

1 **Interfacial oxygen nanobubbles reduce methylmercury**
2 **production ability of sediments in eutrophic waters**

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14 **Abstract**

15 Eutrophication can induce hypoxia/anoxia and rich organic matter at the sediment-water
16 interface in surface waters. When eutrophic waters are impacted with mercury (Hg) pollution,
17 methylmercury (MeHg) production ability (MPA) of surface sediment would increase and
18 more MeHg might be produced. To tackle this risk, this study firstly collected samples of
19 surface sediment and overlying water from a typical eutrophic lake—Taihu Lake. Then from
20 a sediment-water simulation system, we demonstrated that eutrophic waters were able to
21 methylate Hg spontaneously, and that sediment is the major Hg sink in the system. After the
22 addition of HgCl₂ solution (approximately 1 mg L⁻¹ in the slurry), MeHg concentrations in
23 the sediment increased by 11.7 times after 48 h. The subsequent column experiments proved
24 that O₂ nanobubbles could significantly decrease the MPA of surface sediment, by up to 48%.
25 Furthermore, we found that O₂ nanobubbles could remediate anoxia mainly by increasing
26 dissolved oxygen (from 0 to 2.1 mg L⁻¹), oxidation-reduction potentials (by 37% on average),
27 and sulfate (by 31% on average) in the overlying water. In addition, O₂ nanobubbles could
28 also help decrease organic matter concentration, as was revealed by the decline of dissolved
29 organic carbon in the overlying water (by up to 57%) and total organic carbon in surface
30 sediment (by up to 37%). The remediation of anoxia and reduction of organic matter could
31 contribute to the decrease of *hgcA* gene abundance (by up to 86%), and thus result in the
32 reduction of MPA after the addition of O₂ nanobubbles. This study revealed the risk of MeHg
33 production in case Hg pollution occurs in eutrophic waters and proposed a feasible solution
34 for MeHg remediation.

35 **Keywords:** Mercury pollution; Mercury methylation; Anoxia remediation; Organic
36 matter; Mercury microbial methylator

37 **1. Introduction**

38 Mercury (Hg) is a toxic trace metal that can travel globally in atmosphere and enter
39 hydrosphere by deposition (Krabbenhoft and Sunderland, 2013). Human activities, such as
40 chlor-alkali production, fossil-fuel combustion, and mining, have greatly augmented Hg flux
41 into aquatic system (Streets et al., 2011). It is suggested that anthropogenic perturbations
42 have tripled Hg content in surface waters since industrialization (Lamborg et al., 2014). Hg
43 pollution in aquatic environment is emerging globally, either in oceans (Mason, 2013;
44 Sunderland et al., 2009; Gobeil et al., 1999) or in freshwaters (Li et al., 2012; Li et al., 2009;
45 Jiang et al., 2006). **In addition**, Hg content in the sediment far exceeds that in the overlying
46 water. Particularly, certain Hg-contaminated sediments in urban, industrial, or mineralized
47 areas might exhibit high Hg concentrations, some of which could reach up to several hundred
48 $\mu\text{g Hg g}^{-1}$ (Liu et al., 2017; Feng et al., 2006).

49 In surface waters, inorganic Hg can be methylated to methylmercury (MeHg), whose
50 content corresponds with changes of Hg inputs (Harris et al., 2007). As a potential
51 neurotoxin, MeHg can pose a significant health threat to human beings after bioaccumulation
52 and biomagnification (Gilmour et al., 2013). Hg methylation is primarily mediated by
53 anaerobic bacteria carrying *hgcAB* genes, such as **sulfate-reducing bacteria**, and tends to take
54 place in anaerobic conditions (Parks et al., 2013). In addition, organic matter can facilitate
55 the production of MeHg by acting as electron donor and microbial substrate for Hg microbial
56 methylators (Bravo et al., 2017; Gu et al., 2011). It has been reported that niches like aquatic
57 sediments are hotspot areas for MeHg production (Podar et al., 2015). For instance, sediments
58 are able to produce high levels of MeHg if the watershed is impacted with Hg pollution

59 (Balogh et al., 2015; Hachiya, 2012). Generally in sediments, the maximum Hg methylation
60 rates appear at the surface layer (Ullrich et al., 2001).

61 To evaluate MeHg production in different environmental matrices, indexes such as Hg
62 methylation rate constant and ratio of MeHg to total mercury (THg) have been used in a
63 previous study (Drott et al., 2008). Yet, these indexes are less likely to reflect net MeHg
64 production if Hg pollution occurs in different environment niches. Herein, MeHg production
65 ability (MPA) is proposed as an indicator to quantify environmental matrix's ability to
66 produce MeHg after exogenous Hg input. It is calculated as the increase of MeHg
67 concentration after 48 h divided by the initial Hg ion (Hg^{2+}) concentration. Accordingly,
68 areas with high MPAs should be paid close attention to for having substantial risks of MeHg
69 production when Hg pollution occurs.

70 Eutrophication is a widespread water pollution in surface waters, affecting 58% of
71 global lakes since industrialization (Taranu et al., 2015). In eutrophic waters, cyanobacteria
72 can form dense blooms and induce adverse effects on the aquatic ecosystems (Huisman et
73 al., 2018). The degradation of cyanobacterial blooms requires oxygen (O_2) and could lead to
74 a state of hypoxia or anoxia in the system (Wang et al., 2016a). Then the subsequent
75 deposition of the degraded cyanobacteria could result in the accumulation of organic matter
76 on the surface sediment (Conley et al., 2009). As a result, in eutrophic waters, surface
77 sediment is likely to display high MPA (Lei et al., 2019). Furthermore, in hyper-eutrophic
78 waters, cyanobacterial blooms might evolve into black blooms, which could further
79 aggravate hypoxia/anoxia in the system. During the outbreak of black blooms, sulfate-
80 reducing bacteria were suggested to be the primary biological factor (Feng et al., 2014), and

81 they also plays in major role in Hg methylation. Therefore, once impacted with exogenous
82 Hg input, surface sediment in hyper-eutrophic waters is highly possible to produce massive
83 MeHg, which requires immediate concern.

84 Several manipulations have been proposed for MeHg remediation in sediments. For
85 instance, capping sorbents like biochar and activated carbon were evaluated for MeHg
86 stabilization in sediments (Liu et al., 2017; Gilmour et al., 2013). Still, as pyrolyzed carbon,
87 these sorbents are likely to release carbon to aquatic systems and might have unpredictable
88 impacts on MeHg production in the long term (Gilmour et al., 2018; Liu et al., 2018a). In
89 addition, aeration of the anoxic sediment has also been proposed to inhibit MeHg production
90 by eliminating hypoxia/anoxia. Nevertheless, the technical feasibility and economic pressure
91 have been a concern due to the large volume of oxygen required (Mailman et al., 2006).
92 Moreover, the subsequent vertical mixing might result in the release of MeHg from sediment
93 to overlying waters as well, which could do greater harm to the aquatic organisms (Soerensen
94 et al., 2016).

95 Interfacial nanobubbles are nanobubbles with radius of curvature of 100–1000 nm and
96 mainly produced at the solid-liquid interface (Seddon et al., 2012). With nanoscale sizes,
97 interfacial nanobubbles can exhibit unique characteristics like extended lifetime and great
98 gas solubility. Interfacial oxygen nanobubbles can be loaded on natural zeolites (specific
99 gravity of 2.15–2.25 g cm⁻³), which are persistent clay minerals and unlikely to add extra
100 ecological pressure to aquatic ecosystems (Lyu et al., 2019). Moreover, oxygen nanobubble-
101 loaded zeolites can settle naturally to the designated areas like surface sediment, at which
102 they can release oxygen to remediate hypoxia/anoxia (Shi et al., 2018). As Hg methylation

103 tends to occur in the anaerobic conditions, the mitigation of hypoxia/anoxia by O₂
104 nanobubbles might induce a reduction in the MPA of surface sediment. Therefore, interfacial
105 oxygen nanobubble (loaded on zeolites) might provide a feasible solution for MeHg
106 remediation in surface sediment.

107 In this work, we performed the Hg methylation and MPA mitigation experiments to
108 investigate the reduction effects of interfacial O₂ nanobubbles on the probably increased
109 MPA of surface sediment, in case severe Hg pollution occurs in eutrophic waters. Our
110 primary objectives are firstly to reveal the fate of THg and MeHg in eutrophic waters
111 impacted with Hg pollution, and to determine the optimal conditions for MPA analysis;
112 secondly, to examine the effects of interfacial O₂ nanobubbles on MPA of surface sediment;
113 finally, to elucidate the mechanism of the mitigation effects of interfacial O₂ nanobubbles.

114 2. Materials and methods

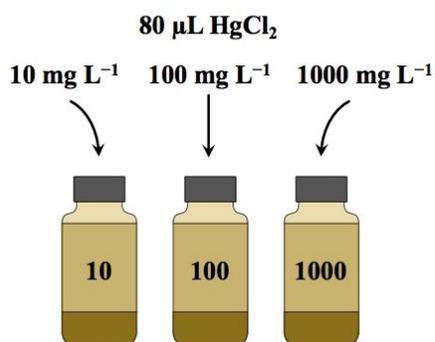
115 2.1 Sampling site

116 The samples of overlying water and surface sediment were collected from an algae
117 accumulation zone in Meiliang Bay (120°09' E, 31°31' N), north of Taihu Lake in December
118 of 2016 and September of 2017. Spatial distribution of the sampling sites is shown in **Fig. S1**
119 in the Supplementary information (SI). Taihu Lake is a typical eutrophic shallow lake located
120 in Wuxi City, Jiangsu Province (China). In recent decades, it has been suffering from severe
121 cyanobacterial blooms nearly every summer (Wang et al., 2016b). Once collected, samples
122 of overlying water and surface sediment were sealed and transferred to the lab at 4 °C in the
123 dark instantly.

124 2.2 Experimental design

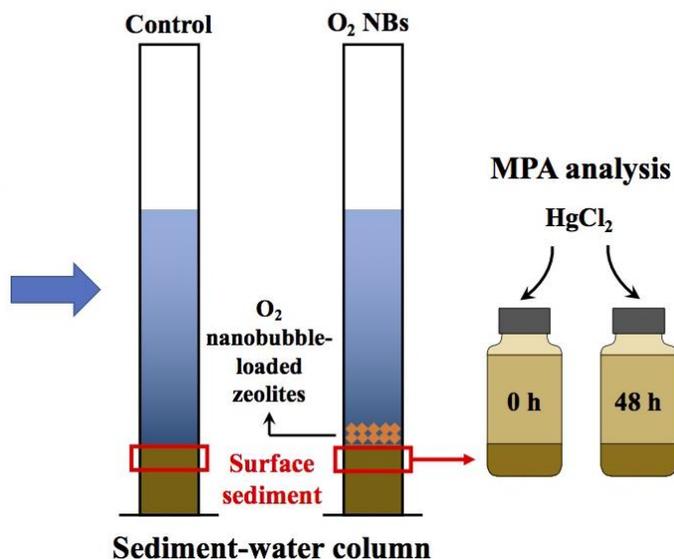
125 The scheme of the whole experimental design is illustrated in **Fig. 1**. The experiment is
126 composed of two phases. The first phase is the Hg methylation experiment performed in 8
127 mL amber glass vials with lake sediment and water (**Fig. 1A**). It is designed to determine the
128 optimal dosage of HgCl₂ solution and incubation time for MPA analysis. **In brief, the MPA**
129 **was calculated by the ratio of the changes in MeHg concentrations after 48h to the initial**
130 **Hg²⁺ concentrations (more details refer to Section 2.5).** The second phase is the MPA
131 mitigation experiment, which combines sediment-water column experiment and MPA
132 analysis in surface sediment from the columns (**Fig 1B**).

Hg methylation experiment



(A)

MPA mitigation experiment



(B)

133 **Fig. 1.** Scheme of the whole experimental design. The experiment is composed of two phases:
134 (A) The Hg methylation experiment. (B) The MPA mitigation experiment.

135 2.2.1 Hg methylation experiment

136 The Hg methylation experiment was carried out in a total of 48 amber glass vials of 8
137 mL (CNW, USA) with Teflon[®] lids. Approximately 110 g sediment were stirred with 330
138 mL deoxygenated lake water (1:3, m:m), both of which were collected from Taihu Lake in
139 2016. Then aliquots of 8 mL were transferred to the vials and spiked with 80 μ L of 10, 100,
140 and 1000 mg L⁻¹ HgCl₂ solutions, leaving the final HgCl₂ concentrations to be around 0.1,
141 1, and 10 mg L⁻¹ in the slurries. The vials were then left to settle after homogenization and
142 incubate in the dark at room temperature. At the same time each day during 7 days, two vials
143 of each spiking concentration were sacrificed for THg and MeHg analysis. During sampling,
144 the overlying water was extracted, filtered with 0.22 μ m syringe filters (ANPEL Laboratory
145 Technologies (Shanghai) Inc., China), then spiked with 30 μ L concentrated hydrochloric acid,
146 and stored at 4 °C for further analysis. The remaining sediment was frozen at -20 °C
147 overnight and freeze dried before analysis. The Hg methylation experiment was carried out
148 in an anaerobic box. All experiments were performed in duplicate.

149 **2.2.2 MPA mitigation experiments**

150 The MPA mitigation experiment was carried out in the plexiglass cylinder columns
151 (Beijing Yinfan Yangming Environmental Protection Technology Co., Ltd., China), which
152 were 5 cm in diameter and 50 cm in height. Each column was filled with 6 cm deep surface
153 sediment and 20 cm deep lake water (ca. 400 mL) collected from Taihu Lake in 2017. The
154 columns were sealed with rubber plugs (applied with Vaseline) and Parafilm[®] M Film (Bemis
155 Company, USA) and put in the dark at 25 °C (average temperature of Taihu Lake water in
156 September) for 30 days without HgCl₂ addition to establish a steady sediment-water interface
157 (Shen et al., 2003). During the last 7 days, dissolved oxygen (DO) concentrations (recorded

158 with a portable DO meter, SI) in the overlying water (about 2 cm above the sediment)
159 maintained at 0. In the meantime, the overlying water in the columns started to turn grey and
160 odorous, which is the typical sign of black blooms.

161 The columns were divided into two groups: i) the Control group was only filled with
162 Taihu sediment and lake water; ii) the O₂ nanobubbles group (O₂ NBs) was based on the
163 Control treatment and added with O₂ nanobubble-loaded zeolites. The preparation and
164 characterization of O₂ nanobubble-loaded zeolites, discussed briefly in the SI, were described
165 in Shi et al., 2018, Zhang et al. 2018 and Wang et al., 2018. and they were put to use
166 immediately after preparation. On day 0 of the incubation of the columns, O₂ nanobubble-
167 loaded zeolites (ca. 2 cm in height in the columns) were sprinkled evenly on the surface
168 sediment of the O₂ NBs group. Then all the columns were sealed except when sampling or
169 analyzing DO and oxidation-reduction potential (ORP) during the experiments. After the
170 addition of O₂ nanobubble-loaded zeolites, the incubation of the columns began and lasted
171 for 20 days.

172 1) MeHg production ability analysis

173 On days 2, 4, 8, 12, and 20 of the incubation, two columns of either treatment were
174 sacrificed for the analysis of MPA and relevant physicochemical parameters. According to
175 the results of the Hg methylation experiment, ~9 g surface sediment samples (2 cm from top)
176 were collected from the columns and spiked with 4 mg L⁻¹ HgCl₂ solutions, forming final
177 HgCl₂ concentration to be around 1 mg L⁻¹ in sediment slurries. After homogeneous mixing,
178 the slurries were divided into two portions. One portion was stored at -20 °C, representing
179 Hg level at t = 0. And the remaining was incubated in the dark at 25 °C for 48 hours,

180 representing Hg level at $t = 48$ h. Spiking and dividing sediment samples were performed in
181 an anaerobic chamber (LAI-3, Longyue, China). Duplicate was employed for both treatments.
182 Thus, for each treatment of the MPA mitigation experiment, there are four parallel samples
183 for MPA analysis. In addition, a portion of surface sediment (without the addition of HgCl_2
184 solution) was stored at -80 °C for microbiological assay.

185 **2) Physicochemical parameter analysis in the columns**

186 Concentrations of DO in the overlying water and ORP at the sediment-water interface
187 were analyzed at regular intervals. On the sampling days, concentrations of dissolved organic
188 carbon (DOC) and sulfate (SO_4^{2-}) in the overlying water, as well as total carbon (C), total
189 nitrogen (N), total sulfide (S), total organic carbon (TOC), and moisture content in surface
190 sediment samples were analyzed respectively. Detailed analytical methods of these
191 parameters can be found in the SI.

192 **2.3 THg and MeHg analysis**

193 For THg analysis in the sediment samples, a Hydra-C mercury analyzer (Teledyne
194 Leeman Labs, USA) following US EPA method 7473 was adopted (USEPA, 2007). For THg
195 analysis in the water samples, the MERX Automatic THg System (Brooks Rand
196 Laboratories, USA) following US EPA method 1631 was adopted (USEPA, 2002). For
197 MeHg analysis in the sediment samples, the pretreatment procedure using $\text{CuSO}_4/\text{HNO}_3$ as
198 leaching solutions was applied (Ji et al., 2019). Then MeHg concentrations were determined
199 with MERX Automatic Methylmercury System (Brooks Rand Laboratories, USA) following
200 US EPA method 1630 (USEPA, 2001).

201 **2.4 DNA extraction and Real-Time Quantitative PCR (qPCR)**

202 Total microbial DNA was extracted from approximately 0.25 g freeze-dried surface
203 sediment samples in the column experiment using DNeasy PowerSoil Kit (QIAGEN Inc.,
204 Germany) following the manufacturer's recommended protocol. Concentrations and quality
205 of the extracted DNA were determined with a Nanodrop UV-Vis spectrophotometer (ND-
206 2000, Thermo-Fisher Scientific, USA), and the abundance of the *hgcA* gene was quantified
207 using an iCycler iQ5 thermocycler (Bio-Rad, USA). The clade-specific degenerate primer
208 pair for Deltaproteobacteria was ORNL-Delta-HgcA (Delta-HgcA-F: GCCAACTACAAG
209 MTGASCTWC; Delta-HgcA-R: CCSGCNGCRCACCAGACRTT) (Liu et al., 2018b).

210 **2.5 Calculation of MeHg production ability (MPA)**

211 The MPA of surface sediment from the columns was calculated according to the
212 modified equation for Hg methylation rate calculation (**Eq. 1**) (Hintelmann et al., 2000).

$$\text{MeHg production ability} = \frac{[\text{MeHg}]_{48} - [\text{MeHg}]_0}{[\text{Hg}^{2+}]_0} \quad (\text{Eq. 1})$$

213 where $[\text{MeHg}]_{48}$ represented MeHg concentration 48 h after the addition of HgCl_2 solution,
214 $[\text{MeHg}]_0$ represented MeHg concentration the instant after the addition of HgCl_2 solution.
215 $[\text{Hg}^{2+}]_0$ represented Hg^{2+} concentration the instant after the addition of HgCl_2 solution, which
216 could be achieved by subtracting the content of MeHg from THg.

217 **2.6 Quality assurance/quality control (QA/QC) and statistical analysis**

218 During MeHg analysis in the sediment, MeHg concentrations in the certified reference
219 material ERM-CC580 (certified MeHg content: $75.5 \pm 3.7 \text{ ng g}^{-1}$, European Reference

220 Materials, Institute for Reference Materials and Measurements, Belgium) were also analyzed,
221 with the average value being $76.0 \pm 7.2 \text{ ng g}^{-1}$ ($n = 3$). The limit of quantification (LOQ) was
222 5 pg Hg in absolute mass, suggesting a good sensibility of the analytical method. Besides,
223 the linear range is from 5 to 800 pg ($r^2 = 0.99$). For THg analysis in the sediment, GSD-10
224 (GBW07310, Institute of Geological and Geophysical Exploration, Chinese Academy of
225 Geological Sciences, China) was used as certified reference material. The average THg
226 concentration measured was $280.02 \pm 0.06 \text{ ng g}^{-1}$ ($n=3$), which agreed well with the certified
227 value ($280 \pm 40 \text{ ng g}^{-1}$).

228 The differences between two groups throughout the incubation were analyzed using a
229 paired-sample t test after the normality test, and differences on every sampling day were
230 assessed by an independent t test. Significance probabilities (p) were also calculated and the
231 difference was declared significantly for $p < 0.05$.

232 3. Results

233 3.1 Characteristics of overlying water and surface sediment

234 As shown in Table 1, the concentrations of TP, TN, and Chlorophyll a in the overlying
235 water of Taihu Lake were 0.13 ± 0.01 , $10.28 \pm 0.29 \text{ mg L}^{-1}$, and $182.90 \pm 10.79 \text{ }\mu\text{g L}^{-1}$.
236 These were consistent with reported results and were typical characteristics of a hyper-
237 eutrophic freshwater (Xu et al., 2017). The average THg concentration in surface sediment
238 was $13.38 \pm 0.31 \text{ ng g}^{-1}$, and the ratio of MeHg to THg was 11.96%. In the overlying water,
239 THg concentration was 1.2‰ of that in the surface sediment, and MeHg concentration was
240 below detection limit. These results fell within the range typically measured in Taihu Lake

241 (Wang et al., 2012). In addition, the THg content in Taihu Lake's surface sediment was much
242 lower than HgCl₂ concentrations added in the Hg methylation experiment (approximately 0.1,
243 1, and 10 mg L⁻¹ in the slurries), which indicated that the potential influence of the
244 background Hg was negligible.

245 **Table 1.** Physiochemical characteristics of the overlying water and surface sediment samples
 246 collected from Taihu Lake (data shown by mean \pm SD, n = 2).

		Overlying water	Surface sediment
TP	mg L ⁻¹	0.13 \pm 0.01	-
PO ₄ ³⁻		0.04 \pm 0.00	-
TN		10.28 \pm 0.29	-
NO ₃ ⁻		0.51 \pm 0.07	-
DOC		33.39 \pm 0.30	-
Chlorophyll <i>a</i>	μ g L ⁻¹	182.90 \pm 10.79	-
THg	ng L ⁻¹	11.34 \pm 1.35	13.38 \pm 0.31
MeHg	(ng g ⁻¹)	ND	0.16 \pm 0.02
C	%	-	0.40 \pm 0.02
N		-	0.06 \pm 0.00
S		-	0.05 \pm 0.00
TOC		-	0.29 \pm 0.01
Moisture content		-	63.90 \pm 0.50
C/N	-	-	7.35 \pm 0.32

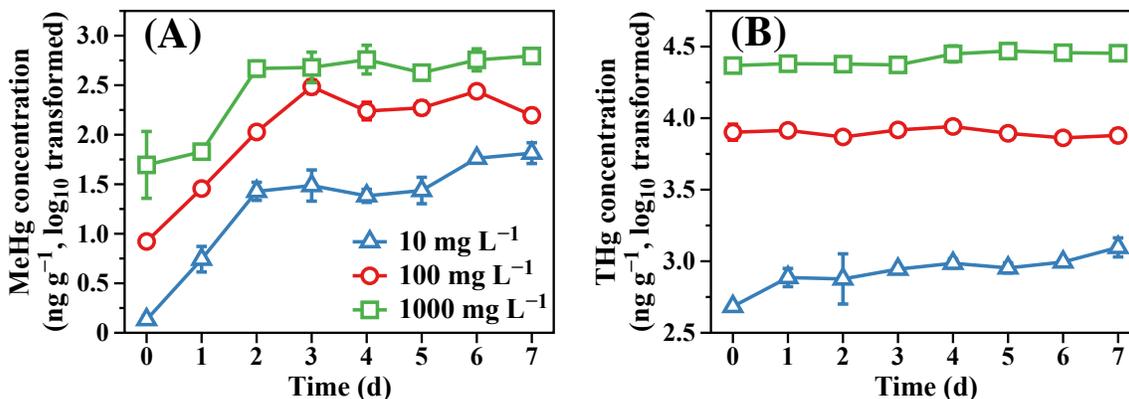
247 Note: ND represented not detected. The unit of "ng L⁻¹" was for concentrations in the
 248 overlying water and "ng g⁻¹" was for sediments.

249 **3.2 Hg fate in simulated Hg-polluted sediment-water system**

250 The Hg methylation experiment was designed to verify MeHg production in the
251 sediment in case abrupt Hg pollution occurs in eutrophic waters. Moreover, in order to
252 investigate the effects of O₂ nanobubbles on MPA, MeHg production in the sediment after
253 the addition of HgCl₂ solution should be sufficient for quantification. Therefore, the optimal
254 dosage of HgCl₂ solution for MPA analysis was determined according to the Hg fate after
255 the addition of three different concentrations of HgCl₂ solutions (10, 100, and 1000 mg L⁻¹)
256 with the final concentrations being around 0.1, 1, and 10 mg L⁻¹ in the slurries. **Fig. 2**
257 illustrated the variations of MeHg and THg in the sediment (after the extraction of overlying
258 water) after the addition of HgCl₂ solutions.

259 As illustrated in **Fig. 2A**, MeHg concentrations in the 10 mg L⁻¹, 100 mg L⁻¹, and 1000
260 mg L⁻¹ groups all increased significantly since day 0, with the maximum increments being
261 47.6, 35.5, and 10.0 times, respectively. This demonstrated the spontaneous MeHg
262 production in the sediment after the outbreak of Hg pollution in eutrophic waters. Particularly,
263 all three groups experienced the most remarkable increase in MeHg concentrations from day
264 0 to 2 (by 18.9, 11.7, and 7.2 times, respectively), and then MeHg concentrations varied
265 minimally till the end of the Hg methylation experiment. According to this, the incubation
266 length of MPA analysis in the sediment was designed as 48 h (**Eq. 1**). Compared with MeHg,
267 THg concentrations varied little over time (**Fig. 2B**). In addition, MeHg and THg
268 concentrations in the overlying water were analyzed as well (SI, **Fig. S2**). We found that for
269 each group, MeHg and THg concentrations in the sediment (1.28–724.56 and 475.09–
270 30864.36 ng g⁻¹) were generally three orders of magnitude higher than those in the overlying

271 water (0–1013.18 and 15.93–1776.46 ng L⁻¹) throughout the experiment. These results
 272 indicated that sediment was the main sink of Hg in surface waters.



273 **Fig. 2.** Variations of (A) MeHg and (B) THg concentrations in the sediment during the 7-day
 274 Hg methylation experiment. All data was transformed to log₁₀ form, and the mean and
 275 standard deviation (SD) were calculated accordingly. Day 0 represented Hg level the instant
 276 after the addition of HgCl₂ solution. Figure legend (i.e. 10 mg L⁻¹, 100 mg L⁻¹, 1000 mg L⁻¹)
 277 referred to the concentrations of the spiking HgCl₂ solution (data shown by mean ± SD, n =
 278 2).

279 Moreover, with different dosages of HgCl₂ solutions, the MeHg fates in the sediment
 280 were significantly ($p < 0.001$) different (**Fig. 2A**). Among the three groups, the 10 mg L⁻¹
 281 group produced the fewest MeHg in the sediment, from 1.36 ng g⁻¹ (day 0) to 27.07 ng g⁻¹
 282 (day 2) and 66.07 ng g⁻¹ (day 7). While in the 100 mg L⁻¹ and 1000 mg L⁻¹ groups, MeHg
 283 concentrations increased maximally from 8.38 to 306.19 ng g⁻¹ (day 0 to 3) and 57.18 to
 284 626.35 ng g⁻¹ (day 0 to 7), respectively. Specifically, the average MeHg concentrations in
 285 the two groups on day 2 were 106.57 and 468.91 ng g⁻¹, far exceeding that in the 10 mg L⁻¹
 286 group. This suggested that 10 mg L⁻¹ (the added HgCl₂ concentration) might not be the

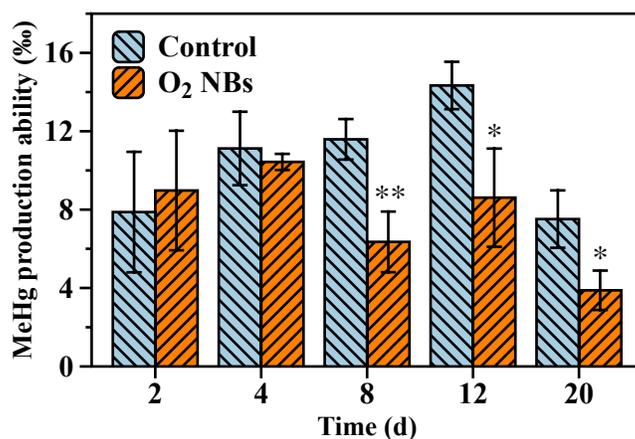
287 optimal Hg dosage for MPA analysis due to its low MeHg production. In addition,
288 differences in MeHg concentrations between the 100 mg L⁻¹ and 1000 mg L⁻¹ groups (3.4
289 times on day 2) were smaller than the difference in the adding concentrations (10 times). This
290 suggested that 100 mg L⁻¹ HgCl₂ solution might produce higher MeHg production in the
291 sediment and was most suitable for the MPA mitigation experiment. In addition, the average
292 THg concentration in the sediment (from day 1 to 7) of the 10 mg L⁻¹ group (852.46 ng g⁻¹)
293 was approximately 1/9 of the 100 mg L⁻¹ group (7519.36 ng g⁻¹) and 1/28 of the 1000 mg
294 L⁻¹ group (23686.35 ng g⁻¹) (**Fig. 2B**), which might be related to the adsorption capacity of
295 Hg on the sediment (Ikingura and Akagi, 1999).

296 In general, there would be spontaneous MeHg production in the Hg-polluted eutrophic
297 waters, and the sediment was the major Hg sink in the system. Moreover, the MPA in the
298 sediment would be analyzed with the production of MeHg within 48 h after the addition of 1
299 mg L⁻¹ HgCl₂ in the sediment-water slurry.

300 **3.3 Variations of MPAs in the surface sediment**

301 Considering that surface sediment in aquatic system has been reported to be the hot spot
302 for Hg methylation (Ullrich et al., 2001), the differences in MPA of surface sediment between
303 the Control and O₂ NBs groups throughout the 20-d incubation period were analyzed and
304 illustrated in **Fig. 3**. The two groups were designed to examine the mitigation effects of
305 interfacial O₂ nanobubbles on the MPA of surface sediment (**Fig. 1B**). Generally, the MPAs
306 in the O₂ NBs group (2.8–12.5‰) were lower than those in the Control group (4.7–15.5‰),
307 particularly from the medium term of the incubation. During the first four days, there was no
308 significant difference in the MPA between the two groups. However, from day 8 to 20, the

309 MPA in the O₂ NBs group decreased significantly compared to the Control group, with the
 310 decrements being 45%, 40%, and 48% on days 8, 12, and 20 ($p < 0.01$, 0.05, and 0.05),
 311 respectively. Moreover, in the Control group, the MPA reached its peak of $14.3 \pm 1.2\%$ on
 312 day 12. This indicated that massive MeHg production in surface sediment would appear
 313 around 12 days after the outbreak of Hg pollution in the eutrophic waters, and that requires
 314 particular concern. While in the O₂ NBs group, the peak of MPA ($10.4 \pm 0.4\%$) appeared on
 315 day 4 and decreased significantly ($p < 0.05$ on day 8 and $p < 0.01$ on day 20) hereafter.



316
 317 **Fig. 3.** Comparison of MeHg production ability of surface sediment from columns with (O₂
 318 NBs) and without (Control) O₂ nanobubbles during the 20-day experiment. The column
 319 experiments and Hg methylation experiments were both performed in duplicate, and the data
 320 was shown by mean \pm SD, $n = 4$. "*" indicates that the significant difference in average MPA
 321 between the Control and O₂ NBs groups is $p < 0.05$; "**" indicates the significant difference
 322 is $p < 0.01$.

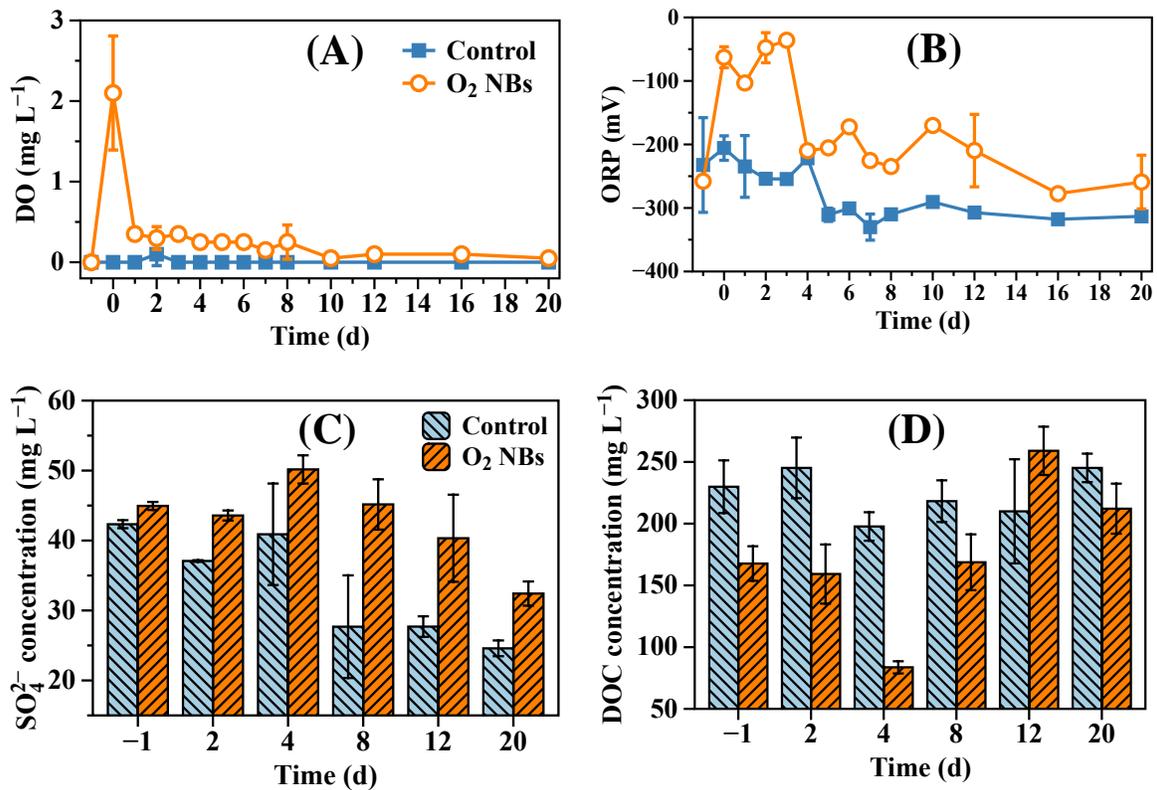
323 As MeHg production in surface sediment was mainly microbially mediated (Yu et al.,
 324 2012), the variations of environmental factors related to the Hg microbial methylator
 325 activities, such as redox conditions and organic matter, might contribute to the reduction

326 effects on MPA by O₂ nanobubbles. Therefore, variations of such factors were analyzed and
327 illustrated in the overlying water (**Fig. 4**) and surface sediment (**Fig. 5**) from the two groups.

328 **3.3.1 Variations of DO, ORP, SO₄²⁻, and DOC in the overlying water**

329 As illustrated in **Fig. 4**, concentrations of DO, ORP, SO₄²⁻, and DOC in the overlying
330 water between the two groups were significantly different during the 20-d incubation period.

331 Among these factors, DO, ORP, and SO₄²⁻ have been suggested to **illustrate** the redox
332 conditions in the overlying water (Duvil et al., 2018; Zhang et al., 2018). First of all, as shown
333 in **Fig. 4A**, the DO concentrations in the overlying water from the O₂ NBs group were
334 significantly higher than those in the Control group throughout the incubation ($p < 0.01$).
335 Specifically, the DO concentrations in both groups began from 0 on day -1, and those in the
336 Control group maintained the level till day 20. However, after the addition of O₂ nanobubbles
337 on day 0, **DO concentrations** in the O₂ NBs group displayed a sudden increase and reached
338 up to 2.1 mg L⁻¹. Even though the DO concentrations in the O₂ NBs group then decreased
339 from day 1, the significant elevation ($p < 0.01$) from the Control group remained until day 8.



340 **Fig. 4.** Concentrations of (A) DO; (B) ORP; (C) SO₄²⁻; and (D) DOC in the overlying water
 341 from columns with (O₂ NBs) and without (Control) O₂ nanobubbles. Day -1 represented the
 342 content before the addition of O₂ nanobubbles. The column experiments were performed in
 343 duplicate and the data was shown by mean ± SD, n = 2.

344 The variations of ORP at the sediment-water interface from both groups are illustrated
 345 in **Fig. 4B**. Throughout the incubation period, the ORP in the Control group was in the range
 346 of -344.7 to -192.0 mV, with the average being -280.9 mV, which might reveal the anoxia
 347 caused by the decomposition of the dead algae. However, the addition of O₂ nanobubbles
 348 significantly ($p < 0.001$) increased the ORP throughout the incubation (in the range of -289.1
 349 to -30.5 mV). From day -1 to day 0, the average ORP in the O₂ NBs group was elevated
 350 from -257.9 to -62.8 mV, by a ratio of 76%. The ORP in the O₂ NBs group was significantly

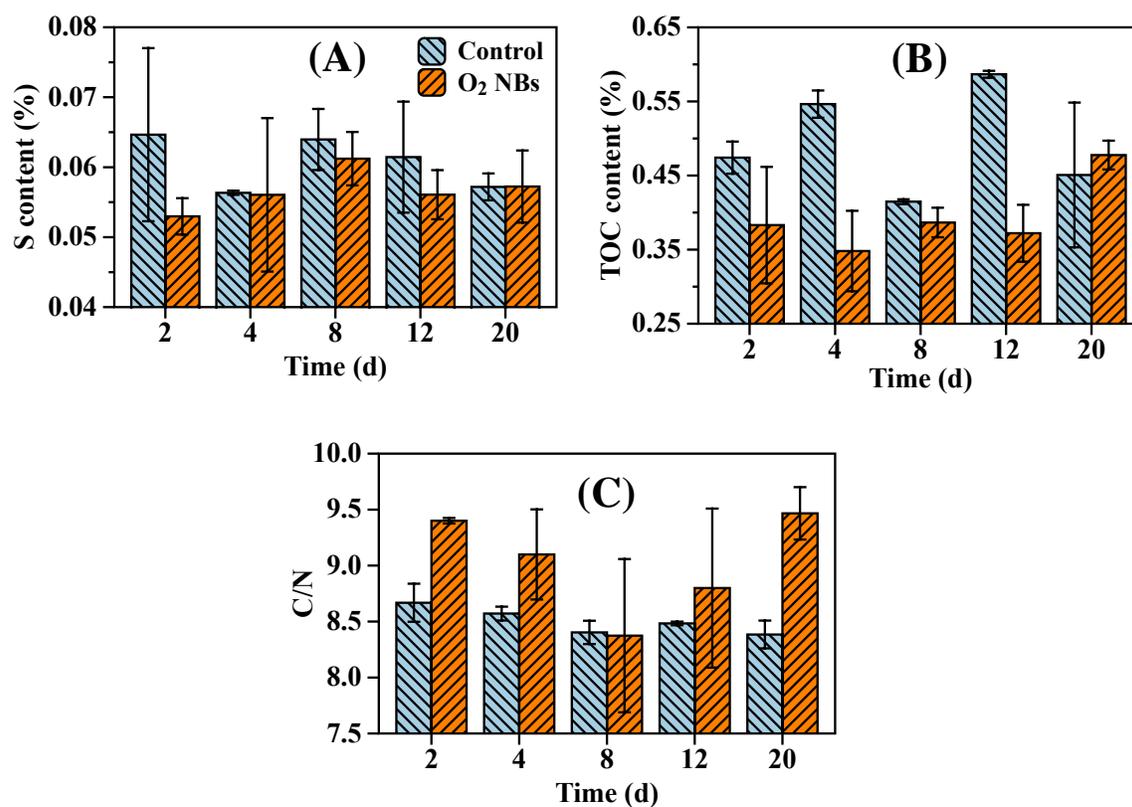
351 higher than that in the Control group from day 0 to 3, with the differences being 69%, 56%,
352 81%, and 86% on each day. From then on to day 20, the gap between the two groups
353 narrowed, but the ORP in the O₂ NBs group was still beyond the Control group.

354 **Fig. 4C** illustrates the comparison result of SO₄²⁻ concentrations in the overlying water
355 between the Control and O₂ NBs groups. The average SO₄²⁻ concentration in all the columns
356 on day -1 was 49.64 ± 6.42 mg L⁻¹. Since the beginning of the experiment, the SO₄²⁻
357 concentrations in the O₂ NBs group (31.20–51.59 mg L⁻¹) were significantly ($p < 0.001$)
358 elevated from to the Control group (22.50–46.03 mg L⁻¹), with the maximum increment
359 being 63% on day 8. The increase of SO₄²⁻ concentrations in the O₂ NBs group corresponded
360 with the increase of DO and ORP content, and all could reveal the enhanced oxidative
361 condition in the columns.

362 Moreover, as shown in **Fig. 4D**, the variation of DOC concentrations in the overlying
363 water after the addition of O₂ nanobubbles was the opposite of DO, ORP, and SO₄²⁻. In
364 general, the DOC concentrations in the O₂ NBs group were significantly lower than the
365 Control group ($p < 0.05$). Compared with the content in Taihu Lake water (33.39 ± 0.30 mg
366 L⁻¹, **Table 1**), the DOC concentrations in the Control and O₂ NBs groups increased
367 significantly on day -1, to 229.88 and 167.70 mg L⁻¹ (by 5.9 and 4.0 times), respectively.
368 On days 2 and 4, the DOC concentrations in the O₂ NBs group decreased significantly ($p <$
369 0.01) compared with the Control group, with the decrements being 35% and 58% respectively.
370 As the DOC content could represent the content of dissolved organic matter (DOM), the
371 decrease in the DOC concentrations in the O₂ NBs group could **indicate a** reduction of DOM
372 in the overlying water.

373 3.3.2 Variations of S, TOC, and ratio of C and N (C/N) in the surface sediment

374 The variations of factors in the surface sediment were likely to bring direct influences
375 on MPA. Thus, relative factors affecting MeHg production, including redox conditions and
376 organic matter content in surface sediment, were analyzed as well. The content of S, TOC,
377 and ratios of C and N (C/N) in the surface sediment is illustrated in **Fig. 5**.



378 **Fig. 5.** Comparison of (A) S content, (B) TOC content, and (C) ratio of C and N content in
379 the surface sediment from columns with (O₂ NBs) and without (Control) O₂ nanobubbles.
380 The column experiments were performed in duplicate and the data was shown by mean ± SD,
381 n = 2.

382 Sulfur content has been suggested to reflect the redox conditions in the sediment and
383 might be influenced by changes in the valence of sulfide and activities of sulfate-reducing

384 bacteria there (Zhu et al., 2017; Gilmour et al., 2011). Accordingly, the S content in the
385 surface sediment from the Control and O₂ NBs groups was analyzed throughout the
386 incubation period. As illustrated in **Fig. 5A**, the S content in both groups varied slightly
387 during the incubation and was similar with the content in the Taihu Lake sediment (**Table 1**).
388 Compared with that in the Control group, the S content in the O₂ NBs group was generally
389 lower, with the largest difference being 18% on day 2. These results might partially suggest
390 the oxidation by O₂ nanobubbles on surface sediment.

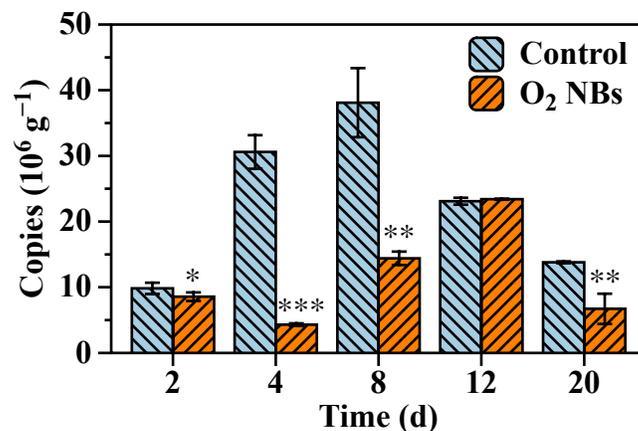
391 In addition, the TOC content could reflect the content of organic matter in surface
392 sediment. As shown in **Fig. 5B**, the TOC concentrations in the O₂ NBs group were
393 significantly lower than the Control group during the incubation ($p < 0.05$). The decrement
394 between the two groups reached its peak by 37% on day 12 (59% and 37% in the Control
395 and O₂ NBs groups, respectively). The TOC content in the surface sediment from the Control
396 group reached its maximum on day 12, well consistent with the variation of MPA. Moreover,
397 the ratio of C and N content could reflect the origin and decomposition of organic matter in
398 the sediment. Compared with the Control group, O₂ nanobubbles significantly increased the
399 ratios of C/N in the surface sediment during the incubation ($p < 0.001$). By comparing the C
400 and N content in the two groups (**Fig. S3**), we discovered that the N content in the O₂ NBs
401 group was generally lower than the Control group from day 2 to 12 with an average decrease
402 by 9.2%. On days 12 and 20, C content in the O₂ NBs group increased by 2.6% and 26.7%
403 on either day.

404 To sum up, after the addition of interfacial O₂ nanobubbles, the MPA of surface
405 sediment decreased significantly during the incubation period. Meanwhile, in the overlying

406 water, O₂ nanobubbles led to the elevation of DO, ORP, and SO₄²⁻ and the decline of DOC.
407 In the surface sediment, the content of S and TOC decreased significantly after the addition
408 of O₂ nanobubbles while the C/N ratios increased conversely. Variations of these factors
409 could contribute to the reduction of MPA, as will be discussed later.

410 3.4 *hgcA* abundances in the surface sediment

411 The *hgcA* gene was reported to indicate the abundances of Hg microbial methylators
412 (Liu et al., 2018b). The variations of *hgcA* abundances might help explain the reduction of
413 MPA after the addition of O₂ nanobubbles from the perspective of microbiology.
414 Accordingly, in the MPA mitigation experiment, abundances of *hgcA* gene in the surface
415 sediment from the Control and O₂ NBs groups were analyzed and illustrated in **Fig. 6**.



416
417 **Fig. 6.** Variation of *hgcA* gene abundance in the surface sediment from columns with (O₂
418 NBs) and without (Control) O₂ nanobubbles. The symbol of "*" represented $p < 0.05$, "***"
419 represented $p < 0.01$, and "****" represented $p < 0.001$.

420 As shown in the figure, the *hgcA* abundances decreased significantly after the addition
421 of O₂ nanobubbles ($p < 0.01$). In the Control and O₂ NBs groups, the *hgcA* abundances were

422 in the range of 9.23–42.67 and 7.04–23.41 $\times 10^6$ copies g^{-1} , respectively. The largest decrease
423 between the two groups was 86% on day 4, from 30.60 $\times 10^6$ to 4.30 $\times 10^6$ copies g^{-1} .
424 Specifically, abundance of *hgcA* in the surface sediment from the Control group reached its
425 peak at 3.81 $\times 10^7$ copies g^{-1} on day 8. The peak appeared slightly ahead of the peak of MPA
426 on day 12 in the Control group (**Fig. 2**). While in the O₂ NBs group, the *hgcA* abundance
427 reached its maximum (23.40 $\times 10^6$ copies g^{-1}) on day 12. The decline in the O₂ NBs group
428 suggested that O₂ nanobubbles might be capable of reducing Hg microbial methylator
429 densities in the surface sediment of eutrophic waters, and the potential causes of the decline
430 will be discussed later.

431 **4. Discussion**

432 **4.1 Hg sink and source in the aquatic system**

433 In the Hg methylation experiment, the THg content in the sediment (**Fig. 2B**) was
434 significantly higher than that in the overlying water (**Fig. S2B**), which is consistent with
435 reported results (Wang et al., 2009). The huge difference (over three orders of magnitude)
436 between the sediment and overlying water implied that sediment could be the major Hg sink
437 if there is abrupt Hg pollution in the surface waters. It has been reported that once introduced
438 to aquatic ecosystem, Hg is primarily complexed with dissolved organic matter and then
439 diffused to the sediment layer, where the complexes might react with sulfide and form β -HgS
440 nanoparticles (Slowey, 2010). Moreover, the effective binding of Hg and particles in
441 sediment might restrain the migration of Hg into overlying water (Stein et al., 1996). This
442 could help explain the difference in THg content between the sediment and overlying water

443 **in this work.** Indeed, high THg content (up to 2010 $\mu\text{g g}^{-1}$) has also been observed in the
444 sediment after massive Hg-polluted effluent was discharged in Minamata Bay from 1932 to
445 1968. In addition, the retention time of Hg in sediment was reported to be quite long. At the
446 time of the dredging operation in 1990, Hg content in the sediment was still as high as 25 μg
447 g^{-1} (Hachiya, 2012).

448 Apart from that, due to hydraulic exchanging at the sediment-water interface, Hg could
449 also be released into overlying water, which made sediment an important Hg source as well
450 (Hester et al., 2009). MeHg has been reported to be the main Hg species entering overlying
451 water from sediments in a bay impacted by Hg discharges (Gill et al., 1999). Considering the
452 much higher content of THg and organic matter in sediment, MeHg production is
453 predominant in the sediment rather than in the overlying water (Qiu et al., 2005; Compeau
454 and Bartha, 1985). Subsequently, MeHg might enter food web via pore water but without
455 substantial photodegradation in deep water (Balogh et al., 2015). In particular, for shallow
456 lakes, the sediment-water exchange has been suggested to remarkably influence the
457 distribution of Hg and MeHg in the overlying water (Choe et al., 2004). Therefore, even
458 though sediment could take up a majority of exogenous Hg once Hg-polluted wastewater
459 was discharged, it could still release a fair **amount** of MeHg into the overlying water and
460 cause adverse effects on it.

461 **4.2 Effects of interfacial oxygen nanobubbles**

462 **In the MPA mitigation experiment, the MPA of surface sediment decreased significantly**
463 **after the addition of O₂ nanobubbles, as shown in Fig. 3. This agreed well with the reported**
464 **results that MeHg content in sediment was lower under aerobic (bubbled with air) than in**

465 anaerobic conditions (bubbled with nitrogen) (Duvil et al., 2018). Hg methylation in natural
466 waters has been reported to be predominantly mediated by microorganisms (Jiang et al.,
467 2018). Therefore, the variations of Hg microbial methylators could largely explain the
468 variations of MPA. The addition of O₂ nanobubbles might induce fluctuations of relative
469 factors in the overlying water and surface sediment. This would trigger changes in activities
470 of Hg microbial methylators, which may be illustrated by the variation of *hgcA* abundances.
471 As a result, the MPA of surface sediment would vary after the addition of O₂ nanobubbles.
472 This might help explain the positive correlation between MPA and *hgcA* abundance (SI, Fig.
473 S4A). Moreover, different environmental factors might induce different effects on Hg
474 microbial methylator activities. Relative factors in the sediment-water columns could be
475 divided into two categories, one was factors regarding redox conditions, and the other was
476 relative to organic matter.

477 **4.2.1 Enhancement of oxidative conditions**

478 The content of DO, ORP, and SO₄²⁻ in the overlying water and S in surface sediment
479 could reflect the variation of redox conditions in the sediment-water columns. Apart from the
480 obviously elevated DO and ORP after the addition of O₂ nanobubbles, the SO₄²⁻
481 concentrations in the overlying water were significantly increased as well. In addition,
482 sulfides (such as FeS and FeS₂) in the sediment might be oxidized to SO₄²⁻, which was likely
483 to enter overlying water (Zhu et al., 2017; Schippers and Jørgensen, 2002). To some extent,
484 the decrease in S content in the O₂ NBs group might reveal the oxidation process in the
485 surface sediment by O₂ nanobubbles. In addition, the *hgcA* abundance was found to be
486 negatively correlated with DO, ORP, and SO₄²⁻ and positively correlated with S (SI, Fig.

487 **S4B–E**). It has been reported that aerobic conditions may inhibit the growth of Hg microbial
488 methylators, and that biotic Hg methylation mainly occurs in the anaerobic conditions
489 (Regnell and Tunlid, 1991). Take sulfate-reducing bacteria for instance, high content of DO
490 (over 1 mg L⁻¹) and ORP (over -100 mV) has been proved to reduce the growth of them
491 (Hao et al., 1996). Accordingly, the significantly elevated content of DO and ORP in the O₂
492 NBs group was suggested to inhibit the growth of sulfate-reducing bacteria, which might
493 limit their abilities to produce MeHg. Apart from that, Hg methylation was reported to be
494 accompanied by the reduction of SO₄²⁻ by sulfate-reducing bacteria (Yu et al., 2012).
495 Therefore, the increase of SO₄²⁻ concentrations in the overlying water and decrease of S
496 content in the surface sediment might also reveal the reduction of sulfate-reducing bacteria's
497 activities after the addition of O₂ nanobubbles.

498 **4.2.2. Reduction of organic matter**

499 Another important factor that could regulate MeHg production is organic matter. The
500 concentrations of DOC in the overlying water and TOC in surface sediment both decreased
501 significantly after the addition of O₂ nanobubbles. In addition, there was an increase in the
502 ratios of C/N in the surface sediment of the O₂ NBs group.

503 **It has been proposed that organic matter can facilitate Hg methylation by manipulating**
504 **activities of Hg microbial methylators** (Drott et al., 2007). In this study, the decrease of DOC
505 and TOC content after the addition of O₂ nanobubbles might **indicate a** reduction of organic
506 matter. In particular, organic matter with lower C/N, usually originated from fresh
507 chlorophyll, has been suggested to be highly labile to Hg microbial methylators (Bravo et al.,
508 2017). The C/N ratio in the O₂ NBs group was comparatively higher than that in the Control

509 group, which suggested the decrease of labile organic matter. The decrease of labile organic
510 matter might be related to the enhanced mineralization in the oxidative condition (Olson and
511 Barbier, 1994), **which** could be induced by O₂ nanobubbles. In addition, in this study,
512 interfacial O₂ nanobubbles were loaded on natural zeolite, which is a common capping
513 material in surface waters. By blocking pollutants from entering the overlying water, O₂
514 nanobubble-loaded zeolites were capable of reducing microbial substrates like organic matter
515 from entering the overlying water; this could also **contribute** to the decrease of DOC
516 concentrations in the overlying water. These results might help explain the correlations
517 between *hgcA* and DOC, TOC, and C/N (SI, **Fig. S4F-H**).

518 As a result, O₂ nanobubbles could **lead to** the enhanced oxidative condition and
519 reduction of labile organic matter, and contribute to the inhibition of Hg microbial
520 methylators. This could in turn lead to the decrease of MeHg production abilities in the
521 surface sediment.

522 Still it is likely that MPA analysis using the surface sediment sampled from the columns
523 might not perfectly reflect *in situ* MeHg production. But since Hg methylation was reported
524 to be mainly mediated by microorganisms, and variations of factors in the overlying water
525 and surface sediment might affect microbial activities, MeHg production abilities could
526 therefore be **altered**.

527 **5. Conclusions**

528 In this work, eutrophic waters were demonstrated to be able to spontaneously produce
529 MeHg if severe Hg pollution occurs, and **Hg were mainly buried in the sediment**. The

530 technology of interfacial oxygen nanobubbles proved to significantly reduce MeHg
531 production abilities of the surface sediment. The alleviation of anoxia and reduction of
532 organic matter induced by O₂ nanobubbles could contribute to the decrease of MPA.
533 Moreover, the abundance of Hg microbial methylators was suggested to decrease
534 significantly after the addition of O₂ nanobubbles. Considering the potentially enhanced
535 MeHg production at the surface sediment of eutrophic waters due to the serious
536 hypoxia/anoxia and organic matter accumulation, this study proposed a promising strategy
537 for MeHg production ability remediation in case Hg pollution occurs.

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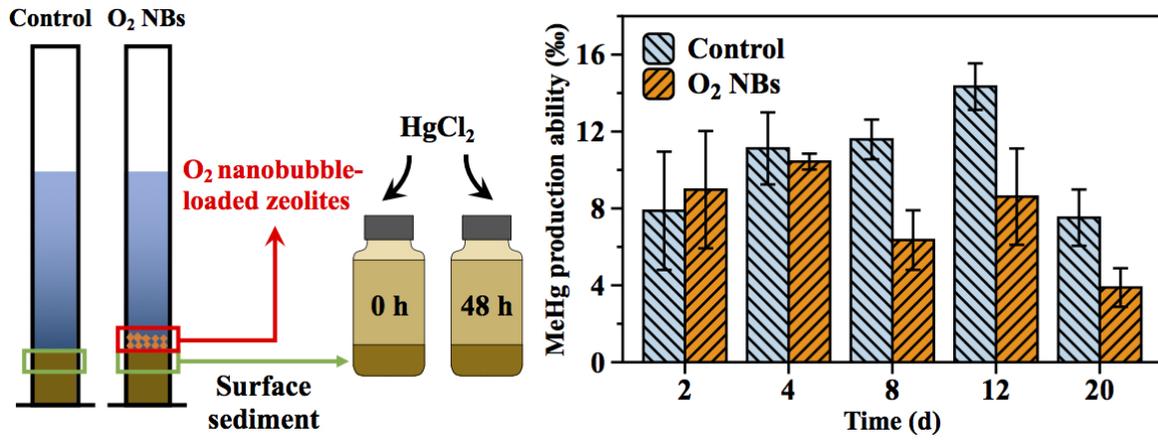
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723 **Graphical abstract**



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