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Benzyldimethyldodecyl Ammonium Chloride Shifts the Proliferation of Functional Genes and Microbial Community in Natural Water from Eutrophic Lake

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Abstract

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

Keywords: *Cyanobacteria; qacE*Δ1; *nirK; Rheinheimera;* microbial diversity

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

1. Introduction

Quaternary ammonium compounds (QACs) are a major class of cationic surfactants in disinfectants, biocides, detergents, and dispersants used across domestic, agricultural, industrial and clinical products { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Benzylalkyldimethylethyl ammonium compounds are one of the most prevalent QACs in natural environments, commonly occurring as effluents from wastewater treatment plants, hospitals, and laundry wastewater { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}. Benzylalkyldimethylethyl ammonium compounds in surface water are often detected in the μg L^{-1} level { ADDIN EN.CITE <EndNote><Cite><Author>Zhang</Author><Year>2015</Year><RecNum>1122</RecNum><DisplayText>(Zhang et al., 2015)</DisplayText><record><rec-number>1122</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1490772298">1122</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zhang, Chang</author><author>Cui, Fang</author><author>Zeng, Guang-ming</author><author>Jiang, Min</author><author>Yang, Zhong-zhu</author><author>Yu, Zhi-gang</author><author>Zhu, Meng-ying</author><author>Shen, Liu-qing</author></authors></contributors><titles><title>Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the

environment</title><secondary-title>Science of The Total Environment</secondary-title></titles><periodical><full-title>Science Of The Total Environment</full-title><abbr-1>Sci Total Environ</abbr-1><abbr-2>Sci. Total. Environ.</abbr-2></periodical><pages>352-362</pages><volume>518–519</volume><keywords><keyword>Quaternary ammonium compounds (QACs)</keyword><keyword>Biodegradation</keyword><keyword>Sorption</keyword><keyword>Toxicity</keyword><keyword>Determination</keyword></keywords><dates><year>2015</year><pub-dates><date>6/15/</date></pub-dates></dates><isbn>0048-9697</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0048969715002727</url><url>http://ac.els-cdn.com/S0048969715002727/1-s2.0-S0048969715002727-main.pdf?_tid=dc0dd0e8-1450-11e7-aa6c-00000aacb35f&acdnat=1490772498_0384763c3f545e833f59411b45eefa4e</url></related-urls></urls><electronic-resource-num>http://dx.doi.org/10.1016/j.scitotenv.2015.03.007</electronic-resource-num></record></Cite></EndNote>**}**. The concentrations of benzyldimethyldodecyl ammonium chloride (BAC), a type of benzylalkyldimethylethyl ammonium compounds, in the surface water downstream from five wastewater treatment plants in the US ranged from 2.7 to 5.8 μg L^{-1} **{** ADDIN EN.CITE <EndNote><Cite><Author>Ferrer</Author><Year>2001</Year><RecNum>1200</RecNum><DisplayText>(Ferrer and Furlong, 2001)</DisplayText><record><rec-number>1200</rec-number><foreign-keys><key app="EN" db-

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Article">17</ref-type><contributors><authors><author>Ferrer,

Imma</author><author>Furlong, Edward

T.</author></authors></contributors><titles><title>Identification of Alkyl

Dimethylbenzylammonium Surfactants in Water Samples by Solid-Phase Extraction

Followed by Ion Trap LC/MS and LC/MS/MS</title><secondary-title>Environmental

Science & Technology</secondary-title></titles><periodical><full-

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2588</pages><volume>35</volume><number>12</number><dates><year>2001</y

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dates></dates><publisher>American Chemical Society</publisher><isbn>0013-

936X</isbn><urls><related-

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urls></urls><electronic-resource-num>10.1021/es001742v</electronic-resource-

num></record></Cite></EndNote>**}**. The concentrations of benzylalkyldimethylethyl

ammonium compounds, including BAC, in Taiwanese river water were detected in the

range 2.5 - 65 μg L^{-1} **{** ADDIN EN.CITE

<EndNote><Cite><Author>Ding</Author><Year>2001</Year><RecNum>1201</Re

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num><urls><related-urls><url><Go to ISI>://WOS:000166262500015</url></related-urls></urls><electronic-resource-num>DOI 10.1021/ac000655i</electronic-resource-num><language>English</language></record></Cite></EndNote>**}**. The total concentration of BAC and other QACs in surface water samples from the Gdańsk City in Poland were found in the range 72.5 - 342 μg L^{-1} **{** ADDIN EN.CITE <EndNote><Cite><Author>Olkowska</Author><Year>2013</Year><RecNum>1203</RecNum><DisplayText>(Olkowska et al., 2013)</DisplayText><record><rec-number>1203</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1491898224">1203</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Olkowska, Ewa</author><author>Polkowska, Żaneta</author><author>Namieśnik, Jacek</author></authors></contributors><titles><title>A solid phase extraction–ion chromatography with conductivity detection procedure for determining cationic surfactants in surface water samples</title><secondary-title>Talanta</secondary-title></titles><periodical><full-title>Talanta</full-title><abbr-1>Talanta</abbr-1><abbr-2>Talanta</abbr-2></periodical><pages>210-216</pages><volume>116</volume><keywords><keyword>Cationic surfactants</keyword><keyword>Solid phase extraction</keyword><keyword>Ion chromatography-conductivity detection</keyword><keyword>Surface water samples</keyword></keywords><dates><year>2013</year><pub-

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num></record></Cite></EndNote>**}**.

Benzylalkyldimethylethyl ammonium compounds could inhibit cell growth via cytoplasmic membrane disruption **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**, so, they can be toxic to aquatic life without target organisms, such as fish **{** ADDIN EN.CITE <EndNote><Cite><Author>Van de Voorde</Author><Year>2012</Year><RecNum>1308</RecNum><DisplayText>(Van de Voorde et al., 2012)</DisplayText><record><rec-number>1308</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1510746316">1308</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Van de Voorde, Antoine</author><author>Lorgeoux, Catherine</author><author>Gromaire, Marie-Christine</author><author>Chebbo, Ghassan</author></authors></contributors><titles><title>Analysis of quaternary ammonium compounds in urban stormwater samples</title><secondary-title>Environmental Pollution</secondary-title></titles><periodical><full-title>Environmental Pollution</full-title><abbr-1>Environ Pollut</abbr-1><abbr-

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

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

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

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

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

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

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

magna are two organisms frequently used to assess the toxicity of

benzylalkyldimethylethyl ammonium compounds on aquatic environments **{** ADDIN

EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The 48-h EC_{50} of *Daphnia magna* after

exposure to BAC was recorded as 0.041 mg L^{-1} **{** ADDIN EN.CITE **{** ADDIN

EN.CITE.DATA **}}** and the 96-h EC_{50} of *Chlorella vulgaris* was 0.203 mg L^{-1} **{**

ADDIN EN.CITE

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

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

number>1144</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1490776349">1144</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zhu, Menjun</author><author>Ge, Fei</author><author>Zhu, Runliang</author><author>Wang, Xueye</author><author>Zheng, Xiaoyan</author></authors></contributors><titles><title>A DFT-based QSAR study of the toxicity of quaternary ammonium compounds on Chlorella vulgaris</title><secondary-title>Chemosphere</secondary-title></titles><periodical><full-title>Chemosphere</full-title><abbr-1>Chemosphere</abbr-1><abbr-2>Chemosphere</abbr-2></periodical><pages>46-52</pages><volume>80</volume><number>1</number><keywords><keyword>Quaternary ammonium compounds</keyword><keyword>QSAR</keyword><keyword>Toxicity</keyword><keyword>Aquatic organism</keyword><keyword>DFT</keyword><keyword>PLS</keyword></keywords><dates><year>2010</year><pub-dates><date>6//</date></pub-dates></dates><isbn>0045-6535</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0045653510003589</url></related-urls></urls><electronic-resource-num>http://dx.doi.org/10.1016/j.chemosphere.2010.03.044</electronic-resource-num></record></Cite></EndNote>**}**. Acute effects of BAC could occur at tens to

hundreds of μg L^{-1} levels for *Daphnia magna* and *Ceriodaphnia dubia*, while genotoxicitical effects at DNA damage level, the lowest adverse effect levels were 0.4 and 4 ng L^{-1} for *D. magna* and *C. dubia*, respectively { ADDIN EN.CITE <EndNote><Cite><Author>Lavorgna</Author><Year>2016</Year><RecNum>1191</RecNum><DisplayText>(Lavorgna et al., 2016)</DisplayText><record><rec-number>1191</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1491811083">1191</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lavorgna, Margherita</author><author>Russo, Chiara</author><author>D'Abrosca, Brigida</author><author>Parrella, Alfredo</author><author>Isidori, Marina</author></authors></contributors><titles><title>Toxicity and genotoxicity of the quaternary ammonium compound benzalkonium chloride (BAC) using Daphnia magna and Ceriodaphnia dubia as model systems</title><secondary-title>Environmental Pollution</secondary-title></titles><periodical><full-title>Environmental Pollution</full-title><abbr-1>Environ Pollut</abbr-1><abbr-2>Environ. Pollut.</abbr-2></periodical><pages>34-39</pages><volume>210</volume><keywords><keyword>Benzalkonium chloride</keyword><keyword>Cationic surfactants</keyword><keyword>Toxicity</keyword><keyword>Genotoxicity</keyword><keyword>Daphnia magna</keyword><keyword>Ceriodaphnia dubia</keyword></keywords><dates><year>2016</year><pub-

dates><date>3//</date></pub-dates></dates><isbn>0269-7491</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0269749115302025</url></related-urls></urls><electronic-resource-num>http://doi.org/10.1016/j.envpol.2015.11.042</electronic-resource-num></record></Cite></EndNote>**}**. It should be paid more attention, because these effective concentrations are much lower than BAC concentrations detected in surface waters.

Benzylalkyldimethylethyl ammonium compounds not only caused negative influence on the organisms but also resulted in changes of antibiotic resistance genes in engineered environment **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. The enhanced selection and spread of antimicrobial genes by these compounds have been regarded as a threat to human health **{** ADDIN EN.CITE <EndNote><Cite><Author>Hegstad</Author><Year>2010</Year><RecNum>1312</RecNum><DisplayText>(Hegstad et al., 2010)</DisplayText><record><rec-number>1312</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1510749339">1312</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hegstad, K.</author><author>Langsrud, S.</author><author>Lunestad, B. T.</author><author>Scheie, A. A.</author><author>Sunde, M.</author><author>Yazdankhah, S. P.</author></authors></contributors><auth-

address>Department of Microbiology and Infection Control, Reference Centre for Detection of Antimicrobial Resistance, University Hospital of North Norway, Tromso, Norway. kristin.hegstad@uit.no</auth-address><titles><title>Does the wide use of quaternary ammonium compounds enhance the selection and spread of antimicrobial resistance and thus threaten our health?</title><secondary-title>Microb Drug Resist</secondary-title><alt-title>Microbial drug resistance (Larchmont, N.Y.)</alt-title></titles><periodical><full-title>Microbial Drug Resistance</full-title><abbr-1>Microb Drug Resist</abbr-1><abbr-2>Microb. Drug Resist.</abbr-2></periodical><pages>91-104</pages><volume>16</volume><number>2</number><edition>2010/04/08</edition><keywords><keyword>Animals</keyword><keyword>Anti-Bacterial Agents/*pharmacology</keyword><keyword>Bacteria/*drug effects</keyword><keyword>*Drug Resistance, Bacterial</keyword><keyword>Humans</keyword><keyword>Industrial Microbiology</keyword><keyword>Microbial Sensitivity Tests</keyword><keyword>Public Health</keyword><keyword>Quaternary Ammonium Compounds/*pharmacology</keyword></keywords><dates><year>2010</year><pub-dates><date>Jun</date></pub-dates></dates><isbn>1076-6294</isbn><accession-num>20370507</accession-num><urls></urls><electronic-resource-num>10.1089/mdr.2009.0120</electronic-resource-num><remote-database-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

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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-

295 81</pages><volume>118</volume><number>Supplement

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

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

dates><date>2012/08/01/</date></pub-dates></dates><isbn>0960-8524</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0960852412007948</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.biortech.2012.05.050</electronic-resource-num></record></Cite></EndNote>}. However, few studies have focused on the effect of benzylalkyldimethylethyl ammonium compounds on microbial diversity and nitrogen cycling genes in natural aquatic environments.

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

2. Materials and Methods

2.1 Materials and setup of freshwater microcosm preparation

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

proliferation of antibiotic resistance genes { ADDIN EN.CITE <EndNote><Cite><Author>Luo</Author><Year>2014</Year><RecNum>428</RecNum><DisplayText>(Luo et al., 2014)</DisplayText><record><rec-number>428</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1406188151">428</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Luo, Yi</author><author>Wang, Qing</author><author>Lu, Qian</author><author>Mu, Quanhua</author><author>Mao, Daqing</author></authors></contributors><titles><title>An Ionic Liquid Facilitates the Proliferation of Antibiotic Resistance Genes Mediated by Class I Integrons</title><secondary-title>Environmental Science & Technology Letters</secondary-title></titles><periodical><full-title>Environmental Science & Technology Letters</full-title><abbr-2>Environ. Sci. Technol. Lett.</abbr-2></periodical><pages>266-270</pages><volume>1</volume><number>5</number><dates><year>2014</year><pub-dates><date>2014/05/13</date></pub-dates></dates><publisher>American Chemical Society</publisher><urls><related-urls><url>http://dx.doi.org/10.1021/ez500103v</url><url>http://pubs.acs.org/doi/abs/10.1021/ez500103v</url></related-urls></urls><electronic-resource-num>10.1021/ez500103v</electronic-resource-num><access-date>2014/07/24</access-date></record></Cite></EndNote>}. Briefly, water samples

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

2.2 DNA extraction

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

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

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

2.4 16S rRNA gene sequence analysis

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

(Axygen Biosciences, Union City, CA, USA). A mixture of the amplicons was then used for sequencing on an Illumina MiSeq platform according to the standard protocols at Majorbio Bioinformatics Technology Co., Ltd. (Shanghai, China). In brief, raw fastq files were quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window. (ii) Sequences whose overlap being longer than 10 bp were merged according to their overlap with mismatch no more than 2 bp. (iii) Sequences of each sample were separated according to barcodes (exactly matching) and primers (allowing 2 nucleotide mismatching), and reads containing ambiguous bases were removed. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) with a novel 'greedy' algorithm that performs chimera filtering and OTU clustering simultaneously { ADDIN EN.CITE <EndNote><Cite><Author>Edgar</Author><Year>2013</Year><RecNum>1100</RecNum><DisplayText>(Edgar, 2013)</DisplayText><record><rec-number>1100</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1488511523">1100</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Edgar, R. C.</author></authors></contributors><titles><title>UPARSE: highly accurate OTU sequences from microbial amplicon reads</title><secondary-title>Nature Methods</secondary-title><alt-title>Nat Methods</alt-title></titles><alt-

411 periodical><full-title>Nat Methods</full-title></alt-periodical><pages>996-
412 +</pages><volume>10</volume><number>10</number><keywords><keyword>div
413 ersity</keyword><keyword>database</keyword></keywords><dates><year>2013</
414 year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1548-
415 7091</isbn><accession-num>WOS:000325073800023</accession-
416 num><urls><related-urls><url><Go to
417 ISI>://WOS:000325073800023</url></related-urls></urls><electronic-resource-
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

Statistical Computing, Vienna, Austria), respectively. The Pearson correlation analysis between different genes and microbial community composition was carried out using SPSS software (IBM Co., USA) and showed by Gephi software v. 0.9.1(Gephi, WebAtlas, France) { ADDIN EN.CITE <EndNote><Cite><Author>Bastian</Author><Year>2009</Year><RecNum>961</RecNum><DisplayText>(Bastian et al., 2009)</DisplayText><record><rec-number>961</rec-number><foreign-keys><key app="EN" db-id="x095edwrr995rve0vtipte09wd9ptz2trr5t" timestamp="1503503495">961</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bastian, Mathieu</author><author>Heymann, Sebastien</author><author>Jacomy, Mathieu</author></authors></contributors><titles><title>Gephi: an open source software for exploring and manipulating networks</title><secondary-title>Icwsm</secondary-title></titles><periodical><full-title>Icwsm</full-title></periodical><pages>361-362</pages><volume>8</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>}. Functional profiling of microbial communities based on 16S rRNA data was carried out using PICRUSt software { ADDIN EN.CITE { ADDIN EN.CITE.DATA }}.

3. Results

3.1 Effect of benzyldimethyldodecyl ammonium chloride on amount of total 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 μg L^{-1}) (Fig. 1). The numbers of 16S rRNA
457 copies for 100, 1000, 10 000 μg 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 μg 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 μg 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 (*sul1*, *tetA*, *tetM*, *qnrD*, *strA*, and $bla_{CTX\text{-}M}$) were
476 assessed in this study (Fig. 1). There was no significant difference between the control

and lowest treatment level (10 μg L^{-1}) for the *qacEΔ1*, *sul1*, *tetM*, *qnrD* and *strA* both on the first day and seventh day. The numbers of *sul1*, *tetA* and *tetM* copies increased as the concentration of BAC increased both on the first and seventh day. *qacA/B* exhibited an increase trend from the control to the highest treatment level on the first day, but no significant differences in the numbers of *qacA/B* copies were found between any of the treatments on Day 7 ($p > 0.05$). The effect of BAC on the proliferation of *strA* and *qacEΔ1* showed similar trend. On the first day, a sharp increase in the abundances of *strA* and *qacEΔ1* exposed to BAC was observed at concentration of 1 000 μg L^{-1}, but no significant difference was found between the abundances of *strA* and *qacEΔ1* at the treatment levels of 100, 1 000 and 10 000 μg L^{-1} on Day 7 ($p > 0.05$). *qnrD* abundances at highest treatment level of 10 000 μg L^{-1} on the seventh day exhibited significant difference with the control, while no significant differences were found between all the other experimental treatments ($p > 0.05$). The effect of BAC on the abundances of bla_{CTX-M} gene increased significantly compared with the control even at the lowest treatment level of 10 μg L^{-1} on Day 1 ($p < 0.05$). However, there was no significant difference in the abundances of bla_{CTX-M} between the control, 10, 100, or 1 000 μg L^{-1} treatments on Day 7.

3.2.2 Nitrogen-cycling genes

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

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

3.3 Effect of benzyldimethyldodecyl ammonium chloride on bacterial community

3.3.1 Bacterial community richness and diversity

The bacterial valid reads obtained from each treatment ranged from 30 963 to 42 619, normalized to 30 900 to compare richness and diversity of bacteria community (Table 2). On Day 1, the control treatment had the highest number of operational taxonomic units (OTUs) with 560, followed by the 10, 100, 1000 and 10 000 μg L^{-1} treatments. After seven days, the 10 and 100 μg L^{-1} treatments had the highest number of OTUs (451), followed by the control, 1000 and 10 000 μg L^{-1} treatments. On Day 1, Shannon diversity indices declined from control to the highest BAC concentration; however, on Day 7, Shannon diversity indices increased as the concentrations increased from the control to 10 000 μg L^{-1}. On Day 7, richness within two high concentration treatments (1 000 and 10 000 μg L^{-1}) remained lower than those of the 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 μg 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 μg 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 μg 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 μg L^{-1} treatment on Day
538 1, respectively (Fig. 2a). The higher concentrations (1000 and 10 000 μg 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. *sul1*, *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

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

4. Discussion

4.1 Effect of BAC on functional genes

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

<EndNote><Cite><Author>Jaglic</Author><Year>2012</Year><RecNum>1233</RecNum><DisplayText>(Jaglic and Cervinkova, 2012)</DisplayText><record><rec-number>1233</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1503521982">1233</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Jaglic, Z</author><author>Cervinkova, D</author></authors></contributors><titles><title>Genetic basis of resistance to quaternary ammonium compounds--the qac genes and their role: a

587 review</title><secondary-title>Veterinarni Medicina</secondary-
588 title></titles><periodical><full-title>Veterinarni Medicina</full-title><abbr-1>Vet
589 Med-czech</abbr-1><abbr-2>Vet. Med-czech.</abbr-
590 2></periodical><volume>57</volume><number>6</number><dates><year>2012</y
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
595 <EndNote><Cite><Author>Edwards</Author><Year>2001</Year><RecNum>1155
596 </RecNum><DisplayText>(Edwards et al., 2001)</DisplayText><record><rec-
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599 timestamp="1491361639">1155</key></foreign-keys><ref-type name="Journal
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601 L.</author><author>Lilley, A. K.</author><author>Timms-Wilson, T.
602 H.</author><author>Thompson, I. P.</author><author>Cooper,
603 I.</author></authors></contributors><auth-address>Natural Environment Research
604 Council, Centre for Ecology and Hydrology-Oxford, Mansfield Road, OX1 3SR,
605 Oxford, UK</auth-address><titles><title>Characterisation of the culturable
606 heterotrophic bacterial community in a small eutrophic lake (Priest
607 Pot)</title><secondary-title>FEMS Microbiol Ecol</secondary-
608 title></titles><periodical><full-title>Fems Microbiology Ecology</full-title><abbr-

609 1>Fems Microbiol Ecol</abbr-1><abbr-2>Fems Microbiol. Ecol.</abbr-

610 2></periodical><pages>295-

611 304</pages><volume>35</volume><number>3</number><dates><year>2001</year

612 ><pub-dates><date>May</date></pub-dates></dates><isbn>1574-6941

613 (Electronic)
0168-6496 (Linking)</isbn><accession-

614 num>11311440</accession-num><urls><related-

615 urls><url>https://www.ncbi.nlm.nih.gov/pubmed/11311440</url></related-

616 urls></urls></record></Cite></EndNote>**}**. In this study, the abundances of *qacEΔ1*

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 *qacEΔ1* 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

624 <EndNote><Cite><Author>Cavaco</Author><Year>2009</Year><RecNum>1161<

625 /RecNum><DisplayText>(Cavaco et al., 2009)</DisplayText><record><rec-

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628 timestamp="1491463727">1161</key></foreign-keys><ref-type name="Journal

629 Article">17</ref-type><contributors><authors><author>Cavaco, L.

630 M.</author><author>Hasman, H.</author><author>Xia,

631 S.</author><author>Aarestrup, F.

632 M.</author></authors></contributors><titles><title>qnrD, a Novel Gene Conferring

633 Transferable Quinolone Resistance in Salmonella enterica Serovar Kentucky and

634 Bovismorbificans Strains of Human Origin</title><secondary-title>Antimicrobial

635 Agents and Chemotherapy</secondary-title></titles><periodical><full-

636 title>Antimicrobial Agents and Chemotherapy</full-title><abbr-1>Antimicrob

637 Agents Ch</abbr-1><abbr-2>Antimicrob. Agents. Ch</abbr-

638 2></periodical><pages>603-

639 608</pages><volume>53</volume><number>2</number><dates><year>2009</year

640 ><pub-dates><date>February 1, 2009</date></pub-dates></dates><urls><related-

641 urls><url>http://aac.asm.org/content/53/2/603.abstract</url></related-

642 urls></urls><electronic-resource-num>10.1128/aac.00997-08</electronic-resource-

643 num></record></Cite></EndNote>**}**, while BAC damaged the phospholipid bilayer

644 of bacterial structures **{** ADDIN EN.CITE

645 <EndNote><Cite><Author>Ioannou</Author><Year>2007</Year><RecNum>1139<

646 /RecNum><DisplayText>(Ioannou et al., 2007)</DisplayText><record><rec-

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649 timestamp="1490775349">1139</key></foreign-keys><ref-type name="Journal

650 Article">17</ref-type><contributors><authors><author>Ioannou, C.

651 J.</author><author>Hanlon, G. W.</author><author>Denyer, S.

652 P.</author></authors></contributors><auth-address>Quest Int, Ashford TN24 0LT,

Kent, England
Univ Brighton, Sch Pharm & Biomol Sci, Brighton BN2 4GJ, E Sussex, England
Univ Cardiff Wales, Welsh Sch Pharm, Cardiff CF1 3XF, Wales</auth-address><titles><title>Action of disinfectant quaternary ammonium compounds against Staphylococcus aureus</title><secondary-title>Antimicrobial Agents and Chemotherapy</secondary-title><alt-title>Antimicrob Agents Ch</alt-title></titles><periodical><full-title>Antimicrobial Agents and Chemotherapy</full-title><abbr-1>Antimicrob Agents Ch</abbr-1><abbr-2>Antimicrob. Agents. Ch</abbr-2></periodical><alt-periodical><full-title>Antimicrobial Agents and Chemotherapy</full-title><abbr-1>Antimicrob Agents Ch</abbr-1><abbr-2>Antimicrob. Agents. Ch</abbr-2></alt-periodical><pages>296-306</pages><volume>51</volume><number>1</number><keywords><keyword>escherichia-coli</keyword><keyword>cytoplasmic constituents</keyword><keyword>antimicrobial action</keyword><keyword>membrane damage</keyword><keyword>resistance</keyword><keyword>mechanisms</keyword><keyword>biocides</keyword><keyword>cell</keyword><keyword>time</keyword><keyword>classification</keyword></keywords><dates><year>2007</year><pub-dates><date>Jan</date></pub-dates></dates><isbn>0066-4804</isbn><accession-num>WOS:000243214200038</accession-num><urls><related-urls><url><Go to ISI>://WOS:000243214200038</url></related-urls></urls><electronic-resource-

num>10.1128/Aac.00375-06</electronic-resource-num><language>English</language></record></Cite></EndNote>}. Hence, different sterilization mechanisms of BAC and quinolones may account for the insensitivity of *qnrD* gene to BAC exposure. An increase of the antibiotic resistance genes encoding efflux pump has been found in the long-term exposure of aerobic microbial communities within engineered, unnatural systems to BAC { ADDIN EN.CITE <EndNote><Cite><Author>Tandukar</Author><Year>2013</Year><RecNum>1157</RecNum><DisplayText>(Tandukar et al., 2013)</DisplayText><record><rec-number>1157</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1491379016">1157</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Tandukar, Madan</author><author>Oh, Seungdae</author><author>Tezel, Ulas</author><author>Konstantinidis, Konstantinos T.</author><author>Pavlostathis, Spyros G.</author></authors></contributors><titles><title>Long-Term Exposure to Benzalkonium Chloride Disinfectants Results in Change of Microbial Community Structure and Increased Antimicrobial Resistance</title><secondary-title>Environmental Science & Technology</secondary-title></titles><periodical><full-title>Environmental Science & Technology</full-title><abbr-1>Environ Sci Technol</abbr-1><abbr-2>Environ. Sci. Technol.</abbr-2></periodical><pages>9730-

9738</pages><volume>47</volume><number>17</number><dates><year>2013</year><pub-dates><date>2013/09/03</date></pub-dates></dates><publisher>American Chemical Society</publisher><isbn>0013-936X</isbn><urls><related-urls><url>http://dx.doi.org/10.1021/es401507k</url><url>http://pubs.acs.org/doi/abs/10.1021/es401507k</url></related-urls></urls><electronic-resource-num>10.1021/es401507k</electronic-resource-num></record></Cite></EndNote>}. In this study, not only did the abundance of efflux pump antibiotic resistance genes increase with increasing concentrations of BAC, but other antibiotic resistance gene types (e.g., ribosomal protection protein, *tetM*) also increased. The majority of plasmids carrying both antibiotic resistance genes and biocide (e.g. BAC) /metal resistance genes (BMRGs) are found to be conjugative (Pal et al., 2015), which may result in the increase in abundances of different kind of antibiotic resistance genes exposed to BAC.

The changes in abundances of eleven genes exposure to BAC could be classified into four groups (Fig. 6). The first group included *tetA*, *qacEΔ1* and *strA*, which showed higher abundances to BAC exposure compared with the control both on Day 1 and Day 7, indicating the selective pressure of BAC on these genes always existed during the experimental period. *amoA* and *nifH* constituted the second group, which exhibited lower abundances to BAC exposure, indicating BAC had negative effect on the proliferation of these two genes and influenced nitrogen cycle in the aquatic 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 **{**

720 ADDIN EN.CITE

721 <EndNote><Cite><Author>Hajaya</Author><Year>2012</Year><RecNum>1321</

722 RecNum><DisplayText>(Hajaya and Pavlostathis,

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

724 app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0"

725 timestamp="1511820333">1321</key></foreign-keys><ref-type name="Journal

726 Article">17</ref-type><contributors><authors><author>Hajaya, Malek

727 G.</author><author>Pavlostathis, Spyros

728 G.</author></authors></contributors><titles><title>Fate and effect of benzalkonium

729 chlorides in a continuous-flow biological nitrogen removal system treating poultry

730 processing wastewater</title><secondary-title>Bioresource Technology</secondary-

731 title></titles><periodical><full-title>Bioresource Technology</full-title><abbr-

732 1>Bioresource Technol</abbr-1><abbr-2>Bioresource. Technol.</abbr-

733 2></periodical><pages>73-

734 81</pages><volume>118</volume><number>Supplement

735 C</number><keywords><keyword>Benzalkonium

736 chlorides</keyword><keyword>Biological nitrogen

737 removal</keyword><keyword>Nitrification</keyword><keyword>Denitrification</k

738 eyword><keyword>Quaternary ammonium

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

740 dates><date>2012/08/01/</date></pub-dates></dates><isbn>0960-

741 8524</isbn><urls><related-

742 urls><url>http://www.sciencedirect.com/science/article/pii/S0960852412007948</url

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

744 num>https://doi.org/10.1016/j.biortech.2012.05.050</electronic-resource-

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

748 <EndNote><Cite><Author>Colloff</Author><Year>2008</Year><RecNum>1171</

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750 number>1171</rec-number><foreign-keys><key app="EN" db-

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754 J.</author><author>Wakelin, S. A.</author><author>Gomez,

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757 in soils for measuring the effects of changes in land use and

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761 1645</pages><volume>40</volume><number>7</number><keywords><keyword>

762 Bacteria</keyword><keyword>amoA</keyword><keyword>napA</keyword><key

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765 production</keyword><keyword>Perturbation</keyword><keyword>Antibiotic</ke

766 yword><keyword>Salinity</keyword><keyword>Australia</keyword></keywords>

767 <dates><year>2008</year><pub-dates><date>7//</date></pub-

768 dates></dates><isbn>0038-0717</isbn><urls><related-

769 urls><url>http://www.sciencedirect.com/science/article/pii/S0038071708000758</url

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

771 num>http://doi.org/10.1016/j.soilbio.2008.01.019</electronic-resource-

772 num></record></Cite></EndNote>**}** and tropical eutrophic freshwater microcosms **{**

773 ADDIN EN.CITE

774 <EndNote><Cite><Author>Rico</Author><Year>2014</Year><RecNum>610</Rec

775 Num><DisplayText>(Rico et al., 2014)</DisplayText><record><rec-

776 number>610</rec-number><foreign-keys><key app="EN" db-

777 id="vzededvvherd97ep2db5pwr1fe5trtad95r0"

778 timestamp="1438068296">610</key></foreign-keys><ref-type name="Journal

779 Article">17</ref-type><contributors><authors><author>Rico,

780 Andreu</author><author>Dimitrov, Mauricio R.</author><author>Van

781 Wijngaarden, René P. A.</author><author>Satapornvanit,

782 Kriengkrai</author><author>Smidt, Hauke</author><author>Van den Brink, Paul

783 J.</author></authors></contributors><titles><title>Effects of the antibiotic

784 enrofloxacin on the ecology of tropical eutrophic freshwater

785 microcosms</title><secondary-title>Aquatic Toxicology</secondary-
786 title></titles><periodical><full-title>Aquatic Toxicology</full-title><abbr-1>Aquat
787 Toxicol</abbr-1><abbr-2>Aquat. Toxicol.</abbr-2></periodical><pages>92-
788 104</pages><volume>147</volume><keywords><keyword>Antibiotics</keyword>
789 <keyword>Enrofloxacin</keyword><keyword>Microcosms</keyword><keyword>E
790 cological risk
791 assessment</keyword><keyword>Tropics</keyword></keywords><dates><year>20
792 14</year><pub-dates><date>2//</date></pub-dates></dates><isbn>0166-
793 445X</isbn><urls><related-
794 urls><url>http://www.sciencedirect.com/science/article/pii/S0166445X13003494</ur
795 l><url>http://ac.els-cdn.com/S0166445X13003494/1-s2.0-S0166445X13003494-
796 main.pdf?_tid=20503948-5c2a-11e5-85da-
797 00000aacb35e&acdnat=1442377350_1cad453f22a48bd36b854abdfa1fa3c2</url
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

808 <EndNote><Cite><Author>Zhou</Author><Year>2011</Year><RecNum>1175</R

809 ecNum><DisplayText>(Zhou et al., 2011)</DisplayText><record><rec-

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811 id="vzededvvherd97ep2db5pwr1fe5trtad95r0"

812 timestamp="1491545066">1175</key></foreign-keys><ref-type name="Journal

813 Article">17</ref-type><contributors><authors><author>Zhou, Zhi-

814 Feng</author><author>Zheng, Yuan-Ming</author><author>Shen, Ju-

815 Pei</author><author>Zhang, Li-Mei</author><author>He, Ji-

816 Zheng</author></authors></contributors><titles><title>Response of denitrification

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

818 soil</title><secondary-title>Environmental Science and Pollution

819 Research</secondary-title></titles><periodical><full-title>Environmental Science

820 and Pollution Research</full-title><abbr-1>Environ Sci Pollut R</abbr-1><abbr-

821 2>Environ. Sci. Pollut. Res.</abbr-2></periodical><pages>1644-

822 1652</pages><volume>18</volume><number>9</number><dates><year>2011</ye

823 ar></dates><isbn>1614-7499</isbn><label>Zhou2011</label><work-type>journal

824 article</work-type><urls><related-urls><url>http://dx.doi.org/10.1007/s11356-011-

825 0482-8</url></related-urls></urls><electronic-resource-num>10.1007/s11356-011-

826 0482-8</electronic-resource-num></record></Cite></EndNote>} and antibiotics {

827 ADDIN EN.CITE

828 <EndNote><Cite><Author>Kleineidam</Author><Year>2010</Year><RecNum>11

829 73</RecNum><DisplayText>(Kleineidam et al., 2010)</DisplayText><record><rec-
830 number>1173</rec-number><foreign-keys><key app="EN" db-
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832 timestamp="1491543991">1173</key></foreign-keys><ref-type name="Journal
833 Article">17</ref-type><contributors><authors><author>Kleineidam,
834 Kristina</author><author>Sharma, Shilpi</author><author>Kotzerke,
835 Anja</author><author>Heuer, Holger</author><author>Thiele-Bruhn,
836 Sören</author><author>Smalla, Kornelia</author><author>Wilke, Berndt-
837 Michael</author><author>Schloter,
838 Michael</author></authors></contributors><titles><title>Effect of Sulfadiazine on
839 Abundance and Diversity of Denitrifying Bacteria by Determining nirK and nirS
840 Genes in Two Arable Soils</title><secondary-title>Microbial Ecology</secondary-
841 title></titles><periodical><full-title>Microbial Ecology</full-title><abbr-
842 1>Microbial Ecol</abbr-1><abbr-2>Microbial Ecol.</abbr-
843 2></periodical><pages>703-
844 707</pages><volume>60</volume><number>4</number><dates><year>2010</year
845 ></dates><isbn>1432-184X</isbn><label>Kleineidam2010</label><work-
846 type>journal article</work-type><urls><related-
847 urls><url>http://dx.doi.org/10.1007/s00248-010-9691-9</url></related-
848 urls></urls><electronic-resource-num>10.1007/s00248-010-9691-9</electronic-
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

to BAC. Hence, BAC had less effect on the proliferation or spread of these three genes (*qnrD*, *tetM* and *nirK*). *qacA/B*, *sul1* and $bla_{CTX\text{-}M}$ were clustered as the four group, which showed higher abundances to BAC exposure compared with control on Day 1 but not on Day 7. These results indicated the BAC could exert different selective pressure on the quaternary ammonium compound-resistant genes, antibiotic resistance genes and nitrogen-cycling genes.

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

Selective enrichment of *Rheinheimera*, *Pseudomonas*, and *Vogesella* was found in the high BAC treatments (Fig. 3), suggesting that these bacteria have resistance or degradation capacity to BAC. In engineered BAC-fed communities, *Pseudomonas* has been identified as the dominant species (over 50%), followed by *Citrobacter* **{** ADDIN EN.CITE **{** ADDIN EN.CITE.DATA **}}**. In domestic drain biofilm microcosms, *Falvorbacterium*, *Sphingobacterium*, *Sediminibacterium*, and *Niabella* were also enriched after exposure to BAC **{** ADDIN EN.CITE <EndNote><Cite><Author>Forbes</Author><Year>2017</Year><RecNum>1183</RecNum><DisplayText>(Forbes et al., 2017)</DisplayText><record><rec-number>1183</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1491791240">1183</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Forbes, Sarah</author><author>Cowley, Nicola</author><author>Humphreys, Gavin</author><author>Mistry, Hitesh</author><author>Amézquita,

873 Alejandro</author><author>McBain, Andrew
874 J.</author></authors></contributors><titles><title>Formulation of Biocides
875 Increases Antimicrobial Potency and Mitigates the Enrichment of Nonsusceptible
876 Bacteria in Multispecies Biofilms</title><secondary-title>Applied and
877 Environmental Microbiology</secondary-title></titles><periodical><full-
878 title>Applied and Environmental Microbiology</full-title><abbr-1>Appl Environ
879 Microb</abbr-1><abbr-2>Appl. Environ. Microb.</abbr-
880 2></periodical><volume>83</volume><number>7</number><dates><year>2017</y
881 ear><pub-dates><date>April 1, 2017</date></pub-dates></dates><urls><related-
882 urls><url>http://aem.asm.org/content/83/7/e03054-
883 16.abstract</url><url>http://aem.asm.org/content/83/7/e03054-16</url></related-
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

<EndNote><Cite><Author>Kazama</Author><Year>1998</Year><RecNum>1185<
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H.</author><author>Hamashima, H.</author><author>Sasatsu,
M.</author><author>Arai, T.</author></authors></contributors><auth-
address>Department of Microbiology, Showa College of Pharmaceutical Sciences,
Tokyo, Japan.</auth-address><titles><title>Distribution of the antiseptic-resistance
genes qacE and qacE delta 1 in gram-negative bacteria</title><secondary-title>FEMS
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0378-1097

917 (Linking)</isbn><accession-num>9503610</accession-num><urls><related-
918 urls><url>https://www.ncbi.nlm.nih.gov/pubmed/9503610</url></related-
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
923 <EndNote><Cite><Author>Yan</Author><Year>2015</Year><RecNum>1187</Rec
924 Num><DisplayText>(Yan et al., 2015)</DisplayText><record><rec-
925 number>1187</rec-number><foreign-keys><key app="EN" db-
926 id="vzededvvherd97ep2db5pwr1fe5trtad95r0"
927 timestamp="1491794132">1187</key></foreign-keys><ref-type name="Journal
928 Article">17</ref-type><contributors><authors><author>Yan,
929 Zaisheng</author><author>Jiang, Helong</author><author>Cai,
930 Haiyuan</author><author>Zhou, Yanli</author><author>Krumholz, Lee
931 R.</author></authors></contributors><titles><title>Complex Interactions Between
932 the Macrophyte Acorus Calamus and Microbial Fuel Cells During Pyrene and
933 Benzo[a]Pyrene Degradation in Sediments</title><secondary-title>Scientific
934 Reports</secondary-title></titles><periodical><full-title>Scientific Reports</full-
935 title><abbr-1>Sci Rep</abbr-1><abbr-2>Sci. Rep.</abbr-
936 2></periodical><pages>10709</pages><volume>5</volume><dates><year>2015</y
937 ear><pub-dates><date>05/29/online</date></pub-dates></dates><publisher>The
938 Author(s)</publisher><work-type>Article</work-type><urls><related-

939 urls><url>http://dx.doi.org/10.1038/srep10709</url></related-
940 urls></urls><electronic-resource-
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 EC_{50} 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 EC_{50} 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
952 <EndNote><Cite><Author>García</Author><Year>2001</Year><RecNum>1189</
953 RecNum><DisplayText>(García et al., 2001)</DisplayText><record><rec-
954 number>1189</rec-number><foreign-keys><key app="EN" db-
955 id="vzededvvherd97ep2db5pwr1fe5trtad95r0"
956 timestamp="1491807867">1189</key></foreign-keys><ref-type name="Journal
957 Article">17</ref-type><contributors><authors><author>García, M.
958 T.</author><author>Ribosa, I.</author><author>Guindulain,
959 T.</author><author>Sánchez-Leal, J.</author><author>Vives-Rego,
960 J.</author></authors></contributors><titles><title>Fate and effect of monoalkyl

961 quaternary ammonium surfactants in the aquatic environment</title><secondary-
962 title>Environmental Pollution</secondary-title></titles><periodical><full-
963 title>Environmental Pollution</full-title><abbr-1>Environ Pollut</abbr-1><abbr-
964 2>Environ. Pollut.</abbr-2></periodical><pages>169-
965 175</pages><volume>111</volume><number>1</number><keywords><keyword>C
966 ationic surfactants</keyword><keyword>Aquatic
967 toxicity</keyword><keyword>Biodegradation</keyword><keyword>Marine
968 bacterioplankton</keyword><keyword>Flow
969 cytometry</keyword></keywords><dates><year>2001</year><pub-
970 dates><date>1//</date></pub-dates></dates><isbn>0269-
971 7491</isbn><urls><related-
972 urls><url>http://www.sciencedirect.com/science/article/pii/S026974919900322X</url
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
985 <EndNote><Cite><Author>Casey</Author><Year>2014</Year><RecNum>1231</R
986 ecNum><DisplayText>(Casey et al., 2014)</DisplayText><record><rec-
987 number>1231</rec-number><foreign-keys><key app="EN" db-
988 id="vzededvvherd97ep2db5pwr1fe5trtad95r0"
989 timestamp="1503400821">1231</key></foreign-keys><ref-type name="Journal
990 Article">17</ref-type><contributors><authors><author>Casey,
991 Aidan</author><author>Fox, Edward M.</author><author>Schmitz-Esser,
992 Stephan</author><author>Coffey, Aidan</author><author>McAuliffe,
993 Olivia</author><author>Jordan,
994 Kieran</author></authors></contributors><titles><title>Transcriptome analysis of
995 Listeria monocytogenes exposed to biocide stress reveals a multi-system response
996 involving cell wall synthesis, sugar uptake, and motility</title><secondary-
997 title>Frontiers in Microbiology</secondary-title></titles><periodical><full-
998 title>Frontiers in Microbiology</full-title><abbr-1>Front. Microbiol.</abbr-1><abbr-
999 2>Front. Microbiol.</abbr-
1000 2></periodical><pages>68</pages><volume>5</volume><dates><year>2014</year
1001 ><pub-dates><date>02/28
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02/07/accepted</date></pub-
1002 dates></dates><publisher>Frontiers Media S.A.</publisher><isbn>1664-
1003 302X</isbn><accession-num>PMC3937556</accession-num><urls><related-
1004 urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3937556/</url></related-

urls></urls><electronic-resource-num>10.3389/fmicb.2014.00068</electronic-resource-num><remote-database-name>PMC</remote-database-name></record></Cite></EndNote>**}**. Energy production, amino acids, carbohydrates and lipids metabolism were involved in the multiple adaptive routes of *Salmonella enterica* for stress of biocide and antibiotic **{** ADDIN EN.CITE <EndNote><Cite><Author>Curiao</Author><Year>2016</Year><RecNum>1293</RecNum><DisplayText>(Curiao et al., 2016)</DisplayText><record><rec-number>1293</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1503524364">1293</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Curiao, Tânia</author><author>Marchi, Emmanuela</author><author>Grandgirard, Denis</author><author>León-Sampedro, Ricardo</author><author>Viti, Carlo</author><author>Leib, Stephen L.</author><author>Baquero, Fernando</author><author>Oggioni, Marco R.</author><author>Martinez, José Luis</author><author>Coque, Teresa M.</author></authors></contributors><titles><title>Multiple adaptive routes of Salmonella enterica Typhimurium to biocide and antibiotic exposure</title><secondary-title>BMC Genomics</secondary-title></titles><periodical><full-title>Bmc Genomics</full-title><abbr-1>BMC Genomics</abbr-1><abbr-2>BMC Genomics</abbr-2></periodical><pages>491</pages><volume>17</volume><number>1</number><

dates><year>2016</year><pub-dates><date>July 13</date></pub-dates></dates><isbn>1471-2164</isbn><label>Curiao2016</label><work-type>journal article</work-type><urls><related-urls><url>https://doi.org/10.1186/s12864-016-2778-z</url></related-urls></urls><electronic-resource-num>10.1186/s12864-016-2778-z</electronic-resource-num></record></Cite></EndNote>**}**. Our results firstly indicated that the functional profiles of aquatic microbial community involved in the adaption process to BAC stress, such as genes related with RNA processing and modification, transcription, lipid transport and metabolism, amino acid transport and metabolism, and cell motility.

4.4 Shift pattern in proliferation of functional genes and microbial community

The functional genes within aquatic microbial community seemed to be more sensitive to BAC exposure. Most of the genes examined in this study, such as *qacEΔ1, sul1*, *tetM*, *strA*, *nifH* and *nirK*, exhibited significantly different abundances in the treatments with over 100 μg L^{-1} BAC on Day 1, compared to the abundances of these genes in the control. The abundances of bla_{CTX-M}, *tetA*, *amoA* and *qacA/B* in the 10 μg L^{-1} treatment on Day 1 were significantly different compared with control. Based on the results of functional genes and microbial diversity, the changes in abundances of functional genes exposure to BAC at lower concentrations were observed before significant changes in microbial community compositions occurred. This may be due to the limitation of 16S rNA gene sequence method. Although 16S rRNA gene sequence can exhibit biases by amplifying species unequally and also capture a

broader range of microbiome diversity, a lower sensitivity and resolution existed for this method { ADDIN EN.CITE <EndNote><Cite><Author>Shah</Author><Year>2011</Year><RecNum>1327</RecNum><DisplayText>(Shah et al., 2011)</DisplayText><record><rec-number>1327</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1511954748">1327</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Shah, Neethu</author><author>Tang, Haixu</author><author>Doak, Thomas G</author><author>Ye, Yuzhen</author></authors></contributors><titles><title>Comparing bacterial communities inferred from 16S rRNA gene sequencing and shotgun metagenomics</title><secondary-title>Biocomputing 2011</secondary-title></titles><pages>165-176</pages><dates><year>2011</year></dates><urls></urls></record></Cite></EndNote>}. This work using qPCR and 16S rRNA gene data analyzed the changes in abundances of specific functional genes and microbial diversity and function exposed to BAC, which were summarized in the Fig. 7 as following: (1) BAC could influence the level of specific functional genes even at low level of BAC (10 μg L^{-1}), such as *bla*$_{CTX\text{-}M}$ and *tetA*; (2) specific bacterial species were enriched due to the stress of BAC, such as *Rheinheimera*, *Pseudomonas*, and *Vogesella*; (3) changes in microbial diversity and function were found significantly at high levels of BAC. The

concentrations of BAC and QACs in most studied surface water were less than 20 μg L^{-1} and 100 μg L^{-1}, respectively { ADDIN EN.CITE <EndNote><Cite><Author>Zhang</Author><Year>2015</Year><RecNum>1122</RecNum><DisplayText>(Zhang et al., 2015)</DisplayText><record><rec-number>1122</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1490772298">1122</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Zhang, Chang</author><author>Cui, Fang</author><author>Zeng, Guang-ming</author><author>Jiang, Min</author><author>Yang, Zhong-zhu</author><author>Yu, Zhi-gang</author><author>Zhu, Meng-ying</author><author>Shen, Liu-qing</author></authors></contributors><titles><title>Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment</title><secondary-title>Science of The Total Environment</secondary-title></titles><periodical><full-title>Science Of The Total Environment</full-title><abbr-1>Sci Total Environ</abbr-1><abbr-2>Sci. Total. Environ.</abbr-2></periodical><pages>352-362</pages><volume>518–519</volume><keywords><keyword>Quaternary ammonium compounds (QACs)</keyword><keyword>Biodegradation</keyword><keyword>Sorption</keyword><keyword>Toxicity</keyword><keyword>Determination</keyword></keywords><dates><year>2015</year><pub-dates><date>6/15/</date></pub-

dates></dates><isbn>0048-9697</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0048969715002727</url><url>http://ac.els-cdn.com/S0048969715002727/1-s2.0-S0048969715002727-main.pdf?_tid=dc0dd0e8-1450-11e7-aa6c-00000aacb35f&acdnat=1490772498_0384763c3f545e833f59411b45eefa4e</url></related-urls></urls><electronic-resource-num>http://dx.doi.org/10.1016/j.scitotenv.2015.03.007</electronic-resource-num></record></Cite></EndNote>**}**, except for QACs in reservoir water with maximum value of 342 μg L^{-1} **{** ADDIN EN.CITE <EndNote><Cite><Author>Olkowska</Author><Year>2013</Year><RecNum>1203</RecNum><DisplayText>(Olkowska et al., 2013)</DisplayText><record><rec-number>1203</rec-number><foreign-keys><key app="EN" db-id="vzededvvherd97ep2db5pwr1fe5trtad95r0" timestamp="1491898224">1203</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Olkowska, Ewa</author><author>Polkowska, Żaneta</author><author>Namieśnik, Jacek</author></authors></contributors><titles><title>A solid phase extraction–ion chromatography with conductivity detection procedure for determining cationic surfactants in surface water samples</title><secondary-title>Talanta</secondary-title></titles><periodical><full-title>Talanta</full-title><abbr-1>Talanta</abbr-1><abbr-2>Talanta</abbr-2></periodical><pages>210-216</pages><volume>116</volume><keywords><keyword>Cationic

surfactants</keyword><keyword>Solid phase extraction</keyword><keyword>Ion chromatography-conductivity detection</keyword><keyword>Surface water samples</keyword></keywords><dates><year>2013</year><pub-dates><date>11/15/</date></pub-dates></dates><isbn>0039-9140</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0039914013004104</url></related-urls></urls><electronic-resource-num>http://doi.org/10.1016/j.talanta.2013.04.083</electronic-resource-num></record></Cite></EndNote>**}**. Combined with the discovery of this study, it could conclude that environmental concentrations of BAC did not obviously influence the aquatic microbial composition and function, only affected the proliferation of specific functional genes. The specific species enriched gave light for isolating bacteria degrading the BAC from natural environment. qPCR and 16SrNA used in this study showed that BAC could shifts the proliferation of specific functional genes and microbial community at DNA level. Further studies are still needed to identify the main pathways of BAC and key players in the nutrient cycling influenced by BAC in aquatic ecosystems, using metatranscriptome at RNA level or functional metaproteomic approach at protein level.

5. Conclusion

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

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

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Conflicts of interest

none

References

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

Variables	*qacΔE*1	*qacA/B*	*sul1*	*tetA*	*tetM*	*qnrD*	*strA*	*blaTXM*	*amoA*	*nifH*	*nirK*
Time	**	***	***	*	ns	ns	***	***	***	***	***
Concentrations	***	**	***	***	***	*	***	***	***	***	***
Time×Concentrations	***	*	**	***	ns	ns	***	**	***	**	**

*p <0.05; **p <0.01; ***p <0.001;

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	Day 1			Day 7		
BAC	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

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Figure captions

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

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

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

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

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

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