in sediments on lake  
98 eutrophication is important to further understanding the biogeochemical cycle of  $P_o$   
99 and improving lake sediment remediation strategies. Accordingly, the objective of the  
100 current study was to establish the responses of sediment  $P_o$  compositional  
101 characteristics to lake trophic status and to investigate possible effects of the major

102 classes of organic molecules on lake eutrophication using  $^{31}\text{P}$  NMR, UV–visible  
103 absorbance, and FT-IR spectroscopy across two set of lakes in China ranging from  
104 oligotrophic to eutrophic.

## 105 **2. Materials and methods**

### 106 *2.1. Study area and background*

107 China has many territories and striking regional differences. The EP and YGP  
108 region are characterized by many lakes that have provided resources to millions of  
109 people and made important contributions to Chinese civilization and socioeconomic  
110 progress. However, these two regions are struggling with the challenge of accelerated  
111 water-quality decline and lake eutrophication following the rapid social-economic  
112 development that has occurred during the past three decades (Yang et al., 2010).  
113 Considering that lake eutrophication in China is primarily a result of anthropogenic  
114 activities (Chen et al., 2014), six lakes were selected in the EP and YGP districts of  
115 China in accordance with their aquatic ecological characteristics, water quality and  
116 intensity of anthropogenic activities. The higher trophic status lakes exhibited more  
117 intense anthropogenic activities, higher concentration of nutrient and higher density of  
118 phytoplankton in the EP and YGP, respectively (Table 1 and Fig. 1).

119 The EP district has a densely distributed river network that includes the middle  
120 and lower reaches of the Yangtze and Huai Rivers, the lower reaches of the Yellow  
121 and Haihe Rivers, and the coastal region of the Grand Canal. The district contains 651  
122 lakes with an area that exceeds 1 km<sup>2</sup> and has a total lake area of 22,900 km<sup>2</sup>. The  
123 lakes, which are generally tectonic and fluvial, evolved from fault depressions and

124 riverbeds (Nanjing Institute of Geography & Limnology Chinese Academy of Science,  
125 2015). Most of the lakes are shallow, with an average water depth of less than 2 m.  
126 The regional climate is subtropical monsoon, with a long-term annual average  
127 temperature of 4°C–17°C and annual precipitation of 600–1,500 mm. The altitude of  
128 the EP district ranges from 5 to 100 m, and it serves as an important engine of  
129 economic growth in China. Lake Poyang, Lake Taihu, and Lake Wuhan-Dong were  
130 selected as research subjects in the EP region. Lake Poyang (28°22′–29° 45′ N,  
131 115°47′–116°45′ E) in the north of Jiangxi Province is a typical overflow lake and an  
132 internationally important wetland with numerous ecological benefits and  
133 environmental regulatory functions. The lake has good water quality because of its  
134 frequent water exchange and relatively low level of human activities (Ni et al., 2015).  
135 Lake Tai (30°56′–31° 33′ N, 119°54′–120°36′ E), which is in the downstream portion  
136 of the Yangtze Delta, is an important drinking water source for surrounding cities,  
137 such as Suzhou and Wuxi. The water quality in the lake has gradually changed with  
138 the rapid economic development in the watershed over the past several decades (Yu et  
139 al., 2013). Lake Wuhan-Dong (30°33′ N, 114°23′ E) in the East of Wuhan is the  
140 largest urban lake in China. This area has been undergoing eutrophication since the  
141 1960s because of domestic sewage being randomly discharged along the lake (Yang  
142 and Chen, 2016).

143 The YGP district is in Guizhou Province and Eastern Yunnan Province, which is  
144 northwest of the Guangxi Zhuang Autonomous Region, and parts of Sichuan, Hubei,  
145 and Hunan Province. This district possesses 60 lakes with areas that exceed 1 km<sup>2</sup> and

146 has a total area of more than 1,199 km<sup>2</sup>. Most of the lakes in the region are deep-water  
147 small areas and closed or semi-closed lakes because they are primarily distributed in  
148 the stratum fracture zone (Nanjing Institute of Geography & Limnology Chinese  
149 Academy of Science, 2015). The regional climate is subject to subtropical monsoons,  
150 with an annual average temperature of 5°C–24°C and a relative uniformity of heat  
151 resources in different seasons. The long-term average annual precipitation is  
152 600–2,000 mm, and the YGP has a high intensity of ultraviolet radiation because of its  
153 high altitude (1,000–4,000 m). Lake Lugu, Lake Erhai, and Lake Dian were selected  
154 as research subjects in the YGP region. Lake Lugu (27°41' N, 27°45' E), which is  
155 situated on the border of Yunnan and Sichuan Provinces, has good water quality  
156 because of the low impact by anthropogenic activities. Lake Erhai (25°35'–25°58' N,  
157 100°05'–100°17' E) in Dali City is currently undergoing a transformation from  
158 mesotrophic to eutrophic status. Lake Dian (24°29'–25°28' N, 102°29'–103°01' E), in  
159 the southwest portion of Kunming City, has been undergoing serious water-quality  
160 deterioration and sustained algal blooms since the mid-1980s (Liu et al., 2014).

161 Lake trophic status can be classified as one of four categories (Fig.1) based on  
162 comprehensive trophic status indexes (TLI) of China (Jin, 2011): oligotrophic (OLI:  
163  $TLI(\Sigma) < 30$ , Lugu Lake), mesotrophic (MES:  $30 \leq TLI(\Sigma) \leq 50$ , Erhai Lake and  
164 Poyang Lake), slightly eutrophic (SLI:  $50 < TLI(\Sigma) < 60$ , Taihu Lake), and moderately  
165 eutrophic (MOD:  $60 \leq TLI(\Sigma) < 70$ , Wuhan-Dong Lake and Dian Lake).

## 166 2.2. Sample collection

167 Thirteen surface sediment samples (5 cm depth) were collected from different



168 lakes using a core sampler (HL-CN, Xihuayi Technology, Beijing, China) in  
169 September 2012 (Fig.1). Sampling number and sites were selected in accordance with  
170 the area and pollution characteristics of the six lakes. Overlying water was also  
171 collected during each session. Prior to analysis, the collected samples were  
172 immediately sealed in plastic bags and stored at 4°C in the dark. Upon arrival to the  
173 laboratory, sediment samples were freeze-dried, then ground and passed through a  
174 100-mesh sieve for homogeneity.

### 175 *2.3. Analysis methods*

#### 176 *2.3.1. Physicochemical analysis*

177 The content of Fe and Al in the sediment was measured using ICP-AES after  
178 microwave digestion by mixing 0.2 g of dried sediments with 10 mL of HNO<sub>3</sub> (68%),  
179 3 mL of H<sub>2</sub>O<sub>2</sub> (30%), and 5 mL of HF (40%). The organic matter (OM) content was  
180 determined with the K<sub>2</sub>CrO<sub>4</sub> external heating method using 0.3 g of dried sediment  
181 (Nanjing Institute of Soil, Chinese Academic of Science, 1978). Contents of TP and  
182 inorganic P were measured using the Standard Measurement and Testing protocol  
183 (Ruban, et al., 2001). Briefly, P was extracted using 1mol L<sup>-1</sup> HCl for 16 h after the  
184 sediment samples were combusted at 500°C for 2 h. The extracts were then analyzed  
185 spectrophotometrically as orthophosphate using the vanadomolybdate method.  
186 Inorganic P was directly extracted using 1mol L<sup>-1</sup> HCl for 16 h, then analyzed  
187 spectrophotometrically as orthophosphate using the vanadomolybdate method. The P<sub>o</sub>  
188 content was defined as the difference between TP and inorganic P.

189 2.3.2. *NaOH–ethylenediaminetetraacetic acid extraction and <sup>31</sup>P NMR analysis*

190 The NaOH–ethylenediaminetetraacetic acid extraction method can efficiently  
191 extract P<sub>o</sub> from the sediment. Briefly, 5 g of sediment was extracted with 50 mL of  
192 NaOH–EDTA solution (0.25 M NaOH-25mM EDTA) at 25°C for 16 h. The mixed  
193 solutions were subsequently centrifuged at 10,000 g for 30 min. The supernatants  
194 were then filtered through a 0.45-µm glass fiber filter, after which 1 mL of the filtrate  
195 was used to analyze the concentrations of NaOH-extracted TP, inorganic P and P<sub>o</sub>.

196 The remaining filtrates were frozen and lyophilized until they completely dried  
197 to a powder. The lyophilized extracts were subsequently re-dissolved in 2.5 mL of  
198 NaOH (1 mol·L<sup>-1</sup>) with 0.1 mL of D<sub>2</sub>O to lock the signal before detection by <sup>31</sup>P  
199 NMR spectroscopy. Next, the solutions were transferred into a 5-mm tube, and the  
200 solution <sup>31</sup>P NMR spectra were determined using a Bruker Avance III 600 analyzer  
201 (Bruker LC, Switzerland) operating at 161.98 MHz for <sup>31</sup>P. Samples were analyzed  
202 using a 12.00 microsecond pulse and a relaxation delay of 2 s, with 24,000 scans  
203 acquired for each sample. All of the chemical shifts of <sup>31</sup>P were analyzed using 85%  
204 H<sub>3</sub>PO<sub>4</sub> as an external criterion. The chemical shifts appeared to differ from the results  
205 of Turner et al. by 0.5 ppm (2008, 2011).

206 2.3.3. *UV–visible absorbance spectroscopy*

207 The compositions of P<sub>o</sub> were analyzed through NaOH extraction using a 1-cm  
208 quartz cuvette in a Hach DR-5000 spectrophotometer at wavelengths ranging  
209 200–700 nm. The analysis parameters included A<sub>253</sub>/A<sub>203</sub>, specific ultraviolet  
210 absorbances at 254 nm (SUVA<sub>254</sub>), and the spectral slope ratio (S<sub>R</sub>). A<sub>253</sub>/A<sub>203</sub> is the

211 ratio of UV–visible absorbance at 253 and 203 nm.  $SUVA_{254}$  is the 100× ratio of the  
212 UV absorbance at 254 nm to the corresponding dissolved organic carbon (DOC)  
213 concentration. Dissolved organic carbon was analyzed using a TOC analyzer  
214 (Shimadzu TOC-500, Japan). The  $S_R$  was the ratio of the spectral slope of a short  
215 wavelength (275–295 nm) to that of a long wavelength (350–400 nm).

#### 216 *2.3.4. FT-IR spectroscopy*

217 One milligram of sediment was mixed with pre-dried KBr (kept under vacuum in  
218 a desiccator until use), then pressed into a mold. The spectra were then analyzed using  
219 a Perkin-Elmer Spectrum 100 FT-IR spectrometer (Waltham, MA, USA), after which  
220 the blank was corrected with a clean KBr pellet. The spectra were evaluated over a  
221 scan range of 400–4000  $cm^{-1}$  with a resolution of 2  $cm^{-1}$ .

#### 222 *2.4. Data analysis and quality control*

223 Solution  $^{31}P$  NMR spectra, UV–visible absorbance spectroscopy, and FT-IR  
224 spectroscopy were analyzed by MesReNova software 9.0 (Forrester Research Inc,  
225 Spain), uvprobe 2.42 software (Shimadzu CO., LTD, Japan) and OMNIC 8.0  
226 (Thermo Nicolet Corporation, USA), respectively. Data were presented and analyzed  
227 using Origin.8 (OriginLab, USA) and SPSS 21 (IBM, USA). Field duplicate samples,  
228 spiked samples, and method blanks were used to control data quality in this study.  
229 Triplicate measurements of each sample were conducted and reported as their  
230 arithmetic mean values. The relative percent difference for each value was <10% in  
231 the duplicate samples. Precision was assured by determining all samples in triplicate,

232 with a relative standard deviation of less than 8%. The spectra were blank subtracted.

### 233 **3. Results**

#### 234 *3.1. Composition of sediment $P_o$ investigated by $^{31}P$ NMR with different trophic status* 235 *lakes*

236 The EP and YGP regions have very high spatial heterogeneity because of the  
237 significant differences in limnological, geographic, and climate characteristics and  
238 anthropogenic activities (Nanjing Institute of Geography & Limnology Chinese  
239 Academy of Science, 2015). Such differences were deemed to result in great  
240 variations in accumulation of nutrients among lakes in the two regions. Therefore, the  
241 dynamics of P in the sediments of different trophic status lake sediment are illustrated  
242 as two separate panels in the figures.

243 As lake trophic status increased, the contents of sediment TP increased in both  
244 regions (Fig. 2). The mean contents of TP were 624, 720 and 1992 mg kg<sup>-1</sup> in the  
245 MES, SLI and MOD from EP lake sediment, while they were 667, 877 and 1910 mg  
246 kg<sup>-1</sup> in the OLI, MES and MOD from YGP lake sediment, respectively. The  $P_o$   
247 showed a similar trend as TP in the sediments, with mean values of 268, 357 and 983  
248 mg kg<sup>-1</sup>, while they accounted for 43%, 50%, and 49% of the TP in the MES, SLI,  
249 and MOD from EP lake sediment, while these were 158, 284, and 1427 mg kg<sup>-1</sup>, and  
250 accounted for 24%, 32%, and 75% of the TP in the OLI, MES, and MOD from YGP  
251 lake sediment, respectively. The contents of NaOH-extractable  $P_o$  were 40, 51, and  
252 114 mg kg<sup>-1</sup> in the MES, SLI, and MOD from EP lake sediment, while they were 67,

253 101, and 116 mg kg<sup>-1</sup> in the OLI, MES, and MOD from YGP lake sediment,  
254 respectively, and these all showed an increasing trend with increasing lake trophic  
255 status.

256 Our analysis using <sup>31</sup>P NMR revealed mainly monoester P, diester P,  
257 phosphonates, orthophosphate, and pyrophosphate in the sediments (SI Fig. S1). The  
258 concentration of P groups in descending order was as follows: orthophosphate >  
259 monoester P > diester P > pyrophosphate > phosphonate. For the inorganic P fraction,  
260 11%–80% was orthophosphate and less than 1.6% was pyrophosphate. For the P<sub>o</sub>  
261 component, only 0.4% or less was phosphonate, while 2%–24% and 0.5%–5% were  
262 monoester P and diester P, respectively. As lake trophic status increased,  
263 orthophosphate, pyrophosphate, monoester P, diester P, and phosphonate all increased  
264 in the sediments (Fig.3 and Fig. S2).

### 265 *3.2. Composition of sediment P<sub>o</sub> investigated by UV–visible absorbance spectroscopy* 266 *with different trophic status lakes*

267 SUVA<sub>254</sub> values have been confirmed to be an effective index for estimation of  
268 the proportion of aromatic compounds in DOM (Weishaar et al., 2003; Yeh et al.,  
269 2014). The A<sub>253</sub>/A<sub>203</sub> values could reflect the concentration of substitution groups of  
270 DOM (Li et al., 2014), while variations in S<sub>R</sub> are related to differences in the  
271 molecular weights of DOM (Helms et al., 2008). In this study, the SUVA<sub>254</sub> values  
272 and A<sub>253</sub>/A<sub>203</sub> ratios ranged from 0.34–1.69 and 0.10–0.31, respectively, and they  
273 increased steadily as lake trophic status increased (Fig. 4a–b). The S<sub>R</sub> value ranged  
274 from 0.58 to 1.17 in the sediments, and it showed a declining trend with increasing

275 lake trophic status (Fig. 4c).

276 *3.3. Composition of sediment  $P_o$  investigated by FT-IR spectroscopy with different*  
277 *trophic status lakes*

278 The FT-IR spectra of the sediments were similar to each other in terms of the  
279 position of the major absorption shoulders and bands in the same region with different  
280 trophic status (Fig. 5). The assignments of the principal peaks of the FT-IR spectra of  
281 sediments in lakes of different trophic status are shown in Table S1. The assignments  
282 of the principal peaks of the FT-IR spectra of sediments in lakes of different trophic  
283 status are shown in *SI* Table S1. There were two intense and sharp peaks observed at  
284 3620 and 1031  $\text{cm}^{-1}$  in all of the sediments reflecting the O-H and P=O in-plane  
285 stretching vibrations. The sediments also showed weak peaks from 2870–2890,  
286 1638–1650, and 779–797  $\text{cm}^{-1}$ , which corresponded to C-H, C=O, and P-O. The weak  
287 peaks at 1427 and 874  $\text{cm}^{-1}$ , which were assigned to C≡C and P-O stretching in  
288 aromatic and arene compounds, only appeared in the YGP lake sediment.

289 *3.4. Relationship of OM and metal to  $P_o$  components*

290 Organic matter and metal elements are key factors in the composition and release  
291 of internal P. In the present study, as lake trophic status increased, the Fe, Al, and OM  
292 contents all increased (Fig. S3), similar to the  $P_o$  and  $P_o$  components in the sediments.  
293 The contents of Fe, Al, and OM were 33–178, 31–37, and 8–94  $\text{g kg}^{-1}$ , respectively.

294 The relationships between  $P_o$  and  $P_o$  components, as well as Fe, Al, and OM  
295 content in the sediments, were analyzed by Pearson's correlation (Table 2). The  $P_o$

296 was significantly positively correlated with monoester P, diester P, orthophosphate,  
297 and pyrophosphate, with the correlative coefficients being 0.724, 0.887, 0.708, and  
298 0.698 ( $p < 0.01$ ), respectively. In addition,  $P_o$  was positively correlated with OM ( $r=$   
299 0.590,  $p < 0.05$ ).

## 300 **4. Discussion**

### 301 *4.1 Relationships between sediment $P_o$ contents and lake trophic status*

302 The content variations of  $P_o$  and  $P_o$  components in lakes of different trophic  
303 status could be identified by the effects of anthropogenic activities and aquatic  
304 ecological characteristics. The levels of sediment  $P_o$  increased with lake trophic status  
305 in both regions, which could be attributed to intensive anthropogenic activities and  
306 intensification of contemporary sedimentation of phytoplankton. Higher trophic status  
307 lakes are usually associated with higher nutrient loading in watersheds as a result of  
308 intensive anthropogenic activities in the watershed, such as domestic pollution, animal  
309 excrement, and planting pollution (Table 1). In addition, the increased trophic status  
310 would enhance the growth of phytoplankton or death of macrophytes debris (Table 1),  
311 thereby increasing sedimentation of  $P_o$ .

312 The results presented above indicate that it is important to explore the  
313 relationship between sediment  $P_o$  content and trophic status of the EP and YGP lakes.  
314 The levels of sediment TP and  $P_o$  in 15 EP lakes and eight YGP lakes from the latest  
315 published studies, and our results are summarized in Table S2. Pearson's correlation  
316 coefficients of the relationships with the TLI were calculated for the EP and YGP

317 regions separately as shown in Fig. 6a and b. For the EP region, TP and  $P_o$  showed a  
318 linear correlation with TLI, with correlation coefficients of 0.8289 ( $p < 0.01$ ) and  
319 0.5720 ( $p < 0.02$ ), respectively (Fig. 6a). For the YGP region, sediment  $P_o$  was  
320 positively correlated with the TLI ( $r = 0.6591$ ,  $p < 0.05$ ). TP is known to be useful as  
321 an indicator of lake trophic status (Vaalgamss, 2004), but its correlation coefficients  
322 with TLI varied significantly in the EP and YGP lakes. This was likely because most  
323 of the P consisted of mobile inorganic fractions, which are adsorbed onto amorphous  
324 iron oxides and will eventually be remineralized and released from the sediment under  
325 certain conditions (Jensen, et al., 1995). In contrast, the linear relationship between  $P_o$   
326 and trophic status was more stable for both the EP and YGP lakes. Sediment  $P_o$   
327 decomposes slowly; therefore, it represents less mobile forms of P and is more closely  
328 related to sedimentation of macrophytes, phytoplankton, and terrestrial organic  
329 detritus (Vaalgamaa, 2004). These findings imply that sediment  $P_o$  may be a superior  
330 indicator of trophic status than TP in China.

#### 331 *4.2 Relationships between sediment $P_o$ components and lake trophic status*

332 With the increase of trophic status, the monoester P, diester P, and phosphonates  
333 contents increased (Fig. 3), similar to the levels of orthophosphate and pyrophosphate  
334 in sediments (SI Fig. S2). The monoester P consisting of labile monoester and  
335 phytate-like P (Jørgensen et al., 2011) was the dominant  $P_o$  in sediments. The  
336 accumulation of monoester P might be closely related to the effects of metal chelates  
337 (Fe, Al) and anthropogenic phytate-like materials input. The significant positive  
338 correlation of monoester P with Fe ( $p < 0.05$ ) and Al ( $p < 0.01$ ) (Table 2) indicates



339 that higher Fe and Al are beneficial to the formation of more monoester P in sediment.  
340 This is because polyvalent cations ( $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ) may increase adsorption onto inositol  
341 phosphate to form insoluble and stable  $\text{Fe}_4$ -phytate that precipitates onto the  
342 sediments, thereby increasing the phytate-like P accumulation. On the other hand,  
343 phytate-like P is primarily derived from indigestible P-bearing materials, including  
344 legumes, triticeae, and cereals, as well as the indigestible excrement of humans and  
345 non-ruminant animals (Ravindran et al., 1994; Lantzsich et al., 1992). Higher trophic  
346 status lakes usually correspond to higher amounts of farming and livestock breeding  
347 and greater population density in watersheds (Table 1), suggesting that loading of  
348 these specific organic materials plays an important role in the accumulation of  
349 monoester P in lake sediment.

350 The diester P increased as lake trophic status increased, which may be closely  
351 related to its source characteristics. Diester P is primarily derived from the  
352 sedimentation of OM as DNA and RNA of microorganisms and degradation products  
353 of phytoplankton (Ahlgren et al., 2006). In this study, higher trophic status lakes were  
354 found to have higher amounts of phytoplankton (Table 1). Diester P was significantly  
355 positively correlated with OM ( $P < 0.01$ ), indicating that OM was essential to  
356 accumulation of diester P (Table 2).

357 Phosphonate, which contains a stable C-P bond (Zhang et al., 2013), and  
358 represents immobile  $\text{P}_o$ . This material showed an increasing trend with increasing lake  
359 trophic status. Sediment phosphonate is primarily derived from the metabolic product  
360 of protozoans (Nowack et al., 2003). However, the biomass of benthos showed

361 decreasing trends as lake trophic status increased (Table 1), illustrating that the  
362 chelation of phosphonate and metal ions might an important factor for accumulation  
363 of phosphonate in the sediments. As trophic status increases, more divalent and  
364 trivalent Fe and Al ions in sediments can integrate more phosphonate, thereby  
365 forming an increased amount of metal bond phosphates that are more stable.

#### 366 *4.3. Relationships between sediment $P_o$ stability and lake trophic status*

##### 367 *(1) UV-visible absorbance spectroscopy*

368 The stability of  $P_o$  is usually closely related to its composition and structural  
369 characteristics in the sediment. Values of DOM in the extracts were significantly  
370 positively correlated with NaOH- $P_o$ , monoester P, and diester P contents, with  
371 correlation coefficients of 0.643, 0.734, and 0.686, respectively (Table 2). These  
372 findings suggest that OM plays a significant role in the compositions of  $P_o$  in the  
373 sediments.  $SUVA_{254}$  values have been confirmed to be an effective index for  
374 estimation of the proportion of aromatic compounds in DOM, with higher  $SUVA_{254}$   
375 values corresponding to greater humification and aromaticity (Weishaar et al., 2003;  
376 Yeh et al., 2014). The  $A_{253}/A_{203}$  values could reflect the concentration of substitution  
377 groups of DOM, with higher ratios indicating higher concentrations of substitution  
378 groups of aromatic rings (Li et al., 2014). Variations in  $S_R$  are related to differences in  
379 the molecular weight of DOM, with higher values corresponding to lower molecular  
380 weight (Helms et al., 2008). The significant positive correlations of  $P_o$  concentration  
381 with  $SUVA_{254}$  and  $A_{253}/A_{203}$  (Fig. 7) indicate that the UV parameters may indirectly  
382 reflect the unidentifiable  $P_o$  that is incorporated into humus.

383 The  $SUVA_{254}$  and  $A_{253}/A_{203}$  values increased steadily as trophic status increased  
384 for both the EP and YGP lakes (Fig. 4a and b), indicating that higher trophic status  
385 lake sediment  $P_o$  contains more substitution groups of aromatic rings and has a higher  
386 degree of humification. This fact is inferred to be attributed to the relatively great  
387 mineralization of OM in the sediment because of somewhat larger numbers of  
388 microorganisms and enzymes in higher trophic status lakes. Carbohydrates and other  
389 labile components of OM are preferred by microorganisms in the mineralization  
390 process, resulting in more aromatic OM with a higher humification degree and  
391 accumulation of  $P_o$  in the sediments (Hur et al., 2011). The  $S_R$  value in the EP and  
392 YGP lakes all decreased with increasing trophic status (Fig. 4c), suggesting that  
393 sediment organic molecules possess a higher molecular weight in higher trophic status  
394 lakes. This might be attributed to the greater mineralization in higher trophic status  
395 lakes, which increases molecular weight of organic molecules (Guggenberger and  
396 Kaiser, 2003).

#### 397 (2) *FT-IR spectroscopy*

398 FT-IR spectroscopy provides information regarding P valence bonds with  
399 functional groups in the OM (Yang et al., 2015). The FT-IR spectra of sediments were  
400 similar to each other in terms of the position of the major absorption shoulders and  
401 bands in the same region of lakes with different trophic status. There were two intense  
402 and sharp peaks observed at  $3620\text{ cm}^{-1}$  and  $1031\text{ cm}^{-1}$  in all sediments because of O-H  
403 and P=O in-plane stretching vibrations, indicating that the sediment  $P_o$  comprises a  
404 large amount of hydroxyls, phospholipids, DNA, and RNA. This was closely related

405 to the large amount of monoester P, diester P, and phosphonate in the sediment  
406 detected by  $^{31}\text{P}$  NMR. The sediments also showed weak peaks in the range of  
407 2870–2890, 1638–1650, and 779–797  $\text{cm}^{-1}$  corresponding to the C-H, C=O, and P-O  
408 in-plane stretching vibrations of proteins, amides, and aromatics, respectively.  
409 Particularly, the weak peaks at 1427 and 874  $\text{cm}^{-1}$ , which were assigned to C $\equiv$ C and  
410 P-O stretching in aromatic and arene compounds, only appeared in the sediment of  
411 YGP lakes, implying that sediment  $\text{P}_o$  in the YGP lakes comprises more aromatic  
412 compounds. UV-radiation-induced degradation of organic molecules is an important  
413 factor in YGP lakes because of their higher altitude (Table 1), which leads to  
414 accumulation of more aromatics into the sediment.

#### 415 *4.4 Possible effects of $\text{P}_o$ composition and stability on lake eutrophication*

416 The release and decomposition of  $\text{P}_o$  are the main routes of recycling of P and  
417 thus an important process determining the level of bioavailable P in both water and  
418 sediment (Ni et al., 2016). Based on the result of  $\text{SUVA}_{254}$  values and  $\text{A}_{253}/\text{A}_{203}$  ratios  
419 reported in this study, higher trophic status lake sediment  $\text{P}_o$  contains more aromatic  
420 substances and functional groups, and the substances have degrees of higher  
421 humification. These functional groups, which include hydroxyl, carboxyl, carbonyl,  
422 and ester groups, can potentially absorb and fix nutrients, heavy metals, and organic  
423 pollutants (Zhang et al., 2016), having a positive effect on lake water quality.  
424 Moreover, higher degree of humification is usually associated with greater  
425 conjugation in aliphatic chains complexes or more complex and condensed aromatic  
426 structures (Li et al., 2015), both of which stabilize the sediment, preventing  $\text{P}_o$  release

427 and degradation. In addition, the  $S_R$  values indicated organic molecules in sediments  
428 of higher trophic status lakes have greater molecular weight. The high molecular  
429 weight usually imparts resistance to biodegradation compared to low molecular  
430 weight (He et al., 2011). Overall, these findings implied that the risk of release of  $P_o$   
431 would be alleviated by increases in lake trophic status because of the more stable  
432 structure of  $P_o$  in the sediment.

433 Alkaline phosphatase, which is extensive in water columns and sediments, is the  
434 most important driver in the biological geochemical cycle of  $P_o$  (Hakulinen et al.,  
435 2005). As lake trophic status increase, increases in microorganisms and phytoplankton  
436 would enhance the amount and activation of alkaline phosphatase, especially during  
437 algal blooms in eutrophic lakes (Barik et al., 2001; Zhou et al., 2002). When  
438 combined with the higher content of monoester P, diester P, and phosphonate in  
439 higher trophic status lakes, the remarkable increase in alkaline phosphatase reflected  
440 that, as an important component of P, the large accumulation of  $P_o$  in sediments can be  
441 mineralized to bioavailable P, facilitating eutrophication. The positive correlation ( $p <$   
442 0.05) between diester P and orthophosphate in sediments (Table 2) indicated that the  
443 mineralization of diester P to bioavailable P by phosphodiesterase would be an  
444 important P source for supporting algal growth and maintaining long-term eutrophic  
445 status, even after external input of P loading has been controlled.

446 The efforts to control lake eutrophication in China began in the mid-1980s. Since  
447 then, great improvements in industrial pollution and erosion and torrent control have  
448 resulted in large-scale declines in lake inorganic P and TP concentrations (Ni and

449 Wang, 2015). However, frequent blooms and serious eutrophication still occur and  
450 have not been fundamentally solved, particularly in Tai Lake, Chao Lake and Dianchi  
451 Lake, which are listed by the Ministry of Environmental Protection of China as  
452 priority lakes in which to achieve significant eutrophication improvements. Thus,  
453 more effective and flexible actions are urgently required to protect these eutrophic  
454 lakes from further deterioration and reverse the process. This study found that the  
455 accumulation of  $P_o$  in lake sediment was closely related to agricultural and domestic  
456 input. Although the risk of sediment  $P_o$  release may be alleviated in eutrophic lakes as  
457 a result of more stable structures of  $P_o$ , the massive storage of sediment bioavailable  
458  $P_o$  (diester and monoester P) will degrade as a result of increased enzyme activity with  
459 increasing trophic status, leading to increased lake eutrophication or resistance of  
460 eutrophic lakes to remediation. Therefore, reducing watershed organic source  
461 materials load, advancing sediment  $P_o$  degradation control for optimization of key  
462 environmental conditions, and strengthening algal removal techniques are essential to  
463 restoration of damaged ecological environments in eutrophic lakes.

## 464 **5. Conclusions**

465  $^{31}P$  NMR, UV-visible absorbance spectroscopy, and FT-IR spectroscopy were  
466 combined to identify the dynamic composition of sediment  $P_o$  across two sets of lakes  
467 ranging from oligotrophic to eutrophic in China. The results showed that sediment  $P_o$   
468 content (accounting for 21%–75% of TP) was positively correlated with trophic level  
469 index in both EP and YJP lakes of China, and the linear relationship was more stable  
470 compared to TP, implying that sediment  $P_o$  may be a superior indicator of trophic

471 status than TP. The  $P_o$  component, phosphonate, accounted for 0.5% or less of  $P_o$ ,  
472 while the monoester P and diester P accounted for 8%–31% and 2%–9% of  $P_o$ ,  
473 respectively, making them the main factors causing  $P_o$  to increase with increasing  
474 trophic status, which is closely related to the enhanced organic sewage load and  
475 intensification of contemporary sedimentation of phytoplankton. Furthermore,  
476 sediment  $P_o$  contained more functional groups and aromatic substances with increased  
477 trophic status, and substances had a higher degree of humification and molecular  
478 weights because of mineralization.

479 The possible effects of  $P_o$  composition on lake eutrophication were also  
480 evaluated. The results showed that sediment  $P_o$  might integrate into more aromatic  
481 substances and functional groups, such as hydroxyl, carboxyl, ester, and carbonyl  
482 groups, in higher trophic-status lakes, which could enhance the stability of  $P_o$  in  
483 sediments. In addition, higher trophic lake sediment exhibited a higher degree of  
484 humification and molecular weights, which imparted resistance to biodegradation, and  
485 thus, reduced the risk of sediment  $P_o$  release. However, the massive accumulation of  
486 bioavailable  $P_o$  remains subject to possible degradation and has the potential to further  
487 enhance eutrophication via dramatic increases in alkaline phosphatase in eutrophic  
488 lakes.

489

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