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Microbial community study in newly established Qingcaosha Reservoir of Shanghai, China

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Abstract Qingcaosha Reservoir located at Yangtze Estuary of China is a newly constructed and one of the largest tidal reservoirs in the world, which will be an important drinking water source of Shanghai. This study aims at investigating microbial community and its shifts corresponding to different water quality during the test running period of Qingcaosha Reservoir. The results showed lower concentrations of total nitrogen (TN) and total phosphorus (TP) in the reservoir than that in Yangtze Estuary. The number of total cultivable bacteria was significantly lower in the reservoir than that of Yangtze Estuary. The denaturing gradient gel electrophoresis (DGGE) analysis showed that the dominant microbes were *α-Proteobacteria*, *β-Proteobacteria*, *Flavobacterium*, *Rheinheimera*, *Prochlorococcus*, and *Synechococcus*. The quantitative PCR (q-PCR) results revealed significantly higher number of cyanobacteria and *Microcystis* in the reservoir during summer season. In addition, bacterial abundance positively correlated with TP concentration inside the reservoir. These results indicated that Qingcaosha Reservoir had ability to reduce the TN and TP in influent and improve the water quality overall. However, it also faced the risk of potential cyanobacteria bloom and eutrophication in Qingcaosha Reservoir where phosphorus will be the nutrient limiting factor.

Z. Huang · B. Xie (✉) · Q. Yuan · W. Xu
Shanghai Key Laboratory on Urbanization Ecological Process and Ecorestoration, Department of Environmental Science & Technology, East China Normal University, Shanghai 200062, China
e-mail: bxie@des.ecnu.edu.cn

J. Lu
Faculty of Health and Environmental Sciences and Institute of Biomedical Technologies, Auckland University of Technology, Private Bag 92006, Box A-25, Auckland, New Zealand

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Introduction

The newly built Qingcaosha Reservoir is located in the middle of the Yangtze Estuary of Shanghai, China, and it is one of the largest tidal reservoirs in the world. It has better influent water quality without the interference of terrestrial sewage and provides significant advantages of freshwater resources. As a strategic water source of Shanghai, Qingcaosha Reservoir provides more than 7 million cubic meter of water daily (Gu et al. 2008; Jiang 2012). Therefore, the water quality of Qingcaosha Reservoir is rather crucial to the drinking water safety of more than 23 million inhabitants in Shanghai.

Qingcaosha Reservoir was built and test run at the end of 2009. It was designed to settle down the suspended solid of influent from Yangtze River, which would lead to better water quality at the outlet of reservoir. However, the reservoir still faces eutrophication problem because of its shallowness and longer hydraulic retention time (HRT) (Lin et al. 2009). It is well known that eutrophication, characterized by excessive nutrient loading and harmful algal blooms, is a global problem in lakes with shallow depth and long HRT (Qin 2009). For example, algal blooms were frequently presented in western and northern parts of Taihu Lake (the third largest freshwater lake in China) located in the Yangtze River Delta, Jiangsu Province whose mean depth is only 1.8 m. The "black spot" events (the decay of numerous algal or other organic materials makes the entire water column black) occurred regularly along the lake shore and triggered serious problems. The 2007 drinking water crisis in the city of Wuxi is an evident example where the water level was lowered drastically (Zhu et al. 2013; Yang et al. 2012). Besides, City Park Lake in Louisiana, USA, is also a shallow urban hyper-eutrophic lake with a 56-day mean HRT

and 1.2-m depth. The lake became highly eutrophic and suffered from frequent and severe algal blooms in late 1970s (Ruley and Rusch 2002). The HRT of Qingcaosha Reservoir is about 50 days, and it may be further extended with salt water intrusion from the East China Sea, which, in turn, may increase the risk of algal bloom in the reservoir (Lin et al. 2009).

Eutrophication in lakes, estuaries, and slow-moving streams all stimulate microbial growth, and thereby, algal bloom occurs if such growth is not restricted, which will cause an ecosystem imbalance and may even pose a detrimental effect to human health (Liang et al. 2013). Cyanobacterial bloom is one of the most severe problems in eutrophic freshwater systems, and it can trigger harmful effects related to drinking water safety and quality (Yen et al. 2012). Many of these blooms are toxic and have capability of producing several potent neurotoxins and hepatotoxins (Rodger et al. 1994). Conventionally, microscopic examination is usually employed to determine the identification, biomass, and cell counts for the cyanobacterial types in the water samples (Chang et al. 2012). In addition, extraction and analysis of photosynthetic pigments, such as chlorophyll *a,* is also widely used in laboratory. However, these methods are not able to rapidly monitor the changes of cyanobacteria community and distribution in water bodies (Zamyadi et al. 2012). With the development of molecular biology, polymerase chain reaction (PCR)-based methods have been commonly used to detect the genotype-specific target genes in samples. The most widely used marker gene is the small subunit ribosomal RNA (rRNA) gene (16S rRNA), and the recent application of molecular techniques in a variety of habitats has produced a large set of sequences from this gene (Zwart et al. 2002). Molecular methods based on 16S rRNA gene analyses like denaturing gradient gel electrophoresis (DGGE) and real-time quantitative PCR (*q*-PCR) represent the powerful tools to study bacterial community structure (Luo et al. 2009; Samant et al. 2012), showing great superiority compared with other traditional methods.

In order to investigate the microbial community composition during the test running period of Qingcaosha Reservoir, PCR-DGGE, q-PCR technique, and the traditional microbiological techniques were used to analyze the temporal and spatial variations of microbial community in this research. This is the first study to report the microbial community status of the Qingcaosha Reservoir. It is expected to provide fundamental information for the management of eutrophication and cyanobacterial bloom control of the reservoir.

Materials and methods

Sampling site layout

The samples were collected individually from Yangtze Estuary and inside Qingcaosha Reservoir from May to December in 2010 during the test run period of the reservoir for the water quality and microbial community analysis. They were collected on-site every month by the plexiglass water harvesting HQM-1 (2.5 L) under 0.5 m of water surface. All field samples were subsequently transferred to lab and then stored after the sampling. Figure 1 showed the Qingcaosha Reservoir in Yangtze Estuary and sampling sites where 11 sites were selected in total. Site R1 to R10 were located inside the reservoir, while R11 is the inlet site, just outside the reservoir. R11-L and R11-H represented samples collected at low tide and high tide, respectively.

Water quality analysis

The water quality parameters, such as pH, total nitrogen (TN), and total phosphorus (TP), were measured according to the Standard Methods (APHA 1998). Values of pH were measured by a digital meter pHS-25 (Shanghai Leici Instrument Co., China) on-site.

Bacterial community composition analysis

Cultivable bacteria counting

Samples were prepared by serial tenfold dilutions and incubated on Luria-Bertani (LB) agar (APHA-AWWA-WEF 1998) for cultivable bacterial counting. The number of colonies was counted and presented as counts per milliliter solution (cfu/mL).

PCR amplification and DGGE

Pretreatment procedure for PCR amplification and DGGE is as follows: 300 mL water samples were filtered through 0.22-μm cellulose acetate membrane for DNA extraction. Genomic DNA of cells on membrane was extracted by the Bacteria Genomic DNA CTAB Miniprep Kit713 (BoCai Biotechnology Inc., China). The extraction procedures were performed following the manufacturer's menu. Extracted DNA was stored at −20 °C for later PCR-DGGE and q-PCR analysis. For total bacteria, the variable V3 region of the 16S rRNA (Muyzer et al. 1993) gene was amplified with the extracted DNA from water as templates. Primers 27f/1492r (Wu et al. 2007) were used for the first 16S rRNA gene fragment amplification. A final volume of 50 μL was used in the assays, which contains 10× PCR buffer (5 μL), 10 mmol/L deoxynucleotide triphosphates (dNTPs; 1 μL), 25 mmol/L $MgCl_2$ (4 μL), 10 μmol/L primers (1 μL), respectively, Taq polymerase (2U), DNA template (2 μL), and double-distilled water. The variable region 3 of the 16S rRNA gene was amplified by using F357-GC and R518 (Muyzer et al. 1993) for the second amplification, which followed a 35-cycle PCR program (initial denaturation at 94 °C for 3 min; subsequent denaturation at 94 °C for 30 s;

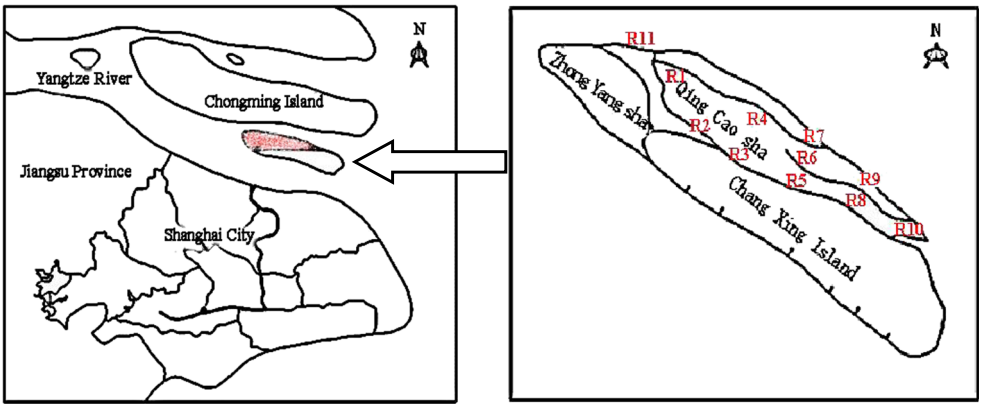

Fig. 1 Sampling site distribution of Qingcaosha Reservoir. The *left picture* shows Qingcaosha Reservoir in Yangtze Estuary, and the *red parts* indicate the reservoir. The *right picture* shows Qingcaosha Reservoir and sampling sites where R1~R10 are the inside reservoirs, and R11 is the outside reservoir

annealing at 57 °C for 45 s; extension at 72 °C for 40 s; and final extension at 72 °C for 7 min). The DNA quality was determined by 1.0 % agarose gel electrophoresis and image analysis. DGGE was done by the Bio-Rad Dcode System (Bio-Rad, Hercules, CA, USA). The PCR products were loaded onto 8 % (*w*/*v*) polyacrylamide gels in 1× TAE (20 mM Tris, 10 mM acetate, 0.5 mM EDTA, pH 8.0). The polyacrylamide gel with a denaturing gradient ranging from 37 to 55 % (where 100 % denaturant contains 7 M urea and 40 % formamide) was prepared by a gradient maker (Shanghai Huxi Instrument Co., China). Electrophoresis was run at 60 °C for 4.5 h with 1× TAE buffer at 180 V. The gel was then stained in 250 mL buffer containing 25 mL of 0.1 mol/L NaCl, and 75 μL of 10,000X GelRed™ for 20 to 30 min. The stained gel was placed in a UV transilluminator and digitized with a gel documentation system (Shanghai Tannon Instrument Co., China). For cyanobacteria, primers 27f/1522r (Suzuki and Giovannoni 1996) were used for the first 16S rRNA gene fragment amplification. A total volume of 50 μL was used in the assays, which contains 10× PCR buffer (5 μL), 10 mmol/L dNTPs (1 μL), 25 mmol/L $MgCl_2$ (4 μL), 50 pmol of each primer, Taq polymerase (2U), DNA template (1 μL), and double-distilled water. The thermal PCR profile was as follows: initial denaturation at 94 °C for 3 min and denaturation at 94 °C for 1 min followed by 35 cycles of primer annealing at 55 °C for 50 s, chain extension for 1 min at 72 °C, and finally extension at 72 °C for 10 min. A total volume of 2 μL of the PCR product was used as template in a subsequent PCR, performed in 50 μL volume under the same conditions as above, except for using different primers: CYA359F-GC and CYA781R (reverse primer CYA781R was an equimolar mixture of CYA781R (a) and CYA781R (b)).

Sequencing

Individual DGGE bands were cut down from gels and put into the tube with 20 μL of sterile distilled water. Three microliters of the eluted band was re-amplified with non-GC-clamped primer pairs. The procedure was followed from the manufacturer's instructions. The products were sequenced, and the sequencing reactions were executed by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. Sequences were blasted in the NCBI GenBank database. The sequences determined in this study are available in GenBank under JF431093 to JF431104.

q-PCR

Total bacterial 16S rRNA genes, cyanobacteria 16S rRNA genes, and *Microcystis* 16S rRNA genes (Harms et al. 2003; Becker et al. 2002) were quantified in duplicate by q-PCR. SYBR green I, a nonspecific fluorescent dye, was selected in this study (Feng et al. 2012). The DNA extractions for each sample were diluted from 20 to 1000 folds to determine the optimum DNA concentration for q-PCR. DNA template (1 μL) and appropriate primers (400 nM) were combined with iQ Supermix PCR reagent (BioRad). All reactions were run with quantitative DNA standards as positive controls and DNA-free as negative control parallel (Xie et al. 2013). Copy numbers were determined with a standard curve, which was obtained by serial plasmid dilutions of a quantitative plasmid DNA containing a 16S rRNA gene fragment (Ye et al. 2011; Jamal et al. 2012). Quantitative results were reported as copies per milliliter of water samples and normalized to 16S rRNA gene copies.

Statistical correlation analysis

Data for variations in physicochemical and biological parameters over time are given as mean±standard deviation (SD). Correlation and regression analysis was used to evaluate relationship between the microbial amount and water quality conditions. The correlation coefficient is a measure of linear association between two variables. All statistical analyses of the data were performed using SPSS 18.0 software

(SPSS, Inc., Chicago, IL, USA), and $P<0.05$ was considered statistically significant.

Multivariate techniques such as multidimensional scaling (MDS) have been proved to be sensitive in detecting the similarity and difference of study objects. MDS can create a multidimensional space perceptual map, the distance of the points react to their similarities or differences. The cluster analysis was used in this study to measure the similarity of bacterial community among different sites.

Results

Temporal variation of the TN and TP

It can be seen from Fig. 2 that TN concentration fluctuated from 0.12 to 0.84 mg/L inside and 0.62 to 2.32 mg/L outside the reservoir. TN concentration reached the peak in June, while dropped to the lowest point in September and November. Besides, the TP content was 0.012 to 0.060 mg/L and 0.050 to 0.172 mg/L inside and outside the reservoir, respectively. Likewise, the highest was detected in August and lowest in November. And, both TN and TP concentration in the reservoir were lower than that in the outside sampling sites. As a result, their mean removal efficiency was 73 and 65 %, respectively. The detected nutrient content reduction could show that Qingcaosha Reservoir can purify itself and thereby significantly improve water quality within the reservoir.

Cultivable microbial counts analysis

Figure 3 displayed the cultivable microbial amount at different sample sites in Qingcaosha Reservoir in four seasons. It can be seen that the number of cultivable microbes in spring and summer was higher than that in autumn and winter, where a two order of magnitude discrepancy was found in outside sampling sites, and this figure also show that bacterial amount

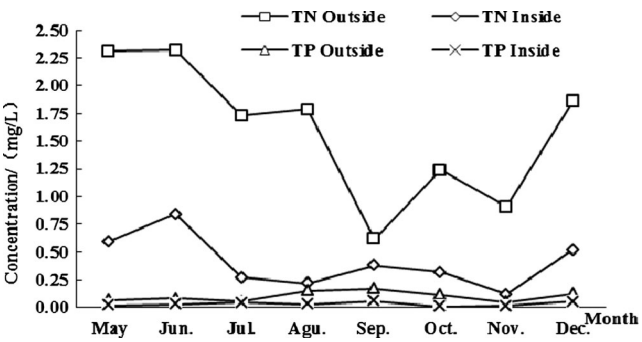

Fig. 2 Trend of TN and TP concentration from May to December of Qingcaosha Reservoir

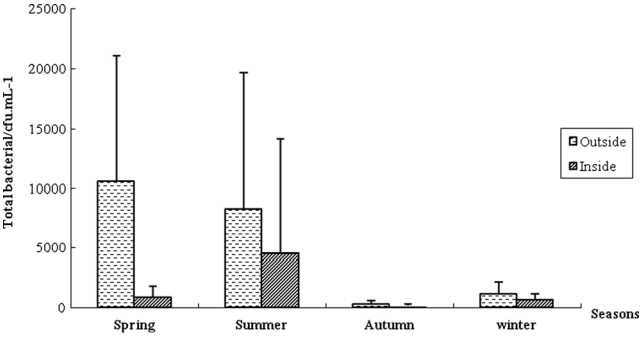

Fig. 3 Results of the average cultivable microbial amount of Qingcaosha Reservoir in different seasons

outside was significantly higher than the one inside. In spring and summer, the average bacterial number is around 10^5 cfu/mL, while in autumn and winter, it decreased to less than 10^3 cfu/ml in the reservoir.

Bacterial community structure analysis

DGGE analysis

As shown in Fig. 4, only DGGE profile of July was presented because it owned relatively higher microbial diversity compared with other months'. It can be seen in Fig. 4 that the common bands were 2, 3, 9, and 11. Band 1 and 4 were the only shared bands in the reservoir, while band 6 was only presented in the influent samples. These main DGGE bands (No. 1 to 12)were cut out and sequenced, and the blast results to gene bank were shown in Table 1.

Most DGGE bands representing the bacteria belongs to the bacterial groups detected from lake and seawater (Zwart et al. 2002), which include *α-Proteobacteria*, *β-Proteobacteria*,

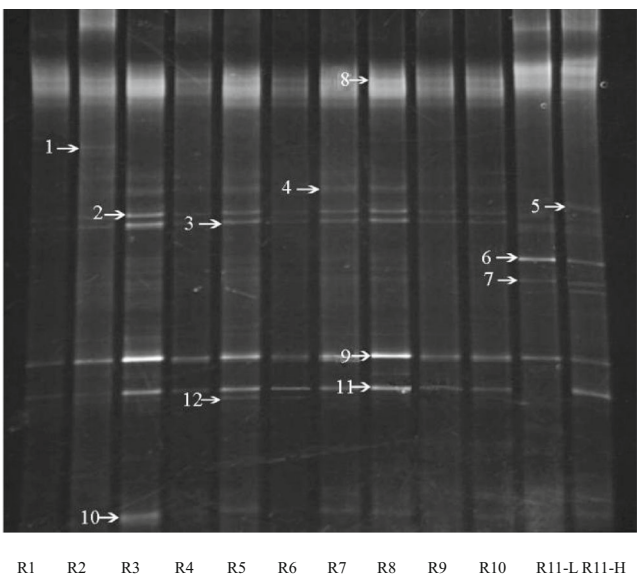

Fig. 4 DGGE sequencing banding profiles of bacteria of Qingcaosha Reservoir

Table 1 Blast results of sequenced DGGE bands

Band			Closest relative			
No.	Accession no.	Similarity (%)	Organism	Accession no.	Phylogenetic affiliation	Source
1	JF431093	99	Uncultured *α-Proteobacterium* clone WA0.2-0d-24 16S rRNA gene	HM153614	Bacteria; *Proteobacteria*; *Alphaproteobacteria*	China: Lake Taihu
2	JF431094	99	Uncultured bacterium clone sw010 16S rRNA gene	GQ258094	Bacteria; environmental samples	Spain: Mediterranean, Punta de Santa Anna, seawater
3	JF431095	94	Uncultured *cyanobacterium* clone FALLScyano01F06 16S rRNA gene	DQ398148	*Cyanobacteria*; environmental samples	USA: Falls Lake, North Carolina
4	JF431096	100	Uncultured bacterium isolate DGGE gel band 0.22-28 16S rRNA gene	GQ342217	Bacteria; environmental samples	China: Hong Kong surface seawater collected from coastal water
5	JF431097	99	*Synechococcus* sp. BAC 106-1 16S ribosomal RNA gene	DQ407518	*Cyanobacteria*; *Chroococcales*; *Synechococcus*	Russia: Lake Baikal
6	JF431098	100 %	*Flavobacterium* sp. BD-b365 16S rRNA gene	EF575563	*Bacteroidetes*; *Flavobacteria*; *Flavobacteriales*	*Flavobacterium* resistens
7	JF431099	89	Uncultured *Rheinheimera* sp. clone 2-42 16S rRNA gene	GQ464399	Bacteria; *Chromatiaceae*; *Rheinheimera*;	Environmental samples
8	JF431100	92	Uncultured *β-Proteobacterium* clone S36.45SM 16S ribosomal RNA gene	AF431284	Bacteria; *Proteobacteria*; *Betaproteobacteria*	Canada: soil from the British Columbia Ministry of Forests
9	JF431101	100	Uncultured bacterium clone DCPA.0912.89	HQ905352	Bacteria; environmental samples	China: Lake Dianchi
10	JF431102	98	*Prochlorococcus* sp. MIT9313 16S ribosomal RNA gene	AF053399	Bacteria; *Cyanobacteria*; *Prochlorococcaceae*	*Prochlorococcus* marinus str. MIT 9313
11	JF431103	99	Uncultured bacterium clone 20bk124 16S rRNA gene	FJ208404	Bacteria; environmental samples	Burkina Faso: Dem reservoir
12	JF431104	98	*Synechococcus* sp. TAG 16S ribosomal RNA	AF448066	*Cyanobacteria*; *Chroococcales*; *Synechococcus*	*Synechococcus* sp. TAG

Bacteroidetes, *Flavobacterium*, *Rheinheimera*, *Prochlorococcus*, and *Synechococcus*. The dominant bacteria are *α-Proteobacteria*, *β-Proteobacteria*, *Flavobacterium*, *Rheinheimera*, *Prochlorococcus*, and *Synechococcus*, and among them, *Prochlorococcus* and *Synechococcus* are cyanobacteria.

Spatial and temporal change of community structure

Four months DGGE profiles of May, July, September, and November were selected to represent the four seasons, and then, their community structures were analyzed (Fig. 5). The distance in the figure represents the similarities of different samples. The shorter the distance, the higher the similarity of the community structure is. On the contrary, longer distance means lower similarity. It can be seen that samples from the same month mostly cluster. However, samples of September and November were clustered closer than that of May and July. Sampling sites inside the reservoir tended to group together but keep distance away from each other outside. The community structure differences among different sites in spring and summer were more obvious than that in autumn and winter. In general, the community structure among different sites within the reservoir owned higher similarity than the outside.

16S rRNA gene abundance analyzed by q-PCR

Figure 6a shows the change of 16S rRNA gene copies of bacteria at different sites in different months. The maximum number of 1.15×10^7 copies/mL appeared in July, and the minimum of 2.38×10^4 copies/mL appeared in October. The former was almost three order magnitude higher than the latter. The average gene copies were 1.83×10^6 copies/mL and 4.16×10^6 copies/mL in and out of the reservoir, respectively.

Figure 6b illustrated cyanobacteria 16S rRNA gene copies in Qingcaosha Reservoir at various sites and in different months. Cyanobacteria amount reached the peak point of 2.35×10^5 copies/mL in September but sharply decreased after September and then stayed at a low level. The lowest amount of cyanobacteria appeared in October. The average cyanobacteria amount was 6.62×10^4 copies/mL outside the reservoir in summer. However, it reached 2.68×10^5

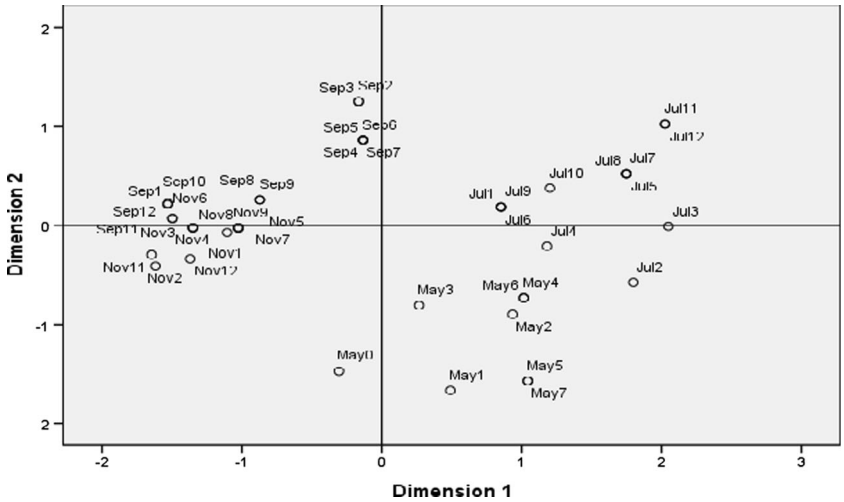

Fig. 5 MDS analysis of bacteria community in different seasons and sites of Qingcaosha Reservoir. May0 is the sampling site outside the reservoir and May1–7 are sampling sites inside the reservoir in May; in other months, No. 1–10 stand for sampling sites R1-R10 respectively, No. 11 for R11-L, No. 12 for R11-H

copies/mL in the reservoir at the same time, which was significantly higher than that of outside the reservoir.

Figure 6 showed that *Microcystis* gene copies had the same trend with cyanobacteria, whose average amount in the reservoir was higher than that of outside the reservoir. And, the mean *Microcystis* amount inside was notably twice higher than the outside reservoir in summer. This may imply that *Microcystis* grew significantly after the water flowing into the reservoir.

Correlation of the microbial gene and water quality parameters

Table 2 shows the relationship between the microbial gene and water TN and TP content in the reservoir. Positive correlations were found between bacteria and TP (r=0.871), *Microcystis*, and cyanobacteria (r=0.965). However, microbial amount had no significant correlation with TN concentration (r=0.447) and *Microcystis* (r=0.441).

Discussion

The concentrations of TN and TP in Qingcaosha Reservoir were lower than that in Yangtze Estuary, which shows that the reservoir could purify itself in terms of TN and TP within certain retention time and thereby improving water quality.

Previous research stated the nitrogen was the limiting factor for the growth of the algae if the N to P (N/P) ratio is less than 16, higher indicates P limitation otherwise (Redfield 1958). In our study, N/P ratio outside the Qingcaosha Reservoir was higher than 29 and 16–29 inside in summer. As in both cases, it was higher than 16, therefore P could be the limiting factor of algal growth (Zhou et al. 2010). Other studies also showed that it risks cyanobacterial occurrence if N/P is less than 29 (Smith 1983). In conclusion, the N/P ratio implies that Qingcaosha Reservoir faces the potential risk of cyanobacterial bloom, especially in long HRT and static water body (Liang et al. 2013). As a result, it is important to understand the status of TN and TP of the reservoir to provide guidance for future reservoir maintenance.

The abundance of total culturable microbes fluctuated with the seasons. It may imply that a large proportion of planktonic bacteria might be deposited or attached with the suspended solid when the flow rate decreased after the water entering reservoir, which therefore decreased the amount of the suspended microorganisms in water body. However, the improved water transparence in warm season would benefit the algal growth.

The DGGE technique can be effectively used to analyze the microbial community structure change and abundance. Each band in the DGGE fingerprint could be considered as one single microbe, and the relative quantities of the certain species in the samples were displayed by DGGE band brightness, namely, the brighter the DGGE band, the higher the bacterial amount (Muyzer et al. 1993). Band 1 was only reoccurred inside the reservoir representing *α-Proteobacteria*, which is closely related to the planktonic bacteria in the Taihu Lake during cyanobacteria bloom stage (Niu et al. 2011). The other major bands (bands 2, 3, 4, 8, 9, 10, and 11) appearing on the inside reservoir samples represented *β-Proteobacteria*, *Bacteroidetes*, and *Prochlorococcus* respectively. *β-Proteobacteria* and *Bacteroidetes* are typically distributed in freshwater lakes. It is widely acknowledged that they mainly exist in natural water bodies, reservoirs, and coastal surface

Fig. 6 Gene copy number of total bacteria, cyanobacteria, and *Microcystis* in Qingcaosha Reservoir based on q-PCR where **a** is total bacteria, **b** is cyanobacteria, and **c** is *Microcystis*; R11-L represented sample of R11 collected at low tide, and R11-H represented sample of R11 collected at high tide

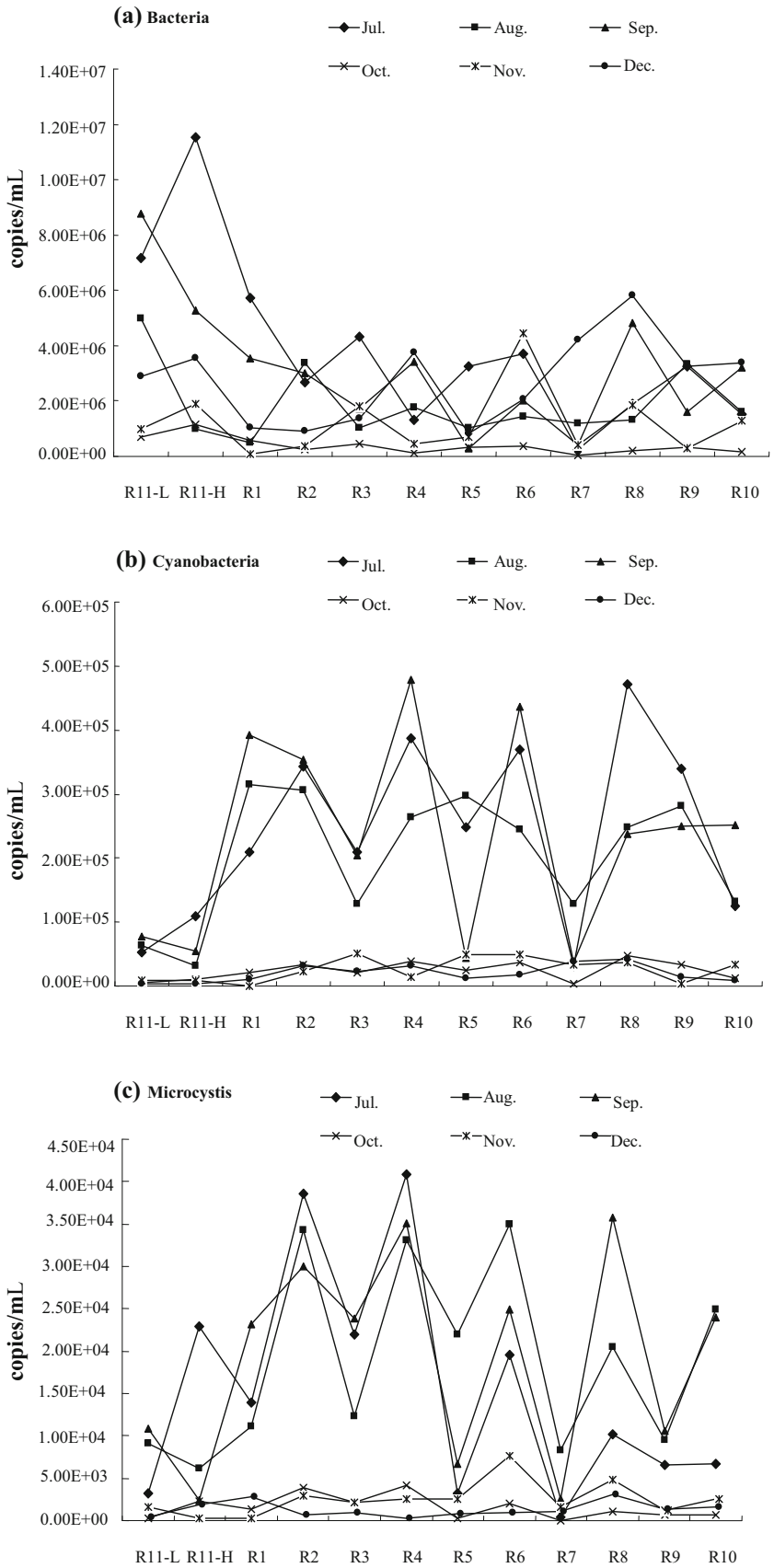

Table 2 Correlations between bacterial gene and TN and TP in Qingcaosha Reservoir

Index	Bacteria	Cyanobacteria	Microcystis	TN	TP
Bacteria	1	0.539	0.441	0.447	0.871*
Cyanobacteria	0.539	1	0.965**	−0.057	0.494
Microcystis	0.441	0.965**	1	−0.065	0.497
TN	0.447	−0.057	−0.065	1	0.681
TP	0.871*	0.494	0.497	0.681	1

*Significant correlation at the 0.05 level, **extreme correlation at the 0.01 level

water bodies where water environment is similar to that of Qingcaosha Reservoir. *Prochlorococcus* is one of the most important representative groups of marine cyanobacteria and main microbe within the global carbon cycle (Rinta-Kanto et al. 2009). The detection of this specie indicates that cyanobacteria are one of the dominant genus in the Qingcaosha Reservoir. However, the difference of the band brightness indicated that abundance of dominant bacteria was not the same in different sampling sites inside the reservoir. Band 6 represented *Flavobacterium*, and it was the dominant species in the influent but less frequently found in the reservoir, which suggests that some bacteria were deposited in the sediment when the flow rate decreased after water enters reservoir. For other bands, band 3, for example, the results showed that it dominated inside the reservoir than outside, indicating that cyanobacteria had significant growth after entering reservoir. Furthermore, band 12 appearing in R1, R2, R3, and R5 representing the *Synechococcus* belongs to cyanobacteria as well. The increase of cyanobacteria species and abundance suggests that Qingcaosha Reservoir has suitable conditions for the cyanobacteria growth in summer and may have potential risk of the bloom occurrence.

The similarity between the communities in the samples taken from the sampling points inside the reservoir suggests efficient mixing of the water in the reservoir; this therefore contributed to even distribution of microbial cells and homogenization of the chemical composition of the water in the reservoir. Moreover, the even hydraulic and nutrient conditions in the reservoir can provide a more stable environment for homogeneous microbial growth (Lin et al. 2009). In Fig. 5, the huge space between samples inside and outside the reservoir in May, July, and September suggested a big difference of bacterial community structure between water samples inside and outside the reservoir. Apart from that, the spacing among these months' samples is significantly farther than that among the different sites'. It means that the impact of the season alternation on bacterial community composition is greater than the spatial site's difference.

The amount of bacteria 16S rRNA gene decreased significantly after entering the reservoir, which is consistent with the culturable microbe count data (Fig. 3). Although there are normally about four 16S rRNA gene copies in a bacterial genome (Case et al. 2007), there existed at least one magnitude difference with that of the cultivable data, which is presumably caused by the unculturability of the majority of environmental microbes (Amann et al. 1995). The q-PCR data showed that cyanobacteria amount was higher in the reservoir in July and September, lower in October though. This may imply that high temperature in summer was suitable for cyanobacteria growth (Cayelan et al. 2012; Wang et al. 2014). The cyanobacterial gene copies are on the contrary of the total bacterial amount. And, this discrepancy reflected that cyanobacteria in water significantly increased after influent flowed into the reservoir. This result was in agreement with the DGGE results, which shows that cyanobacteria are one of the dominated microorganisms at Qingcaosha Reservoir in summer.

The abundance of cyanobacteria to total bacteria 16S rRNA gene copies and *Microcystis* to cyanobacteria gene copies was calculated. It is found that the percentage of cyanobacteria to total bacteria was 15 % in July, August, and September, while below 4 % in November and December. The percentages of *Microcystis* to cyanobacteria were 5 to 10 % in July, August, and September. The cyanobacteria to total bacteria and *Microcystis* to cyanobacteria ratios in summer were significantly higher than other seasons. Similarly, the ratio in the reservoir is higher than outside the reservoir, which suggests that the environment in reservoir may be suitable for the cyanobacteria growth in summer.

Positive correlation (r=0.871, p=0.024) between microbial amount and TP concentration indicates that microbial amount shift has a close correlation with TP changes inside the reservoir. Meanwhile, the bacterial amount correlates to TP concentration inside the reservoir where N/P ratio is over 16. Although the correlation between TP and cyanobateria is less significant (r=0.494), the water quality analysis shows that P could be the limiting factor of algal growth. Further research thus is needed to investigate the rationale behind this. These analyses suggest that TP could be the limiting factor for the growth of microbes inside the reservoir, and controlling TP in the reservoir will be a critical step to maintain water quality (Wang and Wang 2009; Yang and Liu 2010; Nausch et al. 2012).

The abundance of cyanobacteria and *Microcystis* were highly correlated (r=0.965, p=0.002), which is reasonable as *Microcystis* is one of the most dominant species during cyanobacterial bloom and it is also a kind of globally

distributed cyanobacteria and frequently detected in most eutrophic water bodies (Wang et al. 2014). *Microcystis* bloom usually brought about disastrous outcome of water environment (Rinta-Kanto et al. 2009). The occurrence of *Microcystis* in Qingchaosha Reservoir can give prewarning to the reservoir manager of the potential excessive growth and thereby takes the corresponding regulatory measures. The obtained results could provide fundamental information for the control of eutrophication and cyanobacteria bloom during the routine reservoir maintenance.

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