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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
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### 17 Abstract

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

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 <sup>31</sup> 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	<i>al.</i> , 2017). Dissolved inorganic P (e.g., $PO_4^{3-}$ ) 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	PO4 <sup>3-</sup> 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

### 2. Materials and methods 94

#### 95 2.1. Study site and sampling

The study was carried out in Lake Barato, located in the suburban areas of Sapporo and 96 Ishikari, Hokkaido, Japan (Figure 1). Lake Barato is an oxbow lake that was isolated from 97 the Ishikari River when flood protection measures were implemented on the river in 1931. 98 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, 99 respectively. Three rivers, namely the Fushiko, Sousei, and Hassamu, flow into the lake. 100 Treated sewage effluent accounts for 47%, 69%, and 19% of the flows of the Fushiko, 101

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.

# 120 2.2. Determination of phosphorus forms

121	Ultrapure water (18.2 M $\Omega$ cm) 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 $\mu m$ (Whatman <sup>TM</sup>
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 $^{\circ}$ 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

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

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 $^{\circ}$ 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 °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 <sup>31</sup> 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, D <sub>3</sub> PO <sub>4</sub> (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.

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.

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* 

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

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

231

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 <sup>31</sup> P NMR spectroscopy

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orthophosphate diesters (DNA-P), and pyrophosphate (pyro-P) in the samples (Figure 4).
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232

We detected orthophosphate (ortho-P), orthophosphate monoesters (monoester-P),

The recoveries of DP and PP were high (~90%) compared with previous studies (Bai et al., 233

2015 and 2017; Read et al., 2014; Reitzel et al., 2009) (Table 1 and 2). We found large 234

differences in the P compound classes associated with the PP and DP fractions. Pyro-P was 235

only detected in the PP samples, and DNA-P was only detected in the DP samples in the 236

lake. The P compounds also differed between the inflow rivers and the lake. The spectra 237

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 <sub>NaOH-EDTA</sub> ).
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 $P_{NaOH-EDTA}$ . 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 PNaOH-EDTA. Also at Station 3, monoester-P
249	accounted for a higher proportion of PP than ortho-P, and accounted for 44 % of $P_{NaOH-EDTA}$
250	
251	4. Discussion
252	4.1. Interactions between the inflowing rivers and the downstream lake

It is interesting to note the increase in the organic P compound classes (i.e., monoester-P 253

and DNA-P) between the river inflow and the downstream lake (Table 1 and 2). The high 254

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 PO4 <sup>3-</sup> 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 $PO_4^{3-}$ 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 <sup>31</sup> 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	

## *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 $PO_4^{3-}$ and Fe and Mn oxides and hydroxides (Jin <i>et al.</i> ,
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 PO4 <sup>3-</sup> 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  $PO_4^{3-}$  to organic P in DP suggests that  $PO_4^{3-}$  may be available for bacteria in the lake. 324

325 **5. Conclusion** 

In summary, sewage effluents are the ongoing cause of eutrophication of Lake Barato. We 326 investigated the P forms at different places to determine whether sewage effluent affected 327 the lake downstream of the inflows. We found that inorganic P dominated in the river, but 328 there was a shift in the P compound classes to organic P (monoester-P and DNA-P) in the 329 lake, particularly the dissolved P forms. We also found that there was variation in the P 330 331 compounds in PP and DP downstream in the lake; pyrophosphate was detected in PP, but not DNA-P, but the opposite was true for DP. The source of the DNA-P found in the DP in 332 the lake is unclear, but it may be related to bacterial P uptake and release from sediment. 333 The differences in the P forms and compounds in the inflowing river and the downstream 334 lake suggest that sewage effluents supply a large amount of SRP and PIP, but organic P, in 335 particular DNA-P, is rapidly produced in the lake, especially in DP. 336 337

## 338 Supplementary material

339 Supplementary materials are available.

4	0
	4

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348	
349	References
350	1. APHA, AWWA, WEF, 2012. Standard methods for the examination of water and
351	wastewater, 22nd ed., American Public Health Association, Washington, DC.
352	2. Ahlgren, J.; Tranvik, L.; Gogoll, A.; Waldeback, M.; Markides, K.; Rydin, E.
353	Sediment depth attenuation of biogenic phosphorus compounds measured by <sup>31</sup> P NMR.
354	Environ. Sci. Technol. 2005, 39, 867–872.
355	3. Altundogan, H.S.; Tumen, F. Removal of phosphates from aqueous solutions by
356	using bauxite. I: Effect of pH on the adsorption of various phosphates. J. Chem. Technol.

357	Biotechnol.	2002.	77.	77-	-85.
		)	,		

358	4. Bai, X.L.; Sun, J.H.; Zhou, Y.K.; Gu, L.; Zhao, H.Y.; Wang, J.H. Variations of
359	different dissolved and particulate phosphorus classes during an algae bloom in a eutrophic
360	lake by <sup>31</sup> P NMR spectroscopy. <i>Chemosphere</i> . 2017, 169, 577–585.
361	5. Bai, X.L.; Zhou, Y.K.; Sun, J.H.; Ma, J.H.; Zhao, H.Y.; Liu, X.F. Classes of
362	dissolved and particulate phosphorus compounds and their spatial distributions in the water
363	of a eutrophic lake: a <sup>31</sup> P NMR study. <i>Biogeochemistry</i> . <b>2015</b> , 126, 227–240.
364	6. Chomicki, K.M.; Howell, E.T.; Defield, E.; Dumas, A.; Taylor, W.D. Factors
365	influencing the phosphorus distribution near the mouth of the Grand River, Ontario, Lake
366	Erie. J. Great Lakes Res. 2016, 42, 549–564.
367	7. Cosmidis, J.; Benzerara, K.; Morin, G.; Busigny, V.; Lebeau, O.; Jezequel, D.;
368	Noel, V.; Dublet, G.; Othmane, G. Biomineralization of iron-phosphates in the water
369	column of Lake Pavin (Massif Central, France). Geochim. Cosmochim. Acta. 2014, 126,
370	78–96.
371	8. Dell'Anno, A.; Danovaro, R. Extracellular DNA plays a key role in deep-sea
372	ecosystem functioning. Science. 2005, 309, 2179-2179.

9. Feng, W.Y.; Yang, F.; Zhang, C.; Liu, J.; Song, F.H.; Chen, H.Y.; Zhu, Y.R.; Liu,

374	S.S.; Giesy, J.P. Composition characterization and biotransformation of dissolved,
375	particulate and algae organic phosphorus in eutrophic lakes. Environ. Pollut. 2020, 265,
376	114838.
377	10. Han, C.N.; Zheng, B.H.; Qin, Y.W.; Ma, Y.Q.; Yang, C.C.; Liu, Z.C.; Cao, W.; Chi,
378	M.H. Impact of upstream river inputs and reservoir operation on phosphorus fractions in
379	water-particulate phases in the Three Gorges Reservoir. Sci. Total Environ. 2018, 610,
380	1546–1556.
381	11. Harke, M.J.; Steffen, M.M.; Gobler, C.J.; Otten, T.G.; Wilhelm, S.W.; Wood, S.A.;
382	Paerl, H.W. A review of the global ecology, genomics, and biogeography of the toxic
383	cyanobacterium, Microcystis spp. Harmful Algae. 2016, 54, 4-20.
384	12. Jarvie, H.P.; Neal, C.; Withers, P.J.A. Sewage-effluent phosphorus: A greater risk to
385	river eutrophication than agricultural phosphorus? Sci. Total Environ. 2006, 360, 246–253.
386	13. Jin, X.C.; Wang, S.R.; Pang, Y.; Wu, F.C. Phosphorus fractions and the effect of
387	pH on the phosphorus release of the sediments from different trophic areas in Taihu Lake,
388	China. Environ. Pollut. 2006, 139, 288–295.
389	14. Koski-Vahala, J.; Hartikainen, H. Assessment of the risk of phosphorus loading
390	due to resuspended sediment. J. Environ. Qual. 2001, 30, 960-966.

391	15. Kulakovskaya, T.V.; Vagabov, V.M.; Kulaev, I.S. Inorganic polyphosphate in
392	industry, agriculture and medicine: Modern state and outlook. Process Biochem. 2012, 47,
393	1–10.
394	16. Le Moal, M.; Gascuel-Odoux, C.; Menesguen, A.; Souchon, Y.; Etrillard, C.;
395	Levain, A.; Moatar, F.; Pannard, A.; Souchu, P.; Lefebvre, A.; Pinay, G. Eutrophication: A
396	new wine in an old bottle? Sci. Total Environ. 2019, 651, 1-11.
397	17. Li, J.Y.; Reardon, P.; McKinley, J.P.; Joshi, S.R.; Bai, Y.G.; Bear, K.; Jaisi, D.P.
398	Water column particulate matter: A key contributor to phosphorus regeneration in a coastal
399	eutrophic environment, the Chesapeake Bay. J. Geophys. Res. Biogeosci. 2017, 122,
400	737–752.
401	18. Lin, P.; Guo, L.D. Dynamic changes in the abundance and chemical speciation of
402	dissolved and particulate phosphorus across the river-lake interface in southwest Lake
403	Michigan. Limnol. Oceanogr. 2016a, 61, 771–789.
404	19. Lin, P.; Klump, J.V.; Guo, L.D. Dynamics of dissolved and particulate phosphorus
405	influenced by seasonal hypoxia in Green Bay, Lake Michigan. Sci. Total Environ. 2016b,
406	541, 1070–1082.

407 20. Lin, P.; Klump, J.V.; Guo, L.D. Variations in chemical speciation and reactivity of

408	phosphorus between suspended-particles and surface-sediment in seasonal hypoxia-
409	influenced Green Bay. J. Great Lakes Res. 2018, 44, 864–874.
410	21. Liu, J.; Hu, Y.F.; Yang, J.J.; Abdi, D.; Cade-Menun, B.J. Investigation of Soil
411	Legacy Phosphorus Transformation in Long-Term Agricultural Fields Using Sequential
412	Fractionation, P K-edge XANES and Solution P NMR Spectroscopy. Environ. Sci. Technol.
413	<b>2015</b> , 49, 168–176.
414	22. Mingus, K.A.; Liang, X.M.; Massoudieh, A.; Jaisi, D.P. Stable Isotopes and
415	Bayesian Modeling Methods of Tracking Sources and Differentiating Bioavailable and
416	Recalcitrant Phosphorus Pools in Suspended Particulate Matter. Environ. Sci. Technol.
417	<b>2019</b> , 53, 69–76.
418	23. Murphy, J.; Riley, J.P. A Modified Single Solution Method for Determination of
419	Phosphate in Natural Waters Anal. Chim. Acta. 1962, 26, 31-36.
420	24. Olokotum, M.; Mitroi, V.; Troussellier, M.; Semyalo, R.; Bernard, C.; Montuelle,
421	B.; Okello, W.; Quiblier, C.; Humbert, J.F. A review of the socioecological causes and
422	consequences of cyanobacterial blooms in Lake Victoria. Harmful Algae. 2020, 96, 101829.
423	25. Ozkundakci, D.; Hamilton, D.P.; McDowell, R.; Hill, S. Phosphorus dynamics in
424	sediments of a eutrophic lake derived from <sup>31</sup> P nuclear magnetic resonance spectroscopy.

## 425 Mar. Freshw. Res. 2014, 65, 70–80.

426	26. Paerl, H.W.; Paul, V.J. Climate change: Links to global expansion of harmful
427	cyanobacteria. Water Res. 2012, 46, 1349-1363.
428	27. Parsons, C.T.; Rezanezhad, F.; O'Connell, D.W.; Van Cappellen, P. Sediment
429	phosphorus speciation and mobility under dynamic redox conditions. <i>Biogeosciences</i> . 2017,
430	14, 3585–3602.
431	28. Read, E.K.; Ivancic, M.; Hanson, P.; Cade-Menun, B.J.; McMahon, K.D.
432	Phosphorus speciation in a eutrophic lake by <sup>31</sup> P NMR spectroscopy. <i>Water Res.</i> 2014, 62,
433	229–240.
434	29. Reitzel, K.; Ahlgren, J.; DeBrabandere, H.; Waldeback, M.; Gogoll, A.; Tranvik,
435	L.; Rydin, E. Degradation rates of organic phosphorus in lake sediment. <i>Biogeochemistry</i> .
436	<b>2007</b> , 82, 15–28.
437	30. Reitzel, K.; Ahlgren, J.; Rydin, E.; Egemose, S.; Turner, B.L.; Hupfer, M.

438 Diagenesis of settling seston: identity and transformations of organic phosphorus. J.

- 439 Environ. Monit. 2012, 14, 1098–1106.
- 440 31. Reitzel, K.; Jensen, H.S.; Flindt, M.; Andersen, F.O. Identification of Dissolved
- 441 Nonreactive Phosphorus in Freshwater by Precipitation with Aluminum and Subsequent <sup>31</sup>P

- 442 NMR Analysis. Environ. Sci. Technol. 2009, 43, 5391–5397.
- 443 32. Reynolds, C.S.; Davies, P.S. Sources and bioavailability of phosphorus fractions in
- freshwaters: a British perspective. *Biol. Rev.* **2001**, 76, 27–64.
- 445 33. Shi, K.; Zhang, Y.L.; Zhang, Y.B.; Qin, B.Q.; Zhu, G.W. Understanding the long-
- term trend of particulate phosphorus in a cyanobacteria-dominated lake using MODIS-
- 447 Aqua observations. *Sci. Total Environ.* **2020**, 737, 139736.
- 448 34. Shinohara, R.; Imai, A.; Kawasaki, N.; Komatsu, K.; Kohzu, A.; Miura, S.; Sano,
- 449 T.; Satou, T.; Tomioka, N. Biogenic Phosphorus Compounds in Sediment and Suspended
- 450 Particles in a Shallow Eutrophic Lake: A <sup>31</sup>P-Nuclear Magnetic Resonance (<sup>31</sup>P NMR)
- 451 Study. Environ. Sci. Technol. 2012, 46, 10572–10578.
- 452 35. Shinohara, R.; Imai, A.; Kohzu, A.; Tomioka, N.; Furusato, E.; Satou, T.; Sano, T.;
- 453 Komatsu, K.; Miura, S.; Shimotori, K. Dynamics of particulate phosphorus in a shallow
- 454 eutrophic lake. *Sci. Total Environ.* **2016**, 563, 413–423.
- 455 36. Shinohara, R.; Okunishi, T.; Adachi, K.; Viet, L.S.; Mine, H.; Yamashita, T.; Isobe,
- 456 M. Evaluation of the impact of water dilution within the hypereutrophic Lake Barato,
- 457 Japan. *Lake Reserv Manag.* **2008**, 24, 30–1312.
- 458 37. Shinohara, R.; Ouellette, L.; Nowell, P.; Parsons, C.T.; Matsuzaki, S.S.; Voroney,

- 459 R.P. The composition of particulate phosphorus: A case study of the Grand River, Canada.
- 460 J. Great Lakes Res. 2018, 44, 527–534.
- 461 38. Suzumura, M. Persulfate chemical wet oxidation method for the determination of
- 462 particulate phosphorus in comparison with a high-temperature dry combustion method.
- 463 *Limnol. Oceanogr. Methods.* **2008**, 6, 619–629.
- 464 39. The Hokkaido Regional Development Bureau.
- 465 <u>http://www.hkd.mlit.go.jp/sp/kasen\_keikaku/kluhh4000000awvi-</u>
- 466 <u>att/kluhh4000000axhu.pdf</u>. (in Japanese)
- 467 40. Turner, B.L.; Frossard, E.; Baldwin, D.S. 2005. Organic phosphorus in the
- 468 environment, CABI Publishing, Wallingford, UK ; Cambridge, Mass.
- 469 41. Urban, N.R.; Lu, X.F.; Chai, Y.T.; Apul, D.S. Sediment trap studies in Lake
- 470 Superior: Insights into resuspension, cross-margin transport, and carbon cycling. J. Great
- 471 *Lakes Res.* **2004**, 30, 147–161.
- 472 42. Wang, X.L.; Wei, J.X.; Bai, N.; Cha, H.; Cao, C.; Zheng, K.X.; Liu, Y. The
- 473 phosphorus fractions and adsorption-desorption characteristics in the Wuliangsuhai Lake,
- 474 China. Environ. Sci. Pollut. Res. 2018, 25, 20648–20661.
- 475 43. Withers, P.J.A.; Jarvie, H.P. Delivery and cycling of phosphorus in rivers: A

476 review. Sci. Total Environ. 2008, 400, 379–395.

477	44. Xie, F.Z.; Li, L.; Song, K.; Li, G.L.; Wu, F.C.; Giesy, J.P. Characterization of
478	phosphorus forms in a Eutrophic Lake, China. Sci. Total Environ. 2019, 659, 1437–1447.
479	45. Yanai, J.; Okada, T.; Yamada, H. Elemental composition of agricultural soils in
480	Japan in relation to soil type, land use and region. Soil Sci. Plant Nutr. 2012, 58, 1–10.
481	46. Yang, P.; Yang, C.H.; Yin, H.B. Dynamics of phosphorus composition in
482	suspended particulate matter from a turbid eutrophic shallow lake (Lake Chaohu, China):
483	Implications for phosphorus cycling and management. Sci. Total Environ. 2020, 741,
484	140203.
485	47. Yuan, H.Z.; Tai, Z.Q.; Li, Q.; Liu, E.F. In-situ, high-resolution evidence from
486	water-sediment interface for significant role of iron bound phosphorus in eutrophic lake.
487	Sci. Total Environ. 2020, 706, 136040.
488	48. Zangarini, S.; Sciarria, T.P.; Tambone, F.; Adani, F. Phosphorus removal from
489	livestock effluents: recent technologies and new perspectives on low-cost strategies.
490	Environ. Sci. Pollut. Res. 2020, 27, 5730–5743.
491	49. Zhang, R.Y.; Chen, J.A.; Wang, L.Y.; Wu, F.C. Characteristics of phosphorus
492	components in surface sediments from a Chinese shallow eutrophic lake (Lake Taihu): new

493 insights from chemical extraction and <sup>31</sup>P NMR spectroscopy. *Environ. Sci. Pollut. Res.* 

, 24, 23518–23527.





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



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

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

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



- 505 extending lines denote adjacent values (i.e., the most extreme values within 1.5 of the
- <sup>506</sup> 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.



Figure 3 Contributions of (a) SRP, (b) DAHP, (c) DOP, (d) PIP, and (e) POP to TP in the lake (Stations 1, 2, and 3) and in the rivers (Stations 4, 5, and 6). Within each box, the boxes extend from the 25th to the 75th percentile of each group's distribution of values; the vertical extending lines denote adjacent values (i.e., the most extreme values within 1.5 of the interquartile range [IQR] of the 25th and 75th percentiles of each group); the horizontal black lines denote the median values; square dots denote the mean values, circle dots

517 denote observations outside the range of the adjacent values.



**Figure 4** Solution <sup>31</sup>P NMR spectra of (a) PP and (b) DP at Stations 1–6 in August.

Station	DP recovery	P <sub>NaOH-EDTA</sub>	Ortho-P	Monoester-P	DNA-P	Pyro-P	Poly-P	Residual-P
Station	(%)	$(\mu g-P/L)$	$(\mu g - P/L)$	$(\mu g-P/L)$				
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

Table 1 DP recoveries, TP concentrations in the NaOH-EDTA extract (PNaOH-EDTA), and the P concentration of each P compound

523 in the extract determined by solution  ${}^{31}$ P NMR.

524

	PP recovery	P <sub>NaOH-EDTA</sub>	Ortho-P	Monoester-P	DNA-P	Pyro-P	Poly-P	Residual-P
Station	(%)	$(\mu g-P/L)$	$(\mu g - P/L)$	$(\mu g - P/L)$	(µg-P/L)	$(\mu g - P/L)$	$(\mu g - P/L)$	$(\mu g - P/L)$
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	-

Table 2 PP recoveries, TP concentrations in the NaOH-EDTA extract (PNaOH-EDTA), and the P concentrations of each P compound

526 in the extract determined by solution  ${}^{31}$ P NMR.

527