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3 **Phosphorus compounds in the dissolved and particulate phases in urban rivers and a**  
4 **downstream eutrophic lake as analyzed using <sup>31</sup>P NMR**

5

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15

16

17 **Abstract**

18 Phosphorus (P) discharges from human activities result in eutrophication of lakes. We  
19 investigated whether the forms of phosphorus (P) in rivers with high effluent loads flowing  
20 through urban areas of Sapporo, Japan, were transformed when transported downstream  
21 into a eutrophic lake, namely Lake Barato. We hypothesized that the inorganic P supplied  
22 from the rivers might be transformed to organic forms in the lake. The results showed that  
23 soluble reactive phosphorus (SRP) and particulate inorganic phosphorus (PIP) dominated in  
24 the river discharge to the lake. Suspended solids in the rivers were rich in iron (Fe) so PIP  
25 was associated with Fe. A comparison of the concentrations at the river mouth and 4.5 km  
26 downstream showed that the concentrations of SRP and PIP were lower at 4.5 km  
27 downstream than at the river mouth, whereas the concentrations of organic P (i.e., dissolved  
28 organic phosphorus and particulate organic phosphorus) were similar. The results from  
29 solution  $^{31}\text{P}$  nuclear magnetic resonance spectroscopy of lake water showed that  
30 pyrophosphate was only present in the particulate fraction, while orthophosphate diesters  
31 (DNA-P) were only present in the dissolved fraction. Riverine samples contained  
32 orthophosphate (ortho-P) only, while lake samples contained ortho-P, orthophosphate  
33 monoesters, and DNA-P. The results suggest that the P forms, particularly those of

34 dissolved P, shifted from inorganic to organic forms as the water was discharged from the  
35 river to the lake.

36

37 Keywords: Lake Barato, sewage effluent, solution  $^{31}\text{P}$  NMR, phosphorus speciation,  
38 phosphorus fractionation.

39

## 40 **1. Introduction**

41 Sewage effluents discharged into rivers induce eutrophication and phytoplankton blooms in  
42 downstream lakes, especially in highly urbanized areas (Jarvie *et al.*, 2006). Sewage  
43 effluents tend to be rich in phosphorus (P) (Zangarini *et al.*, 2020), and the P in the effluent  
44 has an important role in the eutrophication of freshwater lakes. Eutrophication can cause  
45 several environmental problems, such as cyanobacterial blooms, dissolved oxygen  
46 depletion, and biodiversity loss, and may mean that the water in the lake cannot be used for  
47 recreation and other purposes (Le Moal *et al.*, 2019). Recent studies have shown that  
48 cyanobacterial blooms in freshwater lakes worldwide are still induced by high amounts of P  
49 transported by the inflowing rivers (Olokotum *et al.*, 2020). Hence, it is important to have  
50 information about the bioavailability and fate of the P that is discharged into lakes from

51 rivers.

52

53 The bioavailability of P depends on the P compound (Reynolds and Davies, 2001), and  
54 particulate P (PP) and dissolved P (DP) play different roles in P cycling in eutrophic lakes  
55 (Feng *et al.*, 2020; Lin *et al.*, 2016a). Many studies have examined the P compounds in PP  
56 as they are a source of DP in the water column (Li *et al.*, 2017; Mingus *et al.*, 2019; Shi *et*  
57 *al.*, 2020; Shinohara *et al.*, 2016; Yang *et al.*, 2020). When PP decomposes, dissolved  
58 organic P (e.g., orthophosphate monoesters and diesters, and pyro/polyphosphate) is  
59 released to the water column through phosphomonoesterase/diesterase activities (Parsons *et*  
60 *al.*, 2017). Dissolved inorganic P (e.g.,  $\text{PO}_4^{3-}$ ) is adsorbed onto mineral particles as PP (e.g.,  
61 Fe oxyhydroxides) (Cosmidis *et al.*, 2014), and is desorbed when the pH increases (Jin *et*  
62 *al.*, 2006), or the oxidation-reduction potential is below a certain threshold (Yuan *et al.*,  
63 2020). Researchers have analyzed and identified the P species in particulate matter using  
64 chemical speciation techniques (Chomicki *et al.*, 2016; Shinohara *et al.*, 2016; Xie *et al.*,  
65 2019; Yang *et al.*, 2020; Zhang *et al.*, 2017).

66

67 An abundance of information about the speciation of PP is available, but there is a lack of

68 corresponding information about DP (Bai *et al.*, 2015 and 2017; Feng *et al.*, 2020; Read *et*  
69 *al.*, 2014; Reitzel *et al.*, 2009). Bai *et al.* (2015) reported that the P compounds and their  
70 proportions in DP were different from those in PP. This result is significant in that it  
71 provides evidence that DP in the lake water includes more than the decomposed products of  
72 PP. The DP species may be affected by a variety of factors, such as the P sources, primary  
73 and secondary bacterial production, and the water residence time. The concentrations of  
74  $\text{PO}_4^{3-}$  and poly/pyrophosphate may be high in the water of rivers and lakes that are strongly  
75 influenced by sewage effluents (Altundogan and Tumen, 2002; Kulakovskaya *et al.*, 2021).  
76 However, even though this is a topic of much importance, only limited information is  
77 available about the speciation of DP in the water of rivers that transport large amounts of  
78 sewage effluent and the lakes into which they flow.

79

80 In this study, we investigated the P composition in the water of a lake that received  
81 discharges from sewage-influenced rivers. For our study site, we chose Lake Barato, a  
82 small eutrophic lake located in the suburbs of Sapporo, one of the largest cities in Japan.  
83 Sewage effluents account for approximately 46% of the total inflow and 65% of the total P  
84 load to the lake and, at approximately 2 weeks, the residence time of water in the lake is

85 short (The Hokkaido Regional Development Bureau). We first investigated how a range of  
86 P forms (soluble reactive P [SRP], dissolved organic P [DOP], dissolved acid hydrolysable  
87 P [DAHP], particulate inorganic P [PIP], and particulate organic P [POP]) varied between  
88 the inflowing rivers and Lake Barato. We then analyzed DP and PP species using solution  
89 <sup>31</sup>P nuclear magnetic resonance (NMR) spectroscopy. Before this analysis, we recovered  
90 the DP and PP species almost completely by flocculation or extraction. We tested the  
91 hypothesis that the inorganic P derived from sewage wastewater treatment plants would be  
92 transformed to organic forms in the small eutrophic lake.

93

## 94 **2. Materials and methods**

### 95 *2.1. Study site and sampling*

96 The study was carried out in Lake Barato, located in the suburban areas of Sapporo and  
97 Ishikari, Hokkaido, Japan (Figure 1). Lake Barato is an oxbow lake that was isolated from  
98 the Ishikari River when flood protection measures were implemented on the river in 1931.  
99 The lake has a total area, average depth, and total length of 4.67 km<sup>2</sup>, 2.8 m, and 20.2 km,  
100 respectively. Three rivers, namely the Fushiko, Sousei, and Hassamu, flow into the lake.  
101 Treated sewage effluent accounts for 47%, 69%, and 19% of the flows of the Fushiko,

102 Sousei, and Hassamu, respectively (The Hokkaido Regional Development Bureau). Three  
103 of the sampling points were in the lake and followed the flow direction (Stations 1, 2, and  
104 3). Stations 4, 5, and 6 were on the Fushiko, Sousei, and Hassamu Rivers, respectively. The  
105 Sousei and Hassamu rivers flowed into the Fushiko, which then flowed into the lake.  
106 Station 1 was in upper region of the lake, and so the water quality at Station 1 was not  
107 affected by the river discharge. In contrast, the water quality at Station 2 was affected by  
108 the rivers because it was at the mouth of the Fushiko. Station 3 was in the lake,  
109 approximately 4.5 km downstream from the Fushiko's mouth.

110

111 From April 2020 to November 2020, we collected water samples and information about  
112 other water quality parameters at the six sampling points (Figure 1). We collected the  
113 surface water samples using well-washed polypropylene containers (3 L). The bottles were  
114 stored on ice and transported to the laboratory as soon as possible. The pH, electrical  
115 conductivity, concentrations of dissolved oxygen, and water temperature of the samples  
116 were measured *in situ*. In the laboratory, we determined the concentrations of five P forms  
117 (SRP, DOP, DAHP, PIP, and POP) and other water quality parameters. Detailed information  
118 about the water quality of the lake is provided in the supplementary material.



119

## 120 *2.2. Determination of phosphorus forms*

121 Ultrapure water (18.2 MΩcm) was used to prepare all the aqueous solutions. The samples  
122 were filtered through glass fiber filters with a nominal pore size of 0.7 μm (Whatman™  
123 GF/F, GE Healthcare Life Sciences, Buckinghamshire, England), to separate the particulate  
124 matter from the water samples. The filters had been pre-combusted at 550 °C for 2 h. The  
125 filters and filtrates were stored (for up to a day) until analysis.

126

127 We determined the concentrations of POP and PIP using a previously reported method  
128 (Shinohara *et al.*, 2016). Prior to determining the PP concentrations, the filters and the  
129 particulate materials (i.e., suspended solids [SS]) were dried overnight at 105 °C. The dried  
130 filters were combusted at 550 °C for 2 h and then each filter was soaked in 1 M HCl (5 mL)  
131 for 24 h at room temperature with a mechanical shaker to extract the P. The extract was  
132 filtered through a 0.45-μm pore size membrane (DISMIC® 28HP045AN; Toyo Roshi  
133 Kaisya, Ltd., Tokyo, Japan), and the filtrate was diluted by more than 10 times (Suzumura,  
134 2008). The P concentrations in the filtrate were determined by the molybdenum blue  
135 method (Murphy and Riley, 1962). The PIP concentrations were determined using the same

136 procedure, but without combusting at 550 °C. The POP concentrations were calculated as  
137 the difference between the PP and PIP concentrations.

138

139 The SRP concentrations in the filtered water samples were directly determined by the  
140 molybdenum blue method. The filtered water samples were digested with potassium  
141 peroxodisulfate or strong acid solution before measuring DP or total inorganic P (SRP +  
142 DAHP), respectively (APHA, 2012). The P concentrations of the digested samples were  
143 determined by the molybdenum blue method. The DOP concentrations were calculated by  
144 subtracting the DP and total inorganic P concentrations. The DAHP concentrations were  
145 calculated by subtracting the total inorganic P and SRP concentrations (APHA, 2012).

146

### 147 *2.3. Solution <sup>31</sup>P NMR spectroscopy of particulate and dissolved phosphorus*

148 We collected lake and river water samples for solution <sup>31</sup>P NMR spectroscopy in August  
149 because the biomass can be expected to be high in the summer season. We prepared  
150 samples for <sup>31</sup>P NMR spectroscopy following a modified version of the procedures  
151 previously reported (Shinohara *et al.*, 2016; Bai *et al.*, 2015; Reitzel *et al.*, 2009). We  
152 obtained the PP needed for the analysis by filtering a portion (~10 L) of each water sample

153 through a GF/F glass fiber filter. The filters and the residue were lyophilized for several  
154 days in a freeze dryer. The dried filters were then soaked in a mixed solution (100 mL, 0.25  
155 M NaOH + 0.05 M ethylenediaminetetraacetic acid [EDTA]), and the P compounds were  
156 extracted for 4 h at 20 °C on a shaker. The extracts were filtered through GF/F glass fiber  
157 filters, and the resulting filtrates were lyophilized for several days. The powder produced  
158 was stored in a freezer (-20 °C) until analysis. The recovery of PP was calculated from the  
159 mass of PP in the water sample and the TP in the extract.

160

161 To prepare the DP samples for the NMR analysis, the DP in the water samples was  
162 flocculated using polyaluminum chloride (PACl; Taki Chemical Co., Ltd., Kakogawa,  
163 Japan). The dosage of PACl was determined from the DP concentrations in the water  
164 sample when the molar ratio of Al/P was 10/1. The PACl solution (0.24 mol-Al/L) was  
165 added to the filtered water sample (10 L), and the mixture was mechanically agitated for 3  
166 min at 150 rpm and then for an additional 10 min at 50 rpm. The pH was adjusted to  
167 between 6.5 and 7.0. The generated flocs were centrifuged at 15,000 rpm using a high-  
168 speed refrigerated centrifuge with a continuous flow rotor. The obtained flocs were re-  
169 dissolved in a mixed solution (40 mL, 0.25 M NaOH + 0.05 M EDTA) for 18 h at 20 °C by

170 shaking. The solution was then lyophilized for several days. The powder obtained was  
171 stored in a freezer ( $-20\text{ }^{\circ}\text{C}$ ) until NMR analysis. The recovery of DP was calculated by  
172 subtracting the mass of DP in the water sample before and after flocculation.

173

174 The spectra of the solution  $^{31}\text{P}$  NMR were recorded on an NMR spectrometer (JNM-ECA  
175 500, JEOL Ltd., Tokyo, Japan) at room temperature. Chemical shifts are reported in ppm  
176 relative to the external standard,  $\text{D}_3\text{PO}_4$  (0.0 ppm), and orthophosphate was adjusted to 0  
177 ppm (Liu *et al.*, 2015). The specific ranges of the P compounds were classified as  
178 orthophosphate (6.0 ppm), orthophosphate monoesters (3.7–5.2 ppm), DNA-derived  
179 orthophosphate diesters ( $-1.7$  to  $-0.7$  ppm), pyrophosphate ( $-4.3$  to  $-3.5$  ppm), and  
180 polyphosphate ( $-20$  ppm) (Shinohara *et al.*, 2012 and 2016).

181

#### 182 *2.4. Statistical analysis*

183 We used the Pearson product moment correlation to identify significant correlations  
184 between the metals and PIP in the SS. A paired t-test was applied to compare the  
185 concentrations of each P form. One-way analysis of variance (ANOVA) was used to  
186 compare the ratio of the P forms in the lake and rivers. P values less than 0.05 were

187 regarded as significant. We used Origin Pro 9.8 software for the analysis.

188

### 189 **3. Results**

#### 190 *3.1. Phosphorus forms in the inflowing rivers*

191 The total P load from the Sousei River (Station 5) was the highest, and its load was between

192 3 and 13 times greater than the loads from the other 2 rivers (see Figure S1). The high P

193 loading from the Sousei (Station 5) was the result of the high P concentrations rather than

194 the river flow rate (Figure 2). There is a municipal wastewater treatment plant relatively

195 close to Station 5 (4.5 km upstream), and a study reported that treated wastewater

196 accounted for 65% of the flow volume in the Sousei river (The Hokkaido Regional

197 Development Bureau). In addition, from a survey at the sewage effluent discharge outlet of

198 the wastewater treatment plant in October 2020, we confirmed that the SRP concentration

199 in the Sousei river increased from 0.006 mg-P/L to 0.53 mg-P/L after the effluent inflow.

200 The SRP form of P dominated at Station 5, and the P loading to the lake from this site

201 mainly reflected the high SRP concentrations. The P loads in the Fushiko (Station 4) and

202 Hassamu (Station 6) Rivers were dominated by PIP.

203

204 We found that the PIP and Fe contents in SS at Stations 4 and 6 were strongly and  
205 positively correlated ( $r = 0.77$ ,  $p < 0.01$ ) (Figure S2). The PIP contents were also positively  
206 correlated with the Mn contents ( $r = 0.61$ ,  $p < 0.05$ ). The concentrations of the other  
207 elements (i.e., Al, Ca, Mg, and S) were not correlated with PIP. Interestingly, as shown in  
208 Figure S2, the data for Station 5 were often outside of the ranges for the other stations.

209

### 210 *3.2. Phosphorus forms in the lake*

211 The phosphorus, chlorophyll-*a*, and nitrogen concentrations (Figure S3–S5) indicate that  
212 Lake Barato is eutrophic. The TP concentrations in Lake Barato were high (0.08–0.26 mg  
213 P/L) (Figure 2). The TP concentrations at the river mouth (Station 2) were higher than those  
214 at the upstream station (Station 1), where the effects of the inflowing river were minimal ( $|t|$   
215 = 10.3,  $p < 0.01$ ). The TP concentrations at the river mouth (Station 2) were also  
216 significantly higher than at Station 3 ( $|t| = 6.8$ ,  $p < 0.01$ ). Of the P forms, the SRP and PIP  
217 concentrations were higher at the river mouth (Station 2) than at the upstream station  
218 (Station 1; SRP:  $|t| = 4.5$ ,  $p < 0.01$ ; PIP:  $|t| = 4.9$ ,  $p < 0.01$ ) and were lower further  
219 downstream (Station 3) than at the river mouth (Station 2; SRP:  $|t| = 3.5$ ,  $p < 0.05$ ; PIP:  $|t| =$   
220 3.0,  $p < 0.05$ ). The SRP and PIP concentrations varied considerably between the sampling

221 stations, but there were no significant changes in the concentrations of DAHP, DOP, and  
222 POP.

223

224 The contributions of each P form to TP were different among the sampling sites (Figure 3  
225 and Table S1). The SRP/TP ratio was significantly higher at Station 5 than at the lake  
226 stations ( $p < 0.01$ ). The PIP/TP ratios were higher at Stations 4 and 6 than in the lake. Apart  
227 from Stations 6 and 2, the POP/TP ratios were higher in the lake than in the rivers ( $p <$   
228  $0.01$ ).

229

### 230 3.3. Determining the P compound classes in PP and DP by Solution $^{31}\text{P}$ NMR spectroscopy

231 We detected orthophosphate (ortho-P), orthophosphate monoesters (monoester-P),  
232 orthophosphate diesters (DNA-P), and pyrophosphate (pyro-P) in the samples (Figure 4).  
233 The recoveries of DP and PP were high ( $\sim 90\%$ ) compared with previous studies (Bai *et al.*,  
234 2015 and 2017; Read *et al.*, 2014; Reitzel *et al.*, 2009) (Table 1 and 2). We found large  
235 differences in the P compound classes associated with the PP and DP fractions. Pyro-P was  
236 only detected in the PP samples, and DNA-P was only detected in the DP samples in the  
237 lake. The P compounds also differed between the inflow rivers and the lake. The spectra

238 show that the DP in the discharge from the rivers only contained ortho-P, whereas the lake  
239 contained ortho-P, monoester-P, and DNA-P. Monoester-P and pyro-P, both PP compounds,  
240 were present in the river samples, but represented less than 20 % of the TP concentrations  
241 in the NaOH-EDTA extract ( $P_{\text{NaOH-EDTA}}$ ).

242

243 At Station 1 on the lake, both PP and DP contained organic P (i.e., monoester-P and/or  
244 DNA-P), which accounted for 62 % of  $P_{\text{NaOH-EDTA}}$ . In contrast, at the river mouth station  
245 (Station 2), ortho-P was the dominant P compound of both PP and DP, and accounted for  
246 70 % of  $P_{\text{NaOH-EDTA}}$ . The P compound classes at Station 3, 4.5 km downstream from the  
247 river inflows were similar in DP, with variation only in ortho-P. At Station 3, the sum of  
248 monoester-P and DNA-P accounted for 44 % of  $P_{\text{NaOH-EDTA}}$ . Also at Station 3, monoester-P  
249 accounted for a higher proportion of PP than ortho-P, and accounted for 44 % of  $P_{\text{NaOH-EDTA}}$ .

250

## 251 **4. Discussion**

### 252 *4.1. Interactions between the inflowing rivers and the downstream lake*

253 It is interesting to note the increase in the organic P compound classes (i.e., monoester-P  
254 and DNA-P) between the river inflow and the downstream lake (Table 1 and 2). The high



255 ortho-P concentrations in DP in the river water suggest that the sewage effluent was a major  
256 source of inorganic P to the lake. Both PP and DP included a high proportion of ortho-P at  
257 the river mouth, but once the river water was discharged into the lake, both forms quickly  
258 became enriched in organic P, as shown by the increase in organic P over the 4.5 km  
259 distance between Stations 2 to 3 and the short water residence time (~14 days). This may be  
260 caused by several physical, chemical, and biological processes downstream from the inflow  
261 to the lake, such as sedimentation of PIP, SRP adsorption/desorption, assimilation of SRP  
262 by plankton, and SRP recycling by plankton (Chomicki *et al.*, 2016; Han *et al.*, 2018; Lin *et*  
263 *al.*, 2016b; Yang *et al.*, 2020). Although the concentrations of TP, SRP, and PIP decreased  
264 from Station 2 to Station 3, the concentrations of DOP and POP did not change (Figure 2).  
265 These results suggest dilution, transformation of SRP to POP by phytoplankton, and PIP  
266 sedimentation. Furthermore, the DP fraction in the river discharge was dominated by ortho-  
267 P but monoester-P and DNA-P were present in the downstream lake (Figure 4), which  
268 indicates transformations between the P forms. The transformations may include  
269 assimilation of DP into living organisms to generate monoester-P and DNA-P. Similarly,  
270 organic P molecules are continually synthesized and released through grazing and viral  
271 lysis in lake water (Harke *et al.*, 2016). In Lake Barato, changes in the pH with the trophic

272 status and the high phytoplankton productivity might cause desorption of  $\text{PO}_4^{3-}$  from  
273 particulate matter to the water, which then further promotes phytoplankton production  
274 (Koski-Vahala and Hartikainen, 2001). Further investigations, such as adsorption and  
275 desorption experiments (Wang *et al.*, 2018) and trapping of particulate sediment in traps  
276 (Urban *et al.*, 2004), are needed to identify the processes that control the shift from  
277 inorganic to organic forms.

278

#### 279 *4.2. Different compositions between dissolved and particulate P*

280 It is also interesting to note the difference between the compositions of the P compounds in  
281 PP and DP in Lake Barato. Pyro-P was present in PP, whereas DNA-P was present in DP in  
282 the lake. To the best of our knowledge, this study represents the first time that clear  
283 differences have been identified between the P compound classes in DP and PP. Many  
284 studies have confirmed the presence of DNA-P in particulate matter (e.g., surface  
285 sediments) and that it is responsible for releasing P to the water column (Ahlgren *et al.*,  
286 2005; Reitzel *et al.*, 2012). Reitzel *et al.* (2012) reported that the DNA-P concentrations  
287 increased when seston was decomposed. The lack of detectable DNA-P in the PP fraction in  
288 this study may reflect low amount of biomass decomposed in August, when this study was

289 carried out. We analyzed DP in detail and detected DNA-P in DP in the lake water, but not  
290 in the river water (Table 1). We suspect that bacteria may take up  $\text{PO}_4^{3-}$  and transform it to  
291 DNA-P, as nucleotides in DNA and RNA are the major P compound class in their cells  
292 (Turner *et al.*, 2005). While DNA does not degrade readily, RNA is easily degraded and  
293 RNA-P-derived mononucleotides have been detected in the monoester-P region in  $^{31}\text{P}$  NMR  
294 spectra (Ahlgren *et al.*, 2005; Shinohara *et al.*, 2013; Yang *et al.*, 2020). Alternatively,  
295 DNA-P in DP may be introduced through P release from sediment, as demonstrated by  
296 Dell'Anno and Danovaro (2005), who reported that P was released from sediment to the  
297 water column when DNA-P degraded. Similarly, Ahlgren *et al.* (2005) also showed that  
298 DNA-P in sediment degraded at a faster rate than in the other P forms. Numerous  
299 researchers have reported the degradation of DNA-P in sediment (Ahlgren *et al.*, 2005;  
300 Ozkundakci *et al.*, 2014; Reitzel *et al.*, 2007), so the DNA-P in Lake Barato's water may  
301 have come from the sediments. In addition, DNA-P in the DP fraction could be viruses  
302 and/or small bacteria passed through the GF/F filters. We did not determine the exact  
303 process, but the fact that DNA-P was not detected in the river water and particles but was  
304 present in the lake water implies that DNA-P may have been produced in the lake.

305

306 4.3. Environmental implications and limitations

307 From the Fushiko and the Hassamu Rivers, P was discharged with metal oxyhydroxides.  
308 The significant strong correlations between PIP and Fe and Mn concentrations may have  
309 resulted from interactions between  $\text{PO}_4^{3-}$  and Fe and Mn oxides and hydroxides (Jin *et al.*,  
310 2006; Lin *et al.*, 2018; Withers and Jarvie, 2008). The study area is a Fe-rich peatland  
311 (Yanai *et al.*, 2012), and the Fe concentrations were higher than the Al and Mn  
312 concentrations (see Table S10 and S11). The P forms in the rivers may have been  
313 determined by the geological characteristics (Shinohara *et al.*, 2018). The characteristics of  
314 the Sousei River were different from those of the other two rivers. The Fe and PIP data for  
315 the Sousei (Station 5) were not correlated (Figure S2). We assume that the difference may  
316 reflect the fact that the Sousei (Station 5) has an artificial channel, constructed by concrete.  
317 The SS concentration at Station 5 was lower than those at Station 4 and 6 (Table S8). The  
318 lack of mineral particles, such as Fe, Al, and Ca, that absorb  $\text{PO}_4^{3-}$  may help explain the  
319 high concentration of SRP in the Sousei River. Our study has a management perspective.  
320 Lake Barato was previously diluted and flushed by water from a large river nearby, to  
321 improve the water quality (Shinohara *et al.*, 2008). Dilution and flushing could help to  
322 reduce the cyanobacterial blooms (Paerl and Paul, 2012), but the rapid transformation from

323  $\text{PO}_4^{3-}$  to organic P in DP suggests that  $\text{PO}_4^{3-}$  may be available for bacteria in the lake.

324

## 325 **5. Conclusion**

326 In summary, sewage effluents are the ongoing cause of eutrophication of Lake Barato. We

327 investigated the P forms at different places to determine whether sewage effluent affected

328 the lake downstream of the inflows. We found that inorganic P dominated in the river, but

329 there was a shift in the P compound classes to organic P (monoester-P and DNA-P) in the

330 lake, particularly the dissolved P forms. We also found that there was variation in the P

331 compounds in PP and DP downstream in the lake; pyrophosphate was detected in PP, but

332 not DNA-P, but the opposite was true for DP. The source of the DNA-P found in the DP in

333 the lake is unclear, but it may be related to bacterial P uptake and release from sediment.

334 The differences in the P forms and compounds in the inflowing river and the downstream

335 lake suggest that sewage effluents supply a large amount of SRP and PIP, but organic P, in

336 particular DNA-P, is rapidly produced in the lake, especially in DP.

337

## 338 **Supplementary material**

339 Supplementary materials are available.

340

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348

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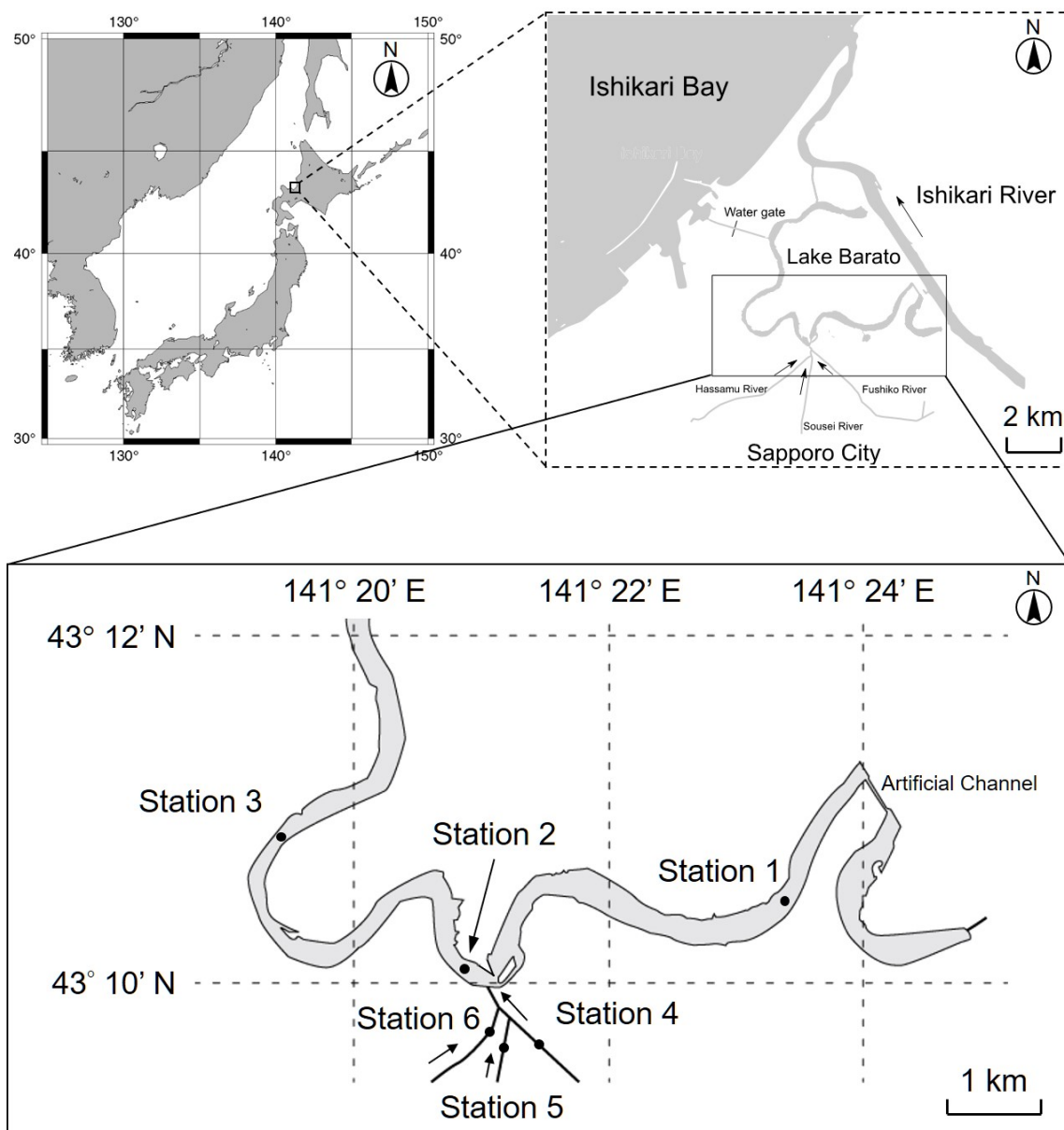
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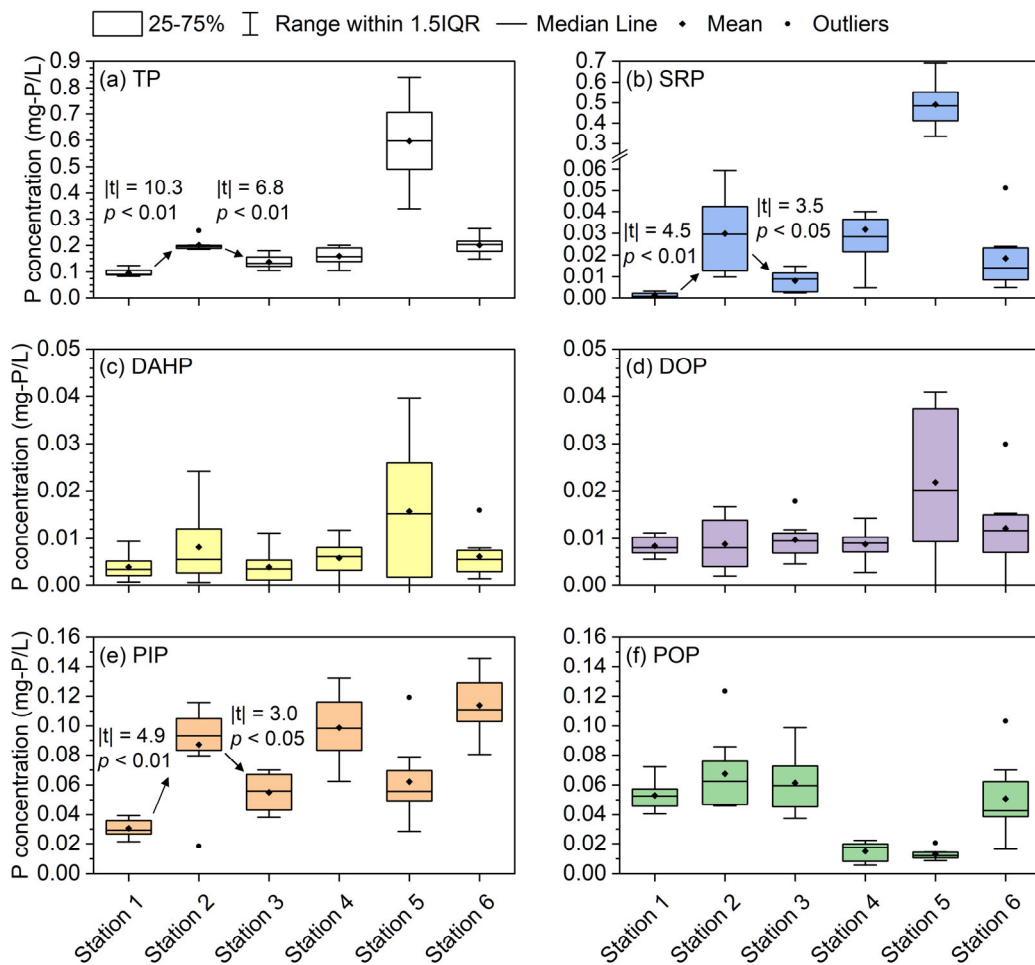
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498 **Figure 1** Location of the sampling sites in Lake Barato (Stations 1, 2, and 3) and inflowing  
499 rivers (Stations 4, 5, and 6).





500

501 **Figure 2** Concentrations of (a) TP, (b) SRP, (c) DAHP, (d) DOP, (e) PIP, and (f) POP in the

502 lake (Stations 1, 2, and 3) and in the rivers (Stations 4, 5, and 6). Arrows indicate

503 significant increases or decreases in the P concentrations. Within each box, the boxes

504 extend from the 25<sup>th</sup> to the 75<sup>th</sup> percentile of each group's distribution of values; the vertical

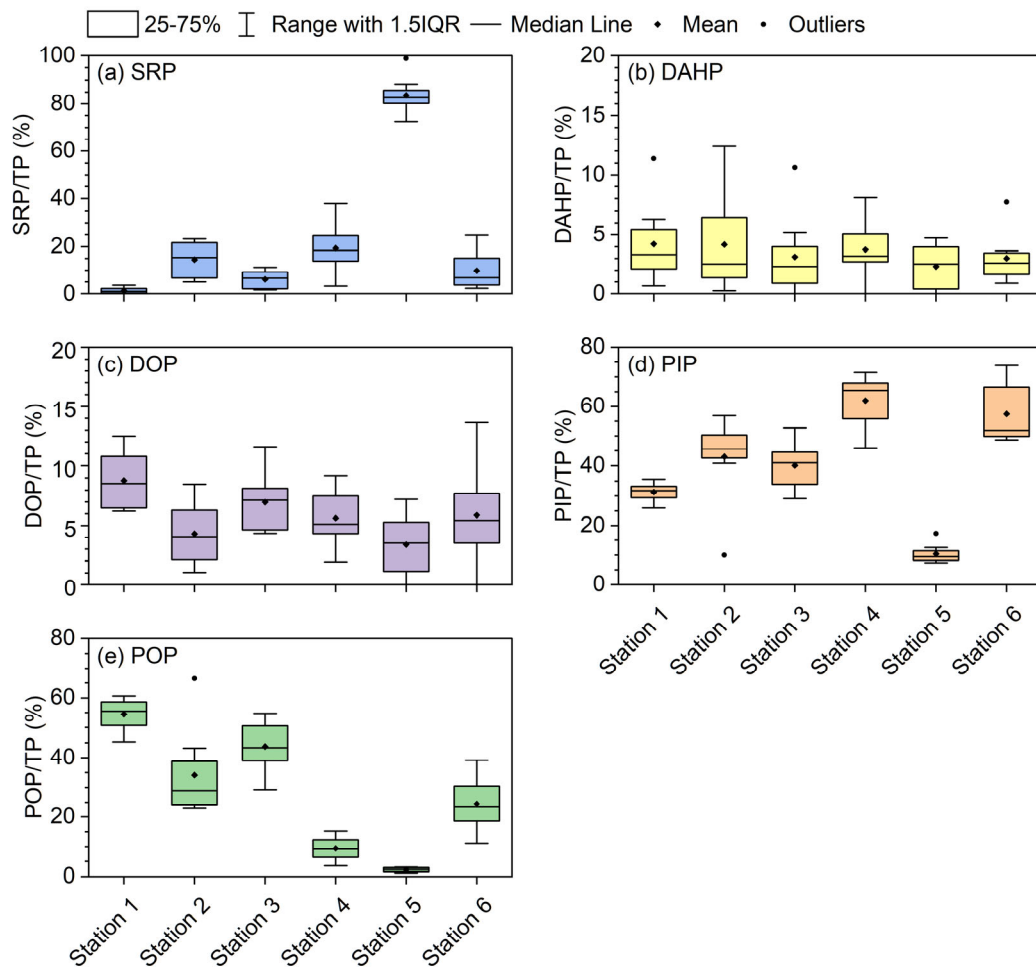
505 extending lines denote adjacent values (i.e., the most extreme values within 1.5 of the

506 interquartile range [IQR] of the 25<sup>th</sup> and 75<sup>th</sup> percentiles of each group); the horizontal

507 black lines denote the median values; square dots denote the mean values, and circle dots

508 denote observations outside the range of the adjacent values.

509



510

511 **Figure 3** Contributions of (a) SRP, (b) DAHP, (c) DOP, (d) PIP, and (e) POP to TP in the

512 lake (Stations 1, 2, and 3) and in the rivers (Stations 4, 5, and 6). Within each box, the

513 boxes extend from the 25th to the 75th percentile of each group's distribution of values; the

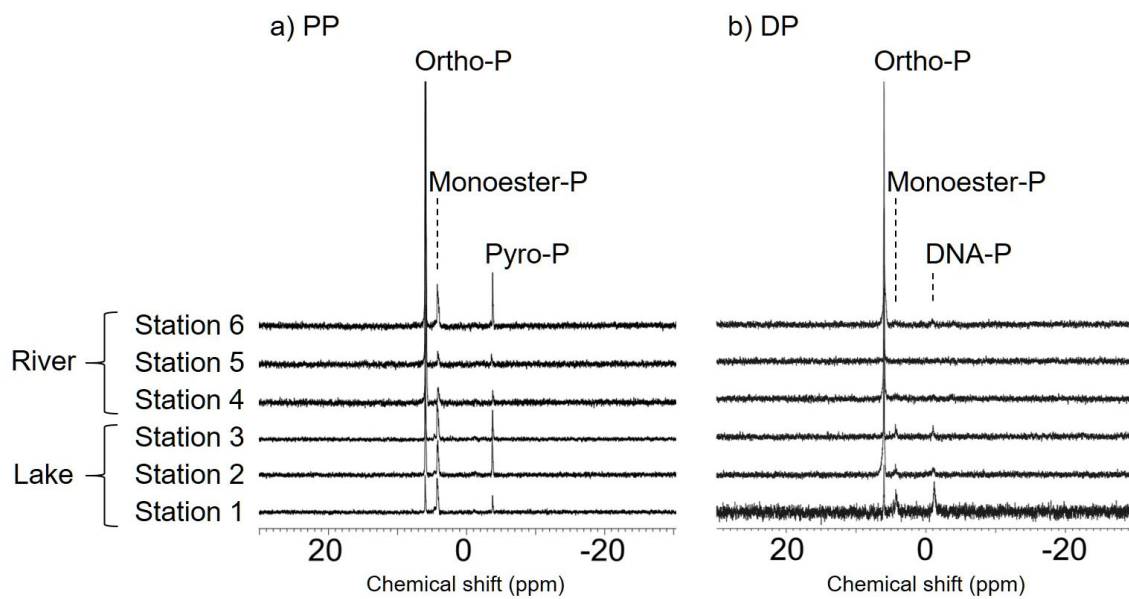
514 vertical extending lines denote adjacent values (i.e., the most extreme values within 1.5 of

515 the interquartile range [IQR] of the 25th and 75th percentiles of each group); the horizontal

516 black lines denote the median values; square dots denote the mean values, circle dots

517 denote observations outside the range of the adjacent values.

518



519

520 **Figure 4** Solution  $^{31}\text{P}$  NMR spectra of (a) PP and (b) DP at Stations 1–6 in August.

521

522 **Table 1** DP recoveries, TP concentrations in the NaOH-EDTA extract ( $P_{\text{NaOH-EDTA}}$ ), and the P concentration of each P compound  
 523 in the extract determined by solution  $^{31}\text{P}$  NMR.

Station	DP recovery (%)	$P_{\text{NaOH-EDTA}}$ ( $\mu\text{g-P/L}$ )	Ortho-P ( $\mu\text{g-P/L}$ )	Monoester-P ( $\mu\text{g-P/L}$ )	DNA-P ( $\mu\text{g-P/L}$ )	Pyro-P ( $\mu\text{g-P/L}$ )	Poly-P ( $\mu\text{g-P/L}$ )	Residual-P ( $\mu\text{g-P/L}$ )
Station 1	88	29	7	10	11	ND	ND	4
Station 2	93	52	47	2	3	ND	ND	4
Station 3	90	36	20	10	6	ND	ND	4
Station 4	94	63	63	ND	ND	ND	ND	4
Station 5	98	562	562	ND	ND	ND	ND	10
Station 6	94	53	53	ND	ND	ND	ND	4

525 **Table 2** PP recoveries, TP concentrations in the NaOH-EDTA extract ( $P_{\text{NaOH-EDTA}}$ ), and the P concentrations of each P compound  
 526 in the extract determined by solution  $^{31}\text{P}$  NMR.

Station	PP recovery (%)	$P_{\text{NaOH-EDTA}}$ ( $\mu\text{g-P/L}$ )	Ortho-P ( $\mu\text{g-P/L}$ )	Monoester-P ( $\mu\text{g-P/L}$ )	DNA-P ( $\mu\text{g-P/L}$ )	Pyro-P ( $\mu\text{g-P/L}$ )	Poly-P ( $\mu\text{g-P/L}$ )	Residual-P ( $\mu\text{g-P/L}$ )
Station 1	82	88	26	51	ND	11	ND	19
Station 2	75	128	79	29	ND	20	ND	44
Station 3	88	108	44	48	ND	17	ND	14
Station 4	98	170	157	9	ND	4	ND	4
Station 5	118	97	91	4	ND	2	ND	-
Station 6	105	183	147	22	ND	14	ND	-