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2 Benzyldimethyldodecyl Ammonium Chloride Shifts the Proliferation of Functional

3 Genes and Microbial Community in Natural Water from Eutrophic Lake

4

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6

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10

11

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12 **Abstract**

13 Benzylalkyldimethylethyl ammonium compounds are pervasive in natural
14 environments and toxic at high concentrations. The changes in functional genes and
15 microbial diversity in eutrophic lake samples exposed to benzyldimethyldodecyl
16 ammonium chloride (BAC) were assessed. BAC exerted negative effects on bacteria
17 abundance, particularly at concentrations of 100 $\mu\text{g L}^{-1}$ and higher. A significant
18 increase in the number of the quaternary ammonium compound-resistant gene *qacA/B*
19 was recorded within the 10 $\mu\text{g L}^{-1}$ treatment after the first day of exposure. Not all
20 antibiotic resistance genes increased in abundance as the concentrations of BAC
21 increased; rather, gene abundances were dependent on the gene type, concentrations
22 of BAC, and contact time. The nitrogen fixation-related gene *nifH* and ammonia
23 monooxygenase gene *amoA* were inhibited by high concentrations of BAC after the
24 first day, whereas an increase of the nitrite reductase gene *nirK* was stimulated by
25 exposure. Microbial communities within higher treatment levels (1 000 and 10 000 μg
26 L^{-1}) exhibited significantly different community composition compared to other
27 treatment levels and the control. Selective enrichment of *Rheinheimera*,
28 *Pseudomonas*, and *Vogesella* were found in the higher treatment levels, suggesting
29 that these bacteria have some resistance or degradation capacity to BAC. Genes
30 related with RNA processing and modification, transcription, lipid transport and
31 metabolism, amino acid transport and metabolism, and cell motility of microbial
32 community function were involved in the process exposed to the BAC stress.

33 **Keywords:** *Cyanobacteria*; *qacEΔ1*; *nirK*; *Rheinheimera*; microbial diversity

34 **Capsule:** Shift pattern in the proliferation of functional genes and microbial
35 community in natural water from eutrophic lake exposed to BAC was assessed.
36

37 **1. Introduction**

38 Quaternary ammonium compounds (QACs) are a major class of cationic
39 surfactants in disinfectants, biocides, detergents, and dispersants used across
40 domestic, agricultural, industrial and clinical products { ADDIN EN.CITE {
41 ADDIN EN.CITE.DATA }}. Benzylalkyldimethylethyl ammonium compounds are
42 one of the most prevalent QACs in natural environments, commonly occurring as
43 effluents from wastewater treatment plants, hospitals, and laundry wastewater {
44 ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Benzylalkyldimethylethyl
45 ammonium compounds in surface water are often detected in the $\mu\text{g L}^{-1}$ level {
46 ADDIN EN.CITE

47 <EndNote><Cite><Author>Zhang</Author><Year>2015</Year><RecNum>1122</R
48 ecNum><DisplayText>(Zhang et al., 2015)</DisplayText><record><rec-
49 number>1122</rec-number><foreign-keys><key app="EN" db-
50 id="vzededvvherd97ep2db5pwr1fe5trtad95r0"
51 timestamp="1490772298">1122</key></foreign-keys><ref-type name="Journal
52 Article">17</ref-type><contributors><authors><author>Zhang,
53 Chang</author><author>Cui, Fang</author><author>Zeng, Guang-
54 ming</author><author>Jiang, Min</author><author>Yang, Zhong-
55 zhu</author><author>Yu, Zhi-gang</author><author>Zhu, Meng-
56 ying</author><author>Shen, Liu-
57 qing</author></authors></contributors><titles><title>Quaternary ammonium
58 compounds (QACs): A review on occurrence, fate and toxicity in the

59 environment</title><secondary-title>Science of The Total Environment</secondary-

60 title></titles><periodical><full-title>Science Of The Total Environment</full-

61 title><abbr-1>Sci Total Environ</abbr-1><abbr-2>Sci. Total. Environ.</abbr-

62 2></periodical><pages>352-362</pages><volume>518-

63 519</volume><keywords><keyword>Quaternary ammonium compounds

64 (QACs)</keyword><keyword>Biodegradation</keyword><keyword>Sorption</key

65 word><keyword>Toxicity</keyword><keyword>Determination</keyword></keywo

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67 dates></dates><isbn>0048-9697</isbn><urls><related-

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70 main.pdf?_tid=dc0dd0e8-1450-11e7-aa6c-

71 0000aacb35f&acdnat=1490772498_0384763c3f545e833f59411b45eefa4e</url

72 ></related-urls></urls><electronic-resource-

73 num>http://dx.doi.org/10.1016/j.scitotenv.2015.03.007</electronic-resource-

74 num></record></Cite></EndNote>}. The concentrations of benzyldimethyldodecyl

75 ammonium chloride (BAC), a type of benzylalkyldimethylethyl ammonium

76 compounds, in the surface water downstream from five wastewater treatment plants in

77 the US ranged from 2.7 to 5.8 $\mu\text{g L}^{-1}$ { ADDIN EN.CITE

78 <EndNote><Cite><Author>Ferrer</Author><Year>2001</Year><RecNum>1200</R

79 ecNum><DisplayText>(Ferrer and Furlong, 2001)</DisplayText><record><rec-

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81 id="vzededvvhherd97ep2db5pwr1fe5trtad95r0"
82 timestamp="1491895239">1200</key></foreign-keys><ref-type name="Journal
83 Article">17</ref-type><contributors><authors><author>Ferrer,
84 Imma</author><author>Furlong, Edward
85 T.</author></authors></contributors><titles><title>Identification of Alkyl
86 Dimethylbenzylammonium Surfactants in Water Samples by Solid-Phase Extraction
87 Followed by Ion Trap LC/MS and LC/MS/MS</title><secondary-title>Environmental
88 Science & Technology</secondary-title></titles><periodical><full-
89 title>Environmental Science & Technology</full-title><abbr-1>Environ Sci
90 Technol</abbr-1><abbr-2>Environ. Sci. Technol.</abbr-
91 2></periodical><pages>2583-
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97 urls></urls><electronic-resource-num>10.1021/es001742v</electronic-resource-
98 num></record></Cite></EndNote>}. The concentrations of benzylalkyldimethylethyl
99 ammonium compounds, including BAC, in Taiwanese river water were detected in the
100 range 2.5 - 65 µg L⁻¹ { ADDIN EN.CITE
101 <EndNote><Cite><Author>Ding</Author><Year>2001</Year><RecNum>1201</Re
102 cNum><DisplayText>(Ding and Liao, 2001)</DisplayText><record><rec-

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105 timestamp="1491895837">1201</key></foreign-keys><ref-type name="Journal
106 Article">17</ref-type><contributors><authors><author>Ding, W.
107 H.</author><author>Liao, Y. H.</author></authors></contributors><auth-
108 address>Natl Cent Univ, Dept Chem, Chungli 32054, Taiwan</auth-
109 address><titles><title>Determination of alkylbenzyldimethylammonium chlorides in
110 river water and sewage effluent by solid phase extraction and gas chromatography
111 mass spectrometry</title><secondary-title>Analytical Chemistry</secondary-
112 title><alt-title>Anal Chem</alt-title></titles><periodical><full-title>Analytical
113 Chemistry</full-title><abbr-1>Anal Chem</abbr-1><abbr-2>Anal. Chem.</abbr-
114 2></periodical><alt-periodical><full-title>Analytical Chemistry</full-title><abbr-
115 1>Anal Chem</abbr-1><abbr-2>Anal. Chem.</abbr-2></alt-periodical><pages>36-
116 40</pages><volume>73</volume><number>1</number><keywords><keyword>qua
117 ternary ammonium-compounds</keyword><keyword>liquid-
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125 num><urls><related-urls><url><Go to
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127 num>DOI 10.1021/ac000655i</electronic-resource-
128 num><language>English</language></record></Cite></EndNote>}. The total
129 concentration of BAC and other QACs in surface water samples from the Gdańsk
130 City in Poland were found in the range 72.5 - 342 µg L⁻¹ { ADDIN EN.CITE
131 <EndNote><Cite><Author>Olkowska</Author><Year>2013</Year><RecNum>120
132 3</RecNum><DisplayText>(Olkowska et al., 2013)</DisplayText><record><rec-
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136 Article">17</ref-type><contributors><authors><author>Olkowska,
137 Ewa</author><author>Polkowska, Żaneta</author><author>Namieśnik,
138 Jacek</author></authors></contributors><titles><title>A solid phase extraction–ion
139 chromatography with conductivity detection procedure for determining cationic
140 surfactants in surface water samples</title><secondary-title>Talanta</secondary-
141 title></titles><periodical><full-title>Talanta</full-title><abbr-1>Talanta</abbr-
142 1><abbr-2>Talanta</abbr-2></periodical><pages>210-
143 216</pages><volume>116</volume><keywords><keyword>Cationic
144 surfactants</keyword><keyword>Solid phase extraction</keyword><keyword>Ion
145 chromatography-conductivity detection</keyword><keyword>Surface water
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150 ></related-urls></urls><electronic-resource-
151 num>http://doi.org/10.1016/j.talanta.2013.04.083</electronic-resource-
152 num></record></Cite></EndNote>},

153 Benzylalkyldimethylethyl ammonium compounds could inhibit cell growth via
154 cytoplasmic membrane disruption { ADDIN EN.CITE { ADDIN EN.CITE.DATA
155 }}, so, they can be toxic to aquatic life without target organisms, such as fish {
156 ADDIN EN.CITE <EndNote><Cite><Author>Van de
157 Voorde</Author><Year>2012</Year><RecNum>1308</RecNum><DisplayText>(Va
158 n de Voorde et al., 2012)</DisplayText><record><rec-number>1308</rec-
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162 Article">17</ref-type><contributors><authors><author>Van de Voorde,
163 Antoine</author><author>Lorgeoux, Catherine</author><author>Gromaire, Marie-
164 Christine</author><author>Chebbo,
165 Ghassan</author></authors></contributors><titles><title>Analysis of quaternary
166 ammonium compounds in urban stormwater samples</title><secondary-
167 title>Environmental Pollution</secondary-title></titles><periodical><full-
168 title>Environmental Pollution</full-title><abbr-1>Environ Pollut</abbr-1><abbr-

169 2>Environ. Pollut.</abbr-2></periodical><pages>150-

170 157</pages><volume>164</volume><number>Supplement

171 C</number><keywords><keyword>Benzalkonium</keyword><keyword>Liquid

172 chromatography</keyword><keyword>Mass

173 spectrometry</keyword><keyword>Water</keyword><keyword>Particles</keyword

174 ><keyword>Stormwater</keyword></keywords><dates><year>2012</year><pub-

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178 ></related-urls></urls><electronic-resource-

179 num>https://doi.org/10.1016/j.envpol.2012.01.037</electronic-resource-

180 num></record></Cite></EndNote>}, algae { ADDIN EN.CITE { ADDIN

181 EN.CITE.DATA }} and microorganisms { ADDIN EN.CITE { ADDIN

182 EN.CITE.DATA }}. The green algae *Chlorella vulgaris* and the water flea *Daphnia*

183 *magna* are two organisms frequently used to assess the toxicity of

184 benzylalkyldimethylethyl ammonium compounds on aquatic environments { ADDIN

185 EN.CITE { ADDIN EN.CITE.DATA }}. The 48-h EC₅₀ of *Daphnia magna* after

186 exposure to BAC was recorded as 0.041 mg L⁻¹ { ADDIN EN.CITE { ADDIN

187 EN.CITE.DATA }} and the 96-h EC₅₀ of *Chlorella vulgaris* was 0.203 mg L⁻¹ {

188 ADDIN EN.CITE

189 <EndNote><Cite><Author>Zhu</Author><Year>2010</Year><RecNum>1144</Rec

190 Num><DisplayText>(Zhu et al., 2010)</DisplayText><record><rec-

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195 Menjun</author><author>Ge, Fei</author><author>Zhu,
196 Runliang</author><author>Wang, Xueye</author><author>Zheng,
197 Xiaoyan</author></authors></contributors><titles><title>A DFT-based QSAR study
198 of the toxicity of quaternary ammonium compounds on *Chlorella*
199 *vulgaris*</title><secondary-title>Chemosphere</secondary-
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201 1>Chemosphere</abbr-1><abbr-2>Chemosphere</abbr-2></periodical><pages>46-
202 52</pages><volume>80</volume><number>1</number><keywords><keyword>Qu
203 aternary ammonium
204 compounds</keyword><keyword>QSAR</keyword><keyword>Toxicity</keyword>
205 <keyword>Aquatic
206 organism</keyword><keyword>DFT</keyword><keyword>PLS</keyword></keyw
207 ords><dates><year>2010</year><pub-dates><date>6//</date></pub-
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210 ></related-urls></urls><electronic-resource-
211 num><http://dx.doi.org/10.1016/j.chemosphere.2010.03.044></electronic-resource-
212 num></record></Cite></EndNote>}. Acute effects of BAC could occur at tens to

213 hundreds of $\mu\text{g L}^{-1}$ levels for *Daphnia magna* and *Ceriodaphnia dubia*, while
 214 genotoxic effects at DNA damage level, the lowest adverse effect levels were 0.4
 215 and 4 ng L^{-1} for *D. magna* and *C. dubia*, respectively { ADDIN EN.CITE
 216 <EndNote><Cite><Author>Lavorgna</Author><Year>2016</Year><RecNum>1191
 217 </RecNum><DisplayText>(Lavorgna et al., 2016)</DisplayText><record><rec-
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 221 Article">17</ref-type><contributors><authors><author>Lavorgna,
 222 Margherita</author><author>Russo, Chiara</author><author>D'Abrosca,
 223 Brigida</author><author>Parrella, Alfredo</author><author>Isidori,
 224 Marina</author></authors></contributors><titles><title>Toxicity and genotoxicity of
 225 the quaternary ammonium compound benzalkonium chloride (BAC) using *Daphnia*
 226 *magna* and *Ceriodaphnia dubia* as model systems</title><secondary-
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 228 title>Environmental Pollution</full-title><abbr-1>Environ Pollut</abbr-1><abbr-
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 230 39</pages><volume>210</volume><keywords><keyword>Benzalkonium
 231 chloride</keyword><keyword>Cationic
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240 num></record></Cite></EndNote>}. It should be paid more attention, because these
241 effective concentrations are much lower than BAC concentrations detected in surface
242 waters.

243 Benzylalkyldimethylethyl ammonium compounds not only caused negative
244 influence on the organisms but also resulted in changes of antibiotic resistance genes
245 in engineered environment { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. The
246 enhanced selection and spread of antimicrobial genes by these compounds have been
247 regarded as a threat to human health { ADDIN EN.CITE

248 <EndNote><Cite><Author>Hegstad</Author><Year>2010</Year><RecNum>1312<
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253 Article">17</ref-type><contributors><authors><author>Hegstad,

254 K.</author><author>Langsrud, S.</author><author>Lunestad, B.

255 T.</author><author>Scheie, A. A.</author><author>Sunde,

256 M.</author><author>Yazdankhah, S. P.</author></authors></contributors><auth-

257 address>Department of Microbiology and Infection Control, Reference Centre for
258 Detection of Antimicrobial Resistance, University Hospital of North Norway, Tromsø,
259 Norway. kristin.hegstad@uit.no</auth-address><titles><title>Does the wide use of
260 quaternary ammonium compounds enhance the selection and spread of antimicrobial
261 resistance and thus threaten our health?</title><secondary-title>Microb Drug
262 Resist</secondary-title><alt-title>Microbial drug resistance (Larchmont, N.Y.)</alt-
263 title></titles><periodical><full-title>Microbial Drug Resistance</full-title><abbr-
264 1>Microb Drug Resist</abbr-1><abbr-2>Microb. Drug Resist.</abbr-
265 2></periodical><pages>91-
266 104</pages><volume>16</volume><number>2</number><edition>2010/04/08</edi-
267 tion><keywords><keyword>Animals</keyword><keyword>Anti-Bacterial
268 Agents/*pharmacology</keyword><keyword>Bacteria/*drug
269 effects</keyword><keyword>*Drug Resistance,
270 Bacterial</keyword><keyword>Humans</keyword><keyword>Industrial
271 Microbiology</keyword><keyword>Microbial Sensitivity
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276 num>20370507</accession-num><urls></urls><electronic-resource-
277 num>10.1089/mdr.2009.0120</electronic-resource-num><remote-database-
278 provider>NLM</remote-database-

279 provider<<language>eng</language></record></Cite></EndNote>}. Besides,
280 denitrification process was also inhibited by BAC in engineered environment {
281 ADDIN EN.CITE
282 <EndNote><Cite><Author>Hajaya</Author><Year>2012</Year><RecNum>1321</
283 RecNum><DisplayText>(Hajaya and Pavlostathis,
284 2012)</DisplayText><record><rec-number>1321</rec-number><foreign-keys><key
285 app="EN" db-id="vzededvvhherd97ep2db5pwr1fe5trtad95r0"
286 timestamp="1511820333">1321</key></foreign-keys><ref-type name="Journal
287 Article">17</ref-type><contributors><authors><author>Hajaya, Malek
288 G.</author><author>Pavlostathis, Spyros
289 G.</author></authors></contributors><titles><title>Fate and effect of benzalkonium
290 chlorides in a continuous-flow biological nitrogen removal system treating poultry
291 processing wastewater</title><secondary-title>Bioresource Technology</secondary-
292 title></titles><periodical><full-title>Bioresource Technology</full-title><abbr-
293 1>Bioresource Technol</abbr-1><abbr-2>Bioresource. Technol.</abbr-
294 2></periodical><pages>73-
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296 C</number><keywords><keyword>Benzalkonium
297 chlorides</keyword><keyword>Biological nitrogen
298 removal</keyword><keyword>Nitrification</keyword><keyword>Denitrification</k
299 eyword><keyword>Quaternary ammonium
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304 ></related-urls></urls><electronic-resource-
305 num><https://doi.org/10.1016/j.biortech.2012.05.050></electronic-resource-
306 num></record></Cite></EndNote>}. However, few studies have focused on the effect
307 of benzylalkyldimethylethyl ammonium compounds on microbial diversity and
308 nitrogen cycling genes in natural aquatic environments.

309 In this study, microcosm tests were constructed from water samples collected
310 from Nanhu Lake, a eutrophic lake in the city of Wuhan, Hubei Province in Central
311 China. In this study, we hypothesized that: (1) BAC influenced the abundance of
312 bacteria, including *Cyanobacteria*, in the eutrophic lake; (2) BAC affected the spread
313 of quaternary ammonium compound-resistant genes and antibiotic resistance genes;
314 (3) BAC may influence abundances of *amoA*, *nifH* and *nirK* and affect the nitrogen
315 cycle; and (4) microbial diversity and community composition adapted to BAC
316 depended on the dose of BAC and contact time. The information provided in this
317 study will be beneficial to understand the effects of BAC on aquatic microbial
318 ecosystem in the natural lake environments.

319 **2. Materials and Methods**

320 **2.1 Materials and setup of freshwater microcosm preparation**

321 To assess the influence of BAC on freshwater microcosms, we followed the
322 research protocol outlined in previous study about effect of ionic liquid on the

323 proliferation of antibiotic resistance genes { ADDIN EN.CITE

324 <EndNote><Cite><Author>Luo</Author><Year>2014</Year><RecNum>428</Rec

325 Num><DisplayText>(Luo et al., 2014)</DisplayText><record><rec-

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330 Yi</author><author>Wang, Qing</author><author>Lu, Qian</author><author>Mu,

331 Quanhua</author><author>Mao,

332 Daqing</author></authors></contributors><titles><title>An Ionic Liquid Facilitates

333 the Proliferation of Antibiotic Resistance Genes Mediated by Class I

334 Integrons</title><secondary-title>Environmental Science & Technology

335 Letters</secondary-title></titles><periodical><full-title>Environmental Science

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337 2></periodical><pages>266-

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339 <pub-dates><date>2014/05/13</date></pub-dates></dates><publisher>American

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341 urls><url>http://dx.doi.org/10.1021/ez500103v</url><url>http://pubs.acs.org/doi/abs/

342 10.1021/ez500103v</url></related-urls></urls><electronic-resource-

343 num>10.1021/ez500103v</electronic-resource-num><access-

344 date>2014/07/24</access-date></record></Cite></EndNote>}. Briefly, water samples

345 were collected from different parts of the eutrophic Nanhu Lake in Wuhan in
346 September 2016, and mixed to get homogenous freshwater microcosms. To remove
347 sediment and collect the supernatant, the water samples were placed in a refrigerator
348 set at 4°C and left undisturbed for 3 h. The water quality of the supernatant was
349 recorded as: pH 7.95, dissolved oxygen 10.38 mg L⁻¹, total nitrogen 11.35 mg L⁻¹ and
350 total phosphorus 0.59 mg L⁻¹. The freshwater microcosms were set up in triplicate in 1
351 L bottles containing 800 mL of freshwater. BAC (CAS No. 139-07-1) with 99%
352 purity was purchased from Sigma-Aldrich Co. LLC. The freshwater microcosms were
353 spiked with BAC at nominal concentrations of 0, 10, 100, 1000 and 10 000 µg L⁻¹. All
354 experiments were carried out outdoors over an interval of seven sequential days
355 without rain (starting on September 5, 2016) to simulate natural exposure. The bottles
356 were open to the environment and the volumes were adjusted every two days with
357 sterilized deionized water.

358 **2.2 DNA extraction**

359 The water samples (800 mL) were pre-filtered through GF/A filters (1.6 µm,
360 Whatman) and collected on polyvinylidene fluoride (PVDF) membrane filters (0.22
361 µm, Millipore) using a vacuum pump. The total genomic DNA was extracted using
362 E.N.Z.A Water DNA Kit (Omega, USA) and purified by using the GeneClean Spin
363 Kit (QBiogene, Carlsbad, CA) as described by the manufacturer. The quality and
364 concentration of DNA was evaluated by 1% agarose gel electrophoresis and
365 spectrophotometer analysis at 260 nm (NanoDrop ND-2000c, Thermo, USA).

366 **2.3 Quantification of bacteria and functional genes by real-time PCR**

367 Real-time polymerase chain reactions (qPCRs) were performed to determine the
368 total abundance of bacteria (i.e., number of 16S rRNA gene sequences) and functional
369 genes, including quaternary ammonium compound-resistant genes, nitrogen-cycling
370 genes, and antibiotic resistance genes, present in the water samples. Plasmids with
371 targeted genes were constructed as the standards according to the methods of previous
372 literatures { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Twenty microliters of
373 the reaction mixture of qPCR were prepared and carried out in a 96 well plate by the
374 7500 Fast real-time PCR system (PE Applied Biosystem, USA) according to the
375 manual and protocol of the 7500 Fast real-time PCR system
376 ([https://www.thermofisher.com/order/catalog/product/4351107?SID=srch-srp-](https://www.thermofisher.com/order/catalog/product/4351107?SID=srch-srp-4351107)
377 [4351107](https://www.thermofisher.com/order/catalog/product/4351107?SID=srch-srp-4351107)). The primers and cycle conditions of total bacteria and functional genes
378 were shown in Table S1 (See supplementary materials). Melting curve analysis was
379 applied to check the purity of the amplified products and performed for temperatures
380 ranging from 60 to 95°C. The abundances of total bacteria and functional genes were
381 calculated by comparing the threshold cycle (C_t) values of each sample with the
382 standard curve.

383 **2.4 16S rRNA gene sequence analysis**

384 The genomic DNA extracts served as a template for the PCR amplification of the
385 V2-V4 region of 16S rRNA using the primer set 338F/806R (5'-
386 ACTCCTACGGGAGGCAGCAG-3' and 5'- GGACTACHVGGGTWTCTAAT-3') {
387 ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. The amplified DNA was subjected
388 to agarose gel electrophoresis and purified using AxyPrep DNA Gel Extraction Kit

389 (Axygen Biosciences, Union City, CA, USA). A mixture of the amplicons was then
 390 used for sequencing on an Illumina MiSeq platform according to the standard
 391 protocols at Majorbio Bioinformatics Technology Co., Ltd. (Shanghai, China). In
 392 brief, raw fastq files were quality-filtered by Trimmomatic and merged by FLASH
 393 with the following criteria: (i) The reads were truncated at any site receiving an
 394 average quality score <20 over a 50 bp sliding window. (ii) Sequences whose overlap
 395 being longer than 10 bp were merged according to their overlap with mismatch no
 396 more than 2 bp. (iii) Sequences of each sample were separated according to barcodes
 397 (exactly matching) and primers (allowing 2 nucleotide mismatching), and reads
 398 containing ambiguous bases were removed. Operational taxonomic units (OTUs)
 399 were clustered with 97% similarity cutoff using UPARSE (version 7.1
 400 <http://drive5.com/uparse/>) with a novel ‘greedy’ algorithm that performs chimera
 401 filtering and OTU clustering simultaneously { ADDIN EN.CITE
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 403 ecNum><DisplayText>(Edgar, 2013)</DisplayText><record><rec-
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 408 C.</author></authors></contributors><titles><title>UPARSE: highly accurate OTU
 409 sequences from microbial amplicon reads</title><secondary-title>Nature
 410 Methods</secondary-title><alt-title>Nat Methods</alt-title></titles><alt-

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418 num>10.1038/Nmeth.2604</electronic-resource-
419 num><language>English</language></record></Cite></EndNote>}. The taxonomy
420 of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm
421 (<http://rdp.cme.msu.edu/>) against the Silva (Release128 <http://www.arb-silva.de>) 16S
422 rRNA database using confidence threshold of 70%. RDP classifier work was done
423 within the QIIME environment.

424

425 **2.5 Statistical analysis**

426 The richness and diversity of the bacterial communities within each treatment
427 were calculated with the Chao1 richness index and Shannon diversity index within
428 Mothur software, respectively { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}.
429 Differences in the number of functional genes within treatments were measured with a
430 series of one-way analysis of variance (ANOVA). Heatmap of tested functional genes
431 and microbial composition in surface water and Wilcoxon rank-sum test were
432 performed by ‘gplots’ and ‘clusrank’ packages using R v. 3.2.5 (R Foundation for

433 Statistical Computing, Vienna, Austria), respectively. The Pearson correlation analysis
434 between different genes and microbial community composition was carried out using
435 SPSS software (IBM Co., USA) and showed by Gephi software v. 0.9.1(Gephi,
436 WebAtlas, France) { ADDIN EN.CITE
437 <EndNote><Cite><Author>Bastian</Author><Year>2009</Year><RecNum>961</R
438 ecNum><DisplayText>(Bastian et al., 2009)</DisplayText><record><rec-
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441 timestamp="1503503495">961</key></foreign-keys><ref-type name="Journal
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443 Mathieu</author><author>Heymann, Sebastien</author><author>Jacomy,
444 Mathieu</author></authors></contributors><titles><title>Gephi: an open source
445 software for exploring and manipulating networks</title><secondary-
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447 title></periodical><pages>361-
448 362</pages><volume>8</volume><dates><year>2009</year></dates><urls></urls>
449 </record></Cite></EndNote>}. Functional profiling of microbial communities based
450 on 16S rRNA data was carried out using PICRUSt software { ADDIN EN.CITE {
451 ADDIN EN.CITE.DATA }}.

452 **3. Results**

453 **3.1 Effect of benzyldimethyldodecyl ammonium chloride on amount of total**

454 **bacteria**

455 The number of 16S rRNA copies in natural water decreased with the presence of
456 BAC, even at low amounts (e.g., 10 $\mu\text{g L}^{-1}$) (Fig. 1). The numbers of 16S rRNA
457 copies for 100, 1000, 10 000 $\mu\text{g L}^{-1}$ of BAC on the first day were $1.50 \pm 0.04 \times 10^9$,
458 $1.49 \pm 0.13 \times 10^9$, and $1.26 \pm 0.04 \times 10^9$ copies per liter, which were significant lower
459 than that of control with value of $2.06 \pm 0.14 \times 10^9$ copies per liter ($p < 0.05$). The
460 numbers of 16S rRNA copies for all the treatments decreased from first day to the
461 seventh day. There were no significant differences between the numbers of 16S rRNA
462 copies with the 100, 1000, or 10 000 $\mu\text{g L}^{-1}$ treatments, but the numbers of 16S rRNA
463 copies within these treatments were lower than that of control ($p < 0.05$). Based on
464 these results, the presence of BAC clearly exerted a negative effect on bacteria
465 growth, especially at concentrations of higher than 100 $\mu\text{g L}^{-1}$.

466 **3.2 Effect of benzyldimethyldodecyl ammonium chloride on abundance of** 467 **functional genes**

468 The exposure time and concentrations of BAC influenced the abundances of
469 most functional genes at different significant levels ($p < 0.05$, $p < 0.01$, and $p < 0.001$),
470 except the effect of time, and interaction effects of time and concentrations of BAC on
471 the proliferation of *tetM* and *qnrD* (Table 1).

472 **3.2.1 Quaternary ammonium compound-resistant genes and antibiotic resistance** 473 **genes**

474 Two quaternary ammonium compound-resistant genes (*qacEΔ1* and *qacA/B*) and
475 six antibiotic resistance genes (*sulI*, *tetA*, *tetM*, *qnrD*, *strA*, and *bla_{CTX-M}*) were
476 assessed in this study (Fig. 1). There was no significant difference between the control

477 and lowest treatment level ($10 \mu\text{g L}^{-1}$) for the *qacEΔ1*, *sull*, *tetM*, *qnrD* and *strA* both
478 on the first day and seventh day. The numbers of *sull*, *tetA* and *tetM* copies increased
479 as the concentration of BAC increased both on the first and seventh day. *qacA/B*
480 exhibited an increase trend from the control to the highest treatment level on the first
481 day, but no significant differences in the numbers of *qacA/B* copies were found
482 between any of the treatments on Day 7 ($p > 0.05$). The effect of BAC on the
483 proliferation of *strA* and *qacEΔ1* showed similar trend. On the first day, a sharp
484 increase in the abundances of *strA* and *qacEΔ1* exposed to BAC was observed at
485 concentration of $1\ 000 \mu\text{g L}^{-1}$, but no significant difference was found between the
486 abundances of *strA* and *qacEΔ1* at the treatment levels of 100, 1 000 and $10\ 000 \mu\text{g L}^{-1}$
487 ¹ on Day 7 ($p > 0.05$). *qnrD* abundances at highest treatment level of $10\ 000 \mu\text{g L}^{-1}$ on
488 the seventh day exhibited significant difference with the control, while no significant
489 differences were found between all the other experimental treatments ($p > 0.05$). The
490 effect of BAC on the abundances of *bla_{CTX-M}* gene increased significantly compared
491 with the control even at the lowest treatment level of $10 \mu\text{g L}^{-1}$ on Day 1 ($p < 0.05$).
492 However, there was no significant difference in the abundances of *bla_{CTX-M}* between
493 the control, 10, 100, or $1\ 000 \mu\text{g L}^{-1}$ treatments on Day 7.

494 **3.2.2 Nitrogen-cycling genes**

495 The response of nitrogen-cycling genes exposed to different concentrations of
496 BAC is shown in the Fig. 1. Generally, the abundances of *nifH* and *amoA* showed a
497 decrease trend as BAC concentrations increased on Day 1, but the *nirK* showed an
498 increase trend. However, no significant difference within the abundances of *amoA*

499 was found between the control and different treatment levels on Day 7. There was no
500 significant difference in the numbers of *nifH* copies between control and the lowest
501 treatment level (10 µg L⁻¹) on Day 1 and Day 7. Yet, on both Day 1 and Day 7, the
502 abundances of *nifH* within the 100, 1000, and 10 000 µg L⁻¹ treatments were
503 significantly lower than that within the control ($p < 0.05$). The abundances of *nirK* on
504 Day 1 exhibited a slow increase from the control to the 1 000 µg L⁻¹ treatment and a
505 sharp increase as BAC concentrations reached 10 000 µg L⁻¹. After seven days, the
506 numbers of *nirK* copies within 1000 and 10 000 µg L⁻¹ treatments were still
507 significant higher than that of the control ($p < 0.05$).

508 **3.3 Effect of benzyldimethyldodecyl ammonium chloride on bacterial community**

509 **3.3.1 Bacterial community richness and diversity**

510 The bacterial valid reads obtained from each treatment ranged from 30 963 to 42
511 619, normalized to 30 900 to compare richness and diversity of bacteria community
512 (Table 2). On Day 1, the control treatment had the highest number of operational
513 taxonomic units (OTUs) with 560, followed by the 10, 100, 1000 and 10 000 µg L⁻¹
514 treatments. After seven days, the 10 and 100 µg L⁻¹ treatments had the highest number
515 of OTUs (451), followed by the control, 1000 and 10 000 µg L⁻¹ treatments. On Day
516 1, Shannon diversity indices declined from control to the highest BAC concentration;
517 however, on Day 7, Shannon diversity indices increased as the concentrations
518 increased from the control to 10 000 µg L⁻¹. On Day 7, richness within two high
519 concentration treatments (1 000 and 10 000 µg L⁻¹) remained lower than those of the
520 control, 10 and 100 µg L⁻¹ treatments. There is an obvious difference in the diversity

521 at Day 1 but not at Day 7, which may be due to the degradation of BAC by the
522 microorganisms as the contact time increased. Evidence showed that the BAC could
523 be mineralized by enriched *Pseudomonas* sp. from returned activated sludge within
524 300 h (Khan et al., 2015).

525 **3.3.2 Bacterial community structure**

526 The compositions and cluster heatmap of bacterial community exposed to BAC
527 were shown in Fig.2. The bacterial compositions within the 10 and 100 $\mu\text{g L}^{-1}$
528 treatment between Day 1 and Day 7 as well as the control were similar (group A),
529 higher concentrations (1000 and 10 000 $\mu\text{g L}^{-1}$) treatments were classified in the other
530 group (group B) (Fig. 2b). Bacteria within the class *Cyanobacteria* was the highest in
531 abundance of group A (Fig. 2a). *Sphigobacteriia*, *Betaproteobacteria*, *Phycisphaerae*,
532 *Acidobacteria* and *Alphaproteobacteria* were also important components in group A
533 (Fig. 2a). The group B could be divided into two small cluster. Cluster I including
534 higher concentrations (1000 and 10 000 $\mu\text{g L}^{-1}$) treatments on Day 1, which showed
535 that *Gammaproteobacteria* was important composition of bacterial community in this
536 group. *Flavobacteriia* and *Betaproteobacteria* were also the large proportion of the
537 composition of bacterial community in the 1 000 and 10 000 $\mu\text{g L}^{-1}$ treatment on Day
538 1, respectively (Fig. 2a). The higher concentrations (1000 and 10 000 $\mu\text{g L}^{-1}$)
539 treatments on Day 7 constituted the Cluster II, in which *Cytophagia*, *Sphigobacteriia*,
540 *Alphaproteobacteria*, and *Betaproteobacteria* were the main composition of bacterial
541 community (Fig. 2a). Genus difference between the group A and group B was
542 analyzed via Wilcoxon rank-sum test (Fig. 3). It is observed that the proportions of

543 *Cyanobacteria*, *Microcytis*, *Synechococcus*, unclassified_f_Family, and CL500-3
544 (*planctomycetes*) in group A were higher than those in group B ($p < 0.05$), indicating
545 these kinds of bacteria were inhibited or killed by high concentrations of BAC. Group
546 B had higher proportion of *Pseudomonas*, *Vogesella* and *Rheinheimera* at $p < 0.1$
547 level.

548 **3.3.3 Functional profile prediction based on the 16S information**

549 The functional community profiles for each sample based on clusters of
550 orthologous groups (COG) were created (Fig. S1a, Supplementary materials). The
551 heatmap also showed that the functional community profiles were classified also into
552 two groups (Fig. S1b, Supplementary materials), which were the same as those
553 clustered based on the community structure. A significant increase in genes associated
554 with RNA processing and modification, transcription, lipid transport and metabolism,
555 amino acid transport and metabolism, and cell motility was found in group A than
556 group B at $p < 0.05$ via t-test (Fig. 4).

557 **3.4 Correlation analysis between the functional genes and microbial community**

558 The Pearson correlation coefficients between the functional genes and bacterial
559 community composition with p values less than 0.05 were shown in Fig. 5 using
560 Gephi software. *sulI*, *qacEΔ1* and *tetM* showed positive significant correlations with
561 most other antibiotic resistance genes. *Cyanobacteria* exhibited significant negative
562 correlations with most antibiotic resistance genes. Bacteria of *Gammaproteobacteria*
563 and *Betaproteobacteria* showed positive correlations with antibiotic resistance genes.
564 *Gammaproteobacteria* and *Betaproteobacteria* showed evidence that they were the

565 important groups of multi-antibiotic resistance bacteria in surface water of the
566 environment { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Hence, our results
567 also confirmed that shifts in proliferation of antibiotic resistances and microbial
568 community were correlated with each other exposure to BAC.

569 **4. Discussion**

570 **4.1 Effect of BAC on functional genes**

571 Selective enrichment of a population, advantageous mutations, and the transfer of
572 ecologically important genes are important mechanisms for the adaptation of
573 microbial communities to toxic pollutants { ADDIN EN.CITE { ADDIN
574 EN.CITE.DATA }}. *qacA/B* has been shown to be wide-spread among gram-positive
575 bacteria, such as *Staphylococci*, while *qacEΔ1* has been wide-spread among gram-
576 negative bacteria, especially *Enterobacteriaceae* and *Pseudomonas* { ADDIN
577 EN.CITE

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586 quaternary ammonium compounds--the qac genes and their role: a

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591 ear></dates><isbn>0375-8427</isbn><urls></urls></record></Cite></EndNote>}.
592 Gram-negative bacteria were shown to have high insensitivity to antimicrobials
593 compared with gram-positive bacteria in shallow urban lakes { ADDIN EN.CITE {
594 ADDIN EN.CITE.DATA }} and eutrophic lake { ADDIN EN.CITE
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606 heterotrophic bacterial community in a small eutrophic lake (Priest
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616 urls></urls></record></Cite></EndNote>}. In this study, the abundances of *qacEAI*
617 were 110-1495 times higher than *qacA/B* and proportion of *Pseudomonas* increased at
618 high concentrations of BAC compared to low levels of BAC (Fig. 2 and 3). These
619 results indicated *qacEAI* may play a more direct role in the adaptation of bacteria
620 exposed to BAC. Among the antibiotic resistance genes examined, *qnrD* was the most
621 insensitive to BAC exposure. Quinolones inhibit the DNA gyrase of bacteria which
622 could be protected by the 214-amino-acid pentapeptide repeat protein encoded by
623 *qnrD* { ADDIN EN.CITE
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633 Transferable Quinolone Resistance in Salmonella enterica Serovar Kentucky and
634 Bovismorbificans Strains of Human Origin</title><secondary-title>Antimicrobial
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643 num></record></Cite></EndNote>}, while BAC damaged the phospholipid bilayer
644 of bacterial structures { ADDIN EN.CITE
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677 sterilization mechanisms of BAC and quinolones may account for the insensitivity of

678 *qnrD* gene to BAC exposure. An increase of the antibiotic resistance genes encoding

679 efflux pump has been found in the long-term exposure of aerobic microbial

680 communities within engineered, unnatural systems to BAC { ADDIN EN.CITE

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702 10.1021/es401507k</url></related-urls></urls><electronic-resource-
703 num>10.1021/es401507k</electronic-resource-num></record></Cite></EndNote>}.
704 In this study, not only did the abundance of efflux pump antibiotic resistance genes
705 increase with increasing concentrations of BAC, but other antibiotic resistance gene
706 types (e.g., ribosomal protection protein, *tetM*) also increased. The majority of
707 plasmids carrying both antibiotic resistance genes and biocide (e.g. BAC) /metal
708 resistance genes (BMRGs) are found to be conjugative (Pal et al., 2015), which may
709 result in the increase in abundances of different kind of antibiotic resistance genes
710 exposed to BAC.

711 The changes in abundances of eleven genes exposure to BAC could be classified
712 into four groups (Fig. 6). The first group included *tetA*, *qacEΔ1* and *strA*, which
713 showed higher abundances to BAC exposure compared with the control both on Day
714 1 and Day 7, indicating the selective pressure of BAC on these genes always existed
715 during the experimental period. *amoA* and *nifH* constituted the second group, which
716 exhibited lower abundances to BAC exposure, indicating BAC had negative effect on
717 the proliferation of these two genes and influenced nitrogen cycle in the aquatic
718 system. BAC has showed evidence that it initially inhibited the nitrification efficiency

719 at a BAC feed concentration of 5 mg/L in a biological nitrogen removal processes {

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723 2012)</DisplayText><record><rec-number>1321</rec-number><foreign-keys><key

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739 compounds</keyword></keywords><dates><year>2012</year><pub-

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745 num></record></Cite></EndNote>}. Similar results have been observed for
746 antibiotics, which have been proved to have a significant and rapid negative impact on
747 the presence of *amoA* in soils { ADDIN EN.CITE
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772 num></record></Cite></EndNote>} and tropical eutrophic freshwater microcosms {
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783 J.</author></authors></contributors><titles><title>Effects of the antibiotic
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798 ></related-urls></urls><electronic-resource-
799 num>http://dx.doi.org/10.1016/j.aquatox.2013.12.008</electronic-resource-
800 num></record></Cite></EndNote>}. More research is still needed to investigate the
801 effect of BAC on the nitrogen cycle based on the changes of nitrogen removal rate
802 and denitrifer community in the natural water system. The third group composed
803 *qnrD*, *nirK* and *tetM*. Although *nirK* and *tetM* abundances showed increase at high
804 concentrations of BAC, a low multiple was observed for *nirK* and *tetM* compared
805 with *tetA*, *qacEΔ1* and *strA*. *nirK* was not only an important nitrogen cycling gene,
806 but also played important role in the response to different pollutants, such as

807 wastewater { ADDIN EN.CITE

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817 genes nirS, nirK, and nosZ to irrigation water quality in a Chinese agricultural

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836 Sören</author><author>Smalla, Kornelia</author><author>Wilke, Berndt-
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839 Abundance and Diversity of Denitrifying Bacteria by Determining nirK and nirS
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849 resource-num></record></Cite></EndNote>}. *nirK* may be a multipurpose gene and
850 not specificity for pollutants, resulting in slight increase in the abundances exposure

851 to BAC. Hence, BAC had less effect on the proliferation or spread of these three
852 genes (*qnrD*, *tetM* and *nirK*). *qacA/B*, *sulI* and *bla_{CTX-M}* were clustered as the four
853 group, which showed higher abundances to BAC exposure compared with control on
854 Day 1 but not on Day 7. These results indicated the BAC could exert different
855 selective pressure on the quaternary ammonium compound-resistant genes, antibiotic
856 resistance genes and nitrogen-cycling genes.

857 **4.2 Selective enrichment of specific bacteria exposure to BAC in aquatic system.**

858 Selective enrichment of *Rheinheimera*, *Pseudomonas*, and *Vogesella* was found in
859 the high BAC treatments (Fig. 3), suggesting that these bacteria have resistance or
860 degradation capacity to BAC. In engineered BAC-fed communities, *Pseudomonas* has
861 been identified as the dominant species (over 50%), followed by *Citrobacter* {
862 ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. In domestic drain biofilm
863 microcosms, *Falvorbacterium*, *Sphingobacterium*, *Sediminibacterium*, and *Niabella*
864 were also enriched after exposure to BAC { ADDIN EN.CITE
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884 urls></urls><electronic-resource-num>10.1128/aem.03054-16</electronic-resource-
885 num></record></Cite></EndNote>}. These highlights similarities and differences in
886 the response of microbial diversity in natural lake water and engineered systems. The
887 occurrence of enriched *Pseudomonas* spp. communities after the introduction of
888 quaternary ammonium compounds has previously been observed by U. Tezel et al {
889 ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Additionally, *Rheinheimera*
890 species isolated from freshwater culture pond and sea sediment exhibited
891 antimicrobial activity { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}.
892 *Flavobacterium* and *Pseudomonas* species from natural and clinical environments
893 have been proven to contain antiseptic-resistance genes *qacE* and *qacEΔ1* { ADDIN
894 EN.CITE

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919 urls></urls></record></Cite></EndNote>}. *Vogesella* has seldom been reported to be
920 related with exposure of BAC, but along with *Pseudomonas* and *Flavobacterium*, was
921 prominent in the toxic organic pollutants (pyrene and benzo[a]pyrene) removed from
922 lake sediments { ADDIN EN.CITE
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941 `num>10.1038/srep10709http://www.nature.com/articles/srep10709#supplemen`
942 `tary-information</electronic-resource-num></record></Cite></EndNote>}`.

943 **4.3 Effect of BAC on microbial community and function**

944 Microbial abundance and diversity were affected by the varying concentrations
945 of BAC. Our study showed that within water samples exposed to the highest BAC
946 concentrations (i.e., 10 000 µg L⁻¹) microorganism abundance decreased by 38.72%
947 on Day 1 (Fig. 1). The EC₅₀ of BAC for these microbial communities, based on
948 quantitation of 16S rRNA, in natural water from eutrophic lake was more than 10 000
949 µg L⁻¹. The acute toxicity on *Photobacterium phosphoreum* obtained an EC₅₀ in the
950 range of 0.1-1 mg L⁻¹ for both alkyl trimethyl ammonium halides (ATMAC C12-16)
951 and alkyl benzyl dimethyl ammonium halides (BAC C12-16) { ADDIN EN.CITE
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973 ></related-urls></urls><electronic-resource-num>http://doi.org/10.1016/S0269-
974 7491(99)00322-X</electronic-resource-num></record></Cite></EndNote>}. The
975 difference in the resistance of quaternary ammonium compounds for *Photobacterium*
976 *phosphoreum* and the total microbial community in the natural environment may be
977 due to the ability of some microbes to tolerate and degrade quaternary ammonium
978 compounds. Microbial communities exposed to quaternary ammonium compounds in
979 engineered system have been characterized by a versatile repertoire of antibiotic
980 resistance genes and cell envelope modification systems { ADDIN EN.CITE {
981 ADDIN EN.CITE.DATA }}. The transcriptome analysis of *Listeria monocytogenes*
982 exposed to quaternary ammonium compound benzethonium chloride revealed cell

983 wall synthesis, sugar uptake, and motility were involved in the response { ADDIN
984 EN.CITE
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995 Listeria monocytogenes exposed to biocide stress reveals a multi-system response
996 involving cell wall synthesis, sugar uptake, and motility</title><secondary-
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1005 <url></url><electronic-resource-num>10.3389/fmicb.2014.00068</electronic-
1006 resource-num><remote-database-name>PMC</remote-database-
1007 name></record></Cite></EndNote>}. Energy production, amino acids, carbohydrates
1008 and lipids metabolism were involved in the multiple adaptive routes of *Salmonella*
1009 *enterica* for stress of biocide and antibiotic { ADDIN EN.CITE
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1022 *Salmonella enterica* Typhimurium to biocide and antibiotic
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1031 urls></urls><electronic-resource-num>10.1186/s12864-016-2778-z</electronic-
1032 resource-num></record></Cite></EndNote>}. Our results firstly indicated that the
1033 functional profiles of aquatic microbial community involved in the adaption process
1034 to BAC stress, such as genes related with RNA processing and modification,
1035 transcription, lipid transport and metabolism, amino acid transport and metabolism,
1036 and cell motility.

1037 **4.4 Shift pattern in proliferation of functional genes and microbial community**

1038 The functional genes within aquatic microbial community seemed to be more
1039 sensitive to BAC exposure. Most of the genes examined in this study, such as *qacEΔ1*,
1040 *sull*, *tetM*, *strA*, *nifH* and *nirK*, exhibited significantly different abundances in the
1041 treatments with over 100 μg L⁻¹ BAC on Day 1, compared to the abundances of these
1042 genes in the control. The abundances of *bla_{CTX-M}*, *tetA*, *amoA* and *qacA/B* in the 10 μg
1043 L⁻¹ treatment on Day 1 were significantly different compared with control. Based on
1044 the results of functional genes and microbial diversity, the changes in abundances of
1045 functional genes exposure to BAC at lower concentrations were observed before
1046 significant changes in microbial community compositions occurred. This may be due
1047 to the limitation of 16S rRNA gene sequence method. Although 16S rRNA gene
1048 sequence can exhibit biases by amplifying species unequally and also capture a

1049 broader range of microbiome diversity, a lower sensitivity and resolution existed for
1050 this method { ADDIN EN.CITE
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1064 dNote>}. This work using qPCR and 16S rRNA gene data analyzed the changes in
1065 abundances of specific functional genes and microbial diversity and function exposed
1066 to BAC, which were summarized in the Fig. 7 as following: (1) BAC could influence
1067 the level of specific functional genes even at low level of BAC (10 µg L⁻¹), such as
1068 *blactX-M* and *tetA*; (2) specific bacterial species were enriched due to the stress of
1069 BAC, such as *Rheinheimera*, *Pseudomonas*, and *Vogesella*; (3) changes in microbial
1070 diversity and function were found significantly at high levels of BAC. The

1071 concentrations of BAC and QACs in most studied surface water were less than 20 μg
1072 L^{-1} and 100 $\mu\text{g L}^{-1}$, respectively { ADDIN EN.CITE
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1101 maximum value of 342 $\mu\text{g L}^{-1}$ { ADDIN EN.CITE
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1122 num>http://doi.org/10.1016/j.talanta.2013.04.083</electronic-resource-
1123 num></record></Cite></EndNote>}. Combined with the discovery of this study, it
1124 could conclude that environmental concentrations of BAC did not obviously influence
1125 the aquatic microbial composition and function, only affected the proliferation of
1126 specific functional genes. The specific species enriched gave light for isolating
1127 bacteria degrading the BAC from natural environment. qPCR and 16SrNA used in
1128 this study showed that BAC could shifts the proliferation of specific functional genes
1129 and microbial community at DNA level. Further studies are still needed to identify the
1130 main pathways of BAC and key players in the nutrient cycling influenced by BAC in
1131 aquatic ecosystems, using metatranscriptome at RNA level or functional
1132 metaproteomic approach at protein level.

1133 **5. Conclusion**

1134 In this study, BAC was applied to discover its effect on abundances of functional
1135 genes and microbial diversity. Changes within important functional genes in natural
1136 water exposed to BAC were different dependent on the gene type, concentrations of

1137 BAC and exposure time. High concentrations of BAC more than 1 000 $\mu\text{g L}^{-1}$
1138 significantly influenced the microbial diversity and community composition. Low
1139 concentrations had significant influence on the abundances of specific genes but less
1140 effect on microbial composition. The changes of BAC transformation and nutrients
1141 were not recorded in this study, hence, metaproteomic and metatranscriptomic may be
1142 needed to discover relationship between the key microbial species and pathway of
1143 BAC in the aquatic microbial ecosystem in the further research.

1144 **Acknowledgements**

1145 This project was supported in part by National Natural Science Foundation of China
1146 [grant number 31400113], and Youth Innovation Promotion Association of Chinese
1147 Academy of Sciences [grant number 2015282].

1148 **Conflicts of interest**

1149 none

1150 **References**

1151 { ADDIN EN.REFLIST }

1152 Table 1 Statistically significant differences of functional genes based on two-way ANOVA

Variables	<i>qacΔE1</i>	<i>qacA/B</i>	<i>sul1</i>	<i>tetA</i>	<i>tetM</i>	<i>qnrD</i>	<i>strA</i>	<i>blaTXM</i>	<i>amoA</i>	<i>nifH</i>	<i>nirK</i>
Time	**	***	***	*	ns	ns	***	***	***	***	***
Concentrations	***	**	***	***	***	*	***	***	***	***	***
Time × Concentrations	***	*	**	***	ns	ns	***	**	***	**	**

1153 * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$;

1154

1155 Table 2. Bacterial community diversity in water microcosms exposed to BAC after
 1156 one day (Day 1) and seven days (Day 7).

Concentration of BAC	Day 1			Day 7		
	OTUs	Chao1	Shannon	OTUs	Chao1	Shannon
Control (0)	560	589.5	3.48	376	460.4	2.51
10	520	553.5	2.81	451	509.9	2.82
100	513	586.1	2.83	451	497.5	2.80
1 000	250	325.0	2.59	366	411.9	3.47
10 000	154	201.5	2.14	256	279.6	3.98

1157

1158 Figure captions

1159 Fig. 1 The copies of 16S rRNA, quaternary ammonium compound-resistant genes
1160 (*qacEΔ1* and *qacA/B*), antibiotic resistance genes (*sul1*, *tetA*, *tetM*, *qnrD*, *strA*, and
1161 *bla_{CTX-M}*) and nitrogen cycling related genes (*amoA*, *nifH* and *nirK*) in water
1162 microcosms exposed to BAC after one day (1 d) and seven days (7 d). Different letters
1163 over the bars indicate statistically significant differences at $p < 0.05$ level in One-way
1164 ANOVA.

1165 Fig. 2 Microbial community composition at the level of the Class in water
1166 microcosms exposed to BAC after one day (D 1) and seven days (D 7) (a), and
1167 clusters were analyzed using heatmap (b). The number after D1 and D7 means the
1168 concentrations of BAC.

1169 Fig. 3 Difference of genus in the two groups was analyzed using Wilcoxon rank-sum
1170 test responded to exposure of BAC.

1171 Fig. 4 The difference between the specific functional profiles of microbial community
1172 based on 16S sequence at $p < 0.05$ level between group A and Group B.

1173 Fig. 5 The Pearson correlation between the functional genes and microbial
1174 composition using Gephi software. The p values showed in the figure were all less
1175 than 0.05. Lines with pink color indicated negative correlation, and lines with green
1176 color indicated positive correlation.

1177 Fig. 6 The heatmap of quaternary ammonium compound-resistant genes, antibiotic
1178 resistance genes and nitrogen-cycling genes exposure to different concentrations of
1179 BAC after one day (D 1) and seven days (D 7). (The log values for each gene were
1180 normalized to the corresponding log values of D1_control).

1181 Fig. 7 The schematic map of shifts in proliferation of functional genes and microbial
1182 community influenced by BAC stress.