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Full Length Research Paper

Bacterial community compositions in response to sediment properties in urban lakes of Nanjing

Dayong Zhao[1,2]*, Meng Wang[1], Jin Zeng[2,3], Wenming Yan[2], Jianqun Wang[1], Ting Ma[1] and Rui Huang[1]

[1]College of Hydrology and Water Resources, Hohai University, Nanjing, 210098, China.
[2]State Key Laboratory of Hydrology-Water Resources and Hydraulic Engineering, Hohai University, Nanjing, 210098, China.
[3]State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China.

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Compared to other lakes, urban lakes are often shallow, highly artificial and hypertrophic due to the higher level of public interface. Bacteria in lake sediment are important participators in the nutrient cyclings in lake ecosystems. In this study, bacterial community compositions in surface sediment of three urban lakes (Lake Xuanwu, Lake Yueya and Lake Pipa) of Nanjing were investigated by using the terminal restriction fragment length polymorphism (T-RFLP) of 16S ribosomal RNA genes followed by cloning and sequencing. At the same time, the response of bacterial community composition to sediment properties was assessed by multivariate analysis. The results indicated that most of the sampling stations in Lake Xuanwu showed similar T-RFLP pattern, suggesting the similar bacterial community compositions in these stations. However, the bacterial T-RFLP patterns varied among different sampling stations in sediments of Lake Yueya and Lake Pipa. *Chloroflexi* were the most dominant bacterial group in the clone library constructed from Lake Yueya (26.0% of the total clones). Whereas *Betaproteobacteria* were the most abundant group in the clone library from Lake Pipa (18.6% of the total clones). The higher abundance of *Chloroflexi* in sediment of Lake Yueya could be attributed to the higher concentrations of organic matters (OM) and total nitrogen (TN) in the sediment. Canonical correspondence analysis (CCA) showed that the bacterial community compositions in lake sediment were significantly related to the concentrations of OM in the sediment, which was associated with the macrophytes and phytoplankton in the lake ecosystems.

Key words: bacterial community compositions, sediment, urban lakes, multivariate analysis.

INTRODUCTION

Bacteria in lake sediment play important ecological and biogeochemical roles in the freshwater ecosystem, which include regulating the decomposition and transformation of organic matters (OM) and biogenic elements such as C, N, P, Fe, O, and S (Nealson, 1997; Zeng et al., 2008). Therefore, elucidating the composition of the bacterial community is a key step for better understanding the metabolic processes in the freshwater ecosystem (Nixdorf and Jander, 2003; Koizumi et al., 2004). In the past decade, the culture-independent methods based on bacterial 16S rRNA sequences, such as terminal-restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis (DGGE) have been widely employed to investigate bacterial diversity in various environments (Muyzer et al.,

*Corresponding author. E-mail: dyzhao@hhu.edu.cn. Fax: +86 25 83787891. Tel: +86 25 83787891.

1993; da C Jesus et al., 2009; Yang et al., 2011; Zhao et al., 2011).

The composition and diversity of bacterial community are closely related to the sediment properties. While both the sediment properties and the bacterial community structure in the freshwater lake ecosystem have been well documented, the two related aspects were often considered separately (Koizumi et al., 2004; Carini et al., 2005). Investigating the differences of bacterial diversities in lake sediments in respond to the variations of sediment characteristics, would provide useful data for better understanding the complex bacterial ecology in freshwater ecosystems. At present, multivariate analysis methods such as principal component analysis (PCA), non-metric multidimensional scaling (NMDS) and canonical correspondence analysis (CCA) have proven to be robust methods for interpreting the relationship between the bacterial community composition and environmental factors (Iwamoto et al., 2000; Salles et al., 2006). Based on these techniques, environmental variables such as salinity (Ikenaga et al., 2010), nutrient concentrations (Rubin and Leff, 2007; Wu et al., 2008), organic matters (OM) (Bissett et al., 2007), pH (Stepanauskas et al., 2003) and plant cover type (Jensen et al., 2007) have been proven as important factors influencing the bacterial community composition in the marine and river ecosystem. However, whether these important environmental factors also have significant effects on the bacterial community in lake sediment has received little attention.

Urban lakes are very different from other rural and natural lakes: they are shallow, highly artificial and often hypertrophic. Meanwhile, urban lakes always receive higher level of public interface, especially in the densely populated cities such as Nanjing and Wuhan in China (Birch and McCaskie, 1999). Lake Xuanwu (surface area: 3.7 km^2; average depth: 1.43 m) in Nanjing is a typical urban, shallow lake, as well as a famous resort lake of China. The increasing amount of domestic wastewater discharged into the lake has caused severe eutrophication and the recreational value of the lake is also affected. Lake Yueya in Nanjing (surface area: 0.17 km^2; average depth: 2.0 m) was originally part moat of the city. Domestic wastewater and tourism were the main pollution source, which caused the severe eutrophic status in this lake [the average concentrations of total nitrogen (TN) and total phosphorus (TP) in 2006 reached to 2.78 and 0.26 mg/L, respectively] (Yao et al., 2009). Lake Pipa is an urban mini-lake, located in the exterior margin of Zhongshan scenic spots (Nanjing City), which has a surface area of 2.66×10^4 m^2. Water quality of Lake Pipa was fine before the discharge of domestic wastewater from a hotel named Pipa Villa. The hotel was demolished in October of 2005, and the water quality is being recovered slowly.

There have been few studies on documenting the spatial variability in nutrient and physicochemical parameters in sediment of these small urban lakes (Xue et al., 2004). However, the distribution and composition of the bacterial community in lake sediment, and whether the changes in microbial assemblages were associated with diverse environmental factors are also unknown. The aims of this study were to examine the diversity and community composition of bacteria in the three different urban lakes of Nanjing. For that, T-RFLP analysis of PCR amplified fragments and constructing clone libraries were employed. At the same time, the contributions of nitrogen, phosphorus, OM and pH (which have been proven to be key factors for bacterial community structures in marine and river ecosystem) on microbial community compositions were assessed using multivariate analysis techniques.

MATERIALS AND METHODS

Sediment sample collection and physicochemical parameter analysis

Sediment samples were taken from several stations of three urban lakes of Nanjing, including Lake Xuanwu (X1-X5), Lake Yueya (Y1-Y3) and Lake Pipa (P1-P2). The location of each sampling site was recorded with GPS (Table 1). Sediment samples were collected with a corer sampler (437405, HYDRO-BIOS, Germany). Undisturbed sediment cores were collected from each sampling station in three replicates. Sediment samples were stored on ice and in dark during transport to the laboratory. The surface 0-1 cm sediment was sectioned using the sterile spatula and each replicate was well mixed and stored in sterile 50 mL collection tubes. The samples were stored at -80℃ prior to further analysis.

Sediment pH was measured with specific electrodes (PHB-5, REX, China). TN, TP and OM concentrations were measured according to (Jin and Tu, 1990) after the sediment samples were dried with a Freeze Dryer (ALPHA 1-2, CHRIST, Germany). Ammonia nitrogen (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) were extracted with 2 M KCl, and their concentrations were determined using a continuous flow analyzer (San++, SKALAR, Netherlands) (Wu et al., 2010).

DNA extraction

Sediment sample from each sampling station was used for DNA extraction. After frozen dried, 0.5 g sediment sample (dry weight) was used for DNA extraction based on the methods described by Zhou et al. (1996). The amounts of DNA extracted from samples were quantified using a BioPhotometer (Eppendorf, Hamburg, Germany).

PCR amplification and T-RFLP analysis

Variable region of the bacterial 16S rRNA gene was amplified by the primer set 8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 926r (5′-CCGTCAATTCCTTTGAGTTT-3′) (Liu et al., 1997). The 5′ end of the forward primer was labeled with Cy5. The 50 µl PCR mixture contained 1 µl of each primer (10 pmol), 0.25 µl (1.25 U) of Ex Taq DNA polymerase (Takara, Otsu, Japan), 5 µl of 10 × Ex Taq buffer, 5 µl of dNTPs (2.5 mM each), 1 µl of DNA template (approximately

10 ng), and the sterilized ultrapure water up to 50 µl. PCR amplification was performed by using a Thermal Cycler (S1000, Bio-Rad, MA, USA) with the amplification program as follows: 94℃ for 3 min, followed by 30 cycles of 94℃ for 1 min, 5 5℃ for 1 min, and 72℃ for 1 min with the final extension step at 72℃ for 8 min. Negative controls (without DNA template) were run in all amplifications.

Three replicate of PCR products were combined and processed by Mung Bean Nuclease (Takara, Otsu, Japan) according to the manufacturer's instructions, after which the products were purified by Axygen PCR cleanup purification kit (Axygen Biotechnology Ltd. Hangzhou, China) and then digested by the restriction enzyme *HhaI* (Takara, Otsu, Japan), purified again by Axygen PCR cleanup purification kit, and analyzed by capillary electrophoresis using a CEQ 8000 Genetic Analyzer (Beckman Coulter, Fullerton, CA, USA). Accounting for the small differences in running time among samples, fragments from different profiles with less than 1 bp difference were considered to be the same. Peaks of less than 60 bp or longer than 600 bp were discarded. Meanwhile, only the terminal restriction fragments (T-RFs) with a relative area percent over 1% were included for further processing.

Cloning, sequencing and phylogenetic analysis

Clone library was constructed with DNA samples extracted from sediments of each lake, respectively. To amplify equal amounts of the samples, DNA extracted from each lake were mixed, respectively. PCR amplification was performed using the same protocol as that mentioned above except that the forward primer was not labeled with Cy5. PCR products were purified and ligated into the pGEM-T vector (Promega, Madison, WI, USA) following the manufacturer's instructions. Plasmids were transformed into competent *Escherichia coli* cells (DH5α, Takara, Japan) and the presence of the 16S rRNA gene in randomly selected positive clones was checked by PCR amplification using vector primers (T7 and SP6). Positive clones were sent to the Shanghai Majorbio Bio-technology Co., Ltd., China for sequencing.

Chimeric sequences were identified using the Mallard software package, and all suspicious sequences were excluded from further analysis (Ashelford et al., 2006). The remaining sequences were compared with GenBank entries using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The Ribosomal Database Project classifier was applied to assign the acquired sequences to the taxonomic hierarchy (Wang et al., 2007).

The 16S rRNA gene sequences were aligned using ClustalX (Thompson et al., 1997). A phylogenetic tree was constructed by the neighbor-joining method based upon distances determined by Jukes and Cantor (1969) with 1,000 bootstraps using the MEGA 4.0 program (Tamura et al., 2007). In order to test the phylogenetic assignments based on in silico T-RF analysis, randomly selected clones were analyzed by *in-vitro* T-RF by finding the first *HhaI* enzymatic digestion site downstream from 8f (Li et al., 2011).

Nucleotide sequence accession number

Sequences obtained in this study were uploaded and are available at the GenBank database under accession numbers JN849235-JN849369.

Statistical analysis

The initial detrended correspondence analysis (DCA) results demonstrated that the data obtained in this study exhibited unimodal rather than linear responses to the environmental variables (Lepš and Šmilauer, 2003); therefore, CCA was performed by CANOCO 4.5 software package (Biometris, Netherlands) to explain the relationship between the bacterial community structure and environmental factors. Both the environmental data and species data were not transformed for analysis. Forward selection was used to identify environmental factors that significantly affecting the bacterial community structure (Ter Braak, 1987; Lepš and Šmilauer, 2003).

Meanwhile, statistically significant differences in the bacterial community composition reflected in the clone libraries from Lake Xuanwu, Lake Yueya and Lake Pipa were identified using the ∫-Libshuff software package with 10000 randomizations (Schloss et al., 2004). This program calculates the integral form of the Cramér-von Mise statistic based on Monte Carlo methods. The calculated *P* values were used to determine whether there were significant differences between the two clone libraries (Schloss et al., 2004).

RESULTS

Properties of the sediment samples collected from the three lakes

The pH, nutrient and OM concentrations of the sediment samples collected from three different lakes are shown in Table 1. There was no large scale variation of pH (6.65-7.14) in sediment of three different lakes. TN concentrations in sediment of Lake Yueya (3.56-4.22 g/kg) were higher than those of the other two lakes. The average concentration of TP in the five sampling station of Lake Xuanwu was 1.14 g/kg, which was lower than those of Lake Yueya (1.67 g/kg) and Lake Pipa (1.63 g/kg). Sampling station P2 maintained the highest concentrations of ammonia (37.41 mg/kg) and nitrate (13.45 mg/kg). The average concentration of OM in sediment of Lake Yueya was 6.52%, which was higher than those of the other two lakes.

T-RFLP analysis of the bacterial community composition in sediment of three lakes

The T-RFLP profiles of bacterial community composition in sediment of three lakes were shown in Figure 1. Distinct differences of the T-RFLP patterns were observed among the three different lakes. Sampling stations of X1-X4 in Lake Xuanwu showed similar pattern. In sediment of Lake Yueya and Lake Pipa, by contrast, the bacterial T-RFLP patterns varied among different sampling stations. In the five samples of Lake Xuanwu, a total of 58 distinct bacterial T-RFs were identified, including the T-RFs of 86, 87, 88, 95, 96, 131, 132, 176 and 218 bp with the relative abundance > 4%. Meanwhile, T-RFs of 110, 129, 134, 155, 156, 199, 202, 205, 211, 221, 223 and 230 bp were only found in Lake Xuanwu. Other specific T-RFs of 60, 92, 513, 93, 201, 203, 365 and 565 bp were only detected in the samples of Lake Yueya, and

Table 1. The chemical characteristics of the sediment samples collected from three lakes.

Sampling stations	Locations recorded by GPS	pH	TN (g/kg)	TP (g/kg)	NH_4^+ (mg/kg)	NO_3^- (mg/kg)	NO_2^- (mg/kg)	OM (%)
X1	32.06928N, 118.78914S	6.65	3.54±0.12	0.62±0.04	16.78±0.45	1.46±0.09	0.26±0.02	6.79±0.06
X2	32.07277N, 118.79202S	6.86	2.31±0.09	1.09±0.05	12.57±0.52	3.32±0.12	0.17±0.02	5.23±0.07
X3	32.07289N, 118.79613S	6.84	2.19±0.10	1.64±0.04	13.32±0.69	5.24±0.24	0.35±0.03	5.25±0.15
X4	32.08010N, 118.78980S	7.14	2.26±0.11	1.22±0.06	13.90±0.48	11.28±0.68	0.88±0.03	4.79±0.15
X5	32.08180N, 118.79436S	6.86	2.41±0.10	1.15±0.07	11.80±0.61	4.44±0.21	0.42±0.02	5.06±0.17
Y1	32.03120N, 118.82149S	6.65	3.56±0.11	1.53±0.06	12.93±0.44	4.29±0.18	0.38±0.05	6.29±0.21
Y2	32.03065N, 118.82213S	6.78	3.91±0.13	1.65±0.13	12.46±0.48	10.06±0.48	1.05±0.08	6.72±0.03
Y3	32.02956N, 118.82219S	6.76	4.22±0.10	1.82±0.11	13.30±0.38	5.87±0.36	0.39±0.02	6.54±0.22
P1	32.05627N, 118.81670S	6.70	1.81±0.06	1.68±0.08	17.63±0.57	5.15±0.24	0.81±0.05	5.83±0.16
P2	32.05516N, 118.81576S	6.99	2.37±0.07	1.58±0.07	37.41±0.68	13.45±0.39	0.42±0.02	3.73±0.11

GPS: global positioning system, TN: total nitrogen, TP: total phosphorus, NH_4^+: ammonia nitrogen, NO_3^-: nitrate, NO_2^-: nitrite, OM: organic matters.

T-RFs of 62, 86, 87, 88, 96, 97 and 208 bp were the dominant fragments with their relative abundance > 4%. T-RFs of 86 bp was the most dominated fragment in sediment of Lake Pipa with the relative abundance of 16.7%, followed by 87 bp (15.2%) and 475 bp (14.8%). Only three T-RFs of 94, 99 and 564 bp were specifically found in Lake Pipa.

Clone library analysis

Three clone libraries were constructed to identify the bacterial species generated from the sediment of Lake Xuanwu (n = 42), Lake Yueya (n = 50) and Lake Pipa (n = 43) (Table 2). At the same time, Phylogenetic tree of the clone sequences was constructed using the neighbor-joining method based on the MEGA 4.0 software package (Figure 2).

Clone libraries constructed from the sediment of Lake Yueya and Lake Pipa showed remarkable differences. The most important difference was the abundance of clones affiliating with both *Betaproteobacteria* and *Chloroflexi*. *Chloroflexi* were the most dominant bacterial group in the clone library from Lake Yueya (26.0% of the total clones) whereas *Betaproteobacteria* were the most abundant group in the clone library from Lake Pipa (18.6% of the total clones) (Table 2). Both *Betaproteobacteria* and *Chloroflexi* were the most dominant groups (16.7%) in the clone library of Lake Xuanwu, followed by the *Gammaproteobacteria* group which covers the 14.3% of the total clones (Table 2). Lower percentages of clones affiliated with *Epsilonproteobacteria* and *Actinobacteria* phyla were observed in the library of Lake Pipa, whereas these groups were not observed in the libraries of Lake Xuanwu and Lake Yueya. Dominated clones affiliated to *Betaproteobacteria* grouped together with Burkholderiales and Rhodocyclales. The majority of the *Chloroflexi* obtained in this study were found affiliated with Anaerolineae and Caldilineae (Figure 2).

LIBSHUFF analysis

Statistical comparison results of homologous and heterologous coverage curves based on the LIBSHUFF program are shown in Table 3. Comparisons between the clone library of Lake Xuanwu and the libraries from the other two lakes did not reveal significant differences ($P > 0.0083$). At the same time, result of the calculation between Lake Pipa and Lake Yueya libraries yielded a P value of 0.8354 (the Lake Yueya library is homologous), suggesting some overlap between the two libraries. However, a P value of 0.0036 (the Lake Yueya library is heterologous) was also observed, suggesting that the Lake Pipa library contained more taxa that were not found in the Lake Yueya library.

Assignment of T-RFs

Additionally, the randomly selected clones were analyzed by *in-vitro* T-RF by finding the first *HhaI* enzymatic digestion site downstream from 8f to investigate the phylogenetic assignments of T-RFs. As shown in Table 4, many detected T-RFs could be assigned to defined taxonomic groups. At the same time, several T-RFs including 83 and 99 bp

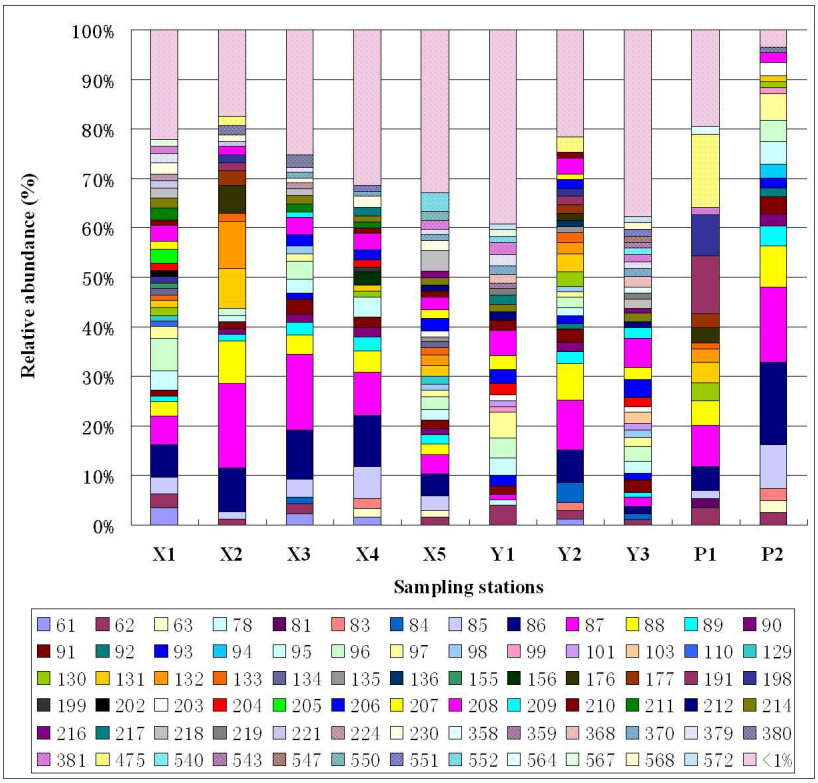

Figure 1. Relative abundance of the bacterial 16S rRNA amplicons recovered from the sediment samples. Those T-RFs of less than 1% of the total abundance were combined together as a single group termed as <1%.

Table 2. Phylogenetic analysis of the clone libraries constructed from the sediment of three lakes.

Phylogenetic group	Number of clones		
	Lake Xuanwu	Lake Yueya	Lake Pipa
Acidobacteria	1	2	2
Actinobacteria	N.D.	N.D.	1
Bacteroidetes	5	5	4
Chloroflexi	7	13	1
Cyanobacteria	N.D.	1	1
Firmicutes	1	N.D.	2
Gemmatimonadetes	2	N.D.	N.D.
OD1	N.D.	2	N.D.
Planctomycete	3	3	1
Proteobacteria (Alpha-)	1	N.D.	3
Proteobacteria (Beta-)	7	7	8
Proteobacteria (Delta-)	2	4	5
Proteobacteria (Epsilon-)	N.D.	N.D.	1
Proteobacteria (Gamma-)	6	6	6
Verrucomicrobia	2	N.D.	3
Unclassified Bacteria	5	7	5
Total	42	50	43

N.D.: not detected.

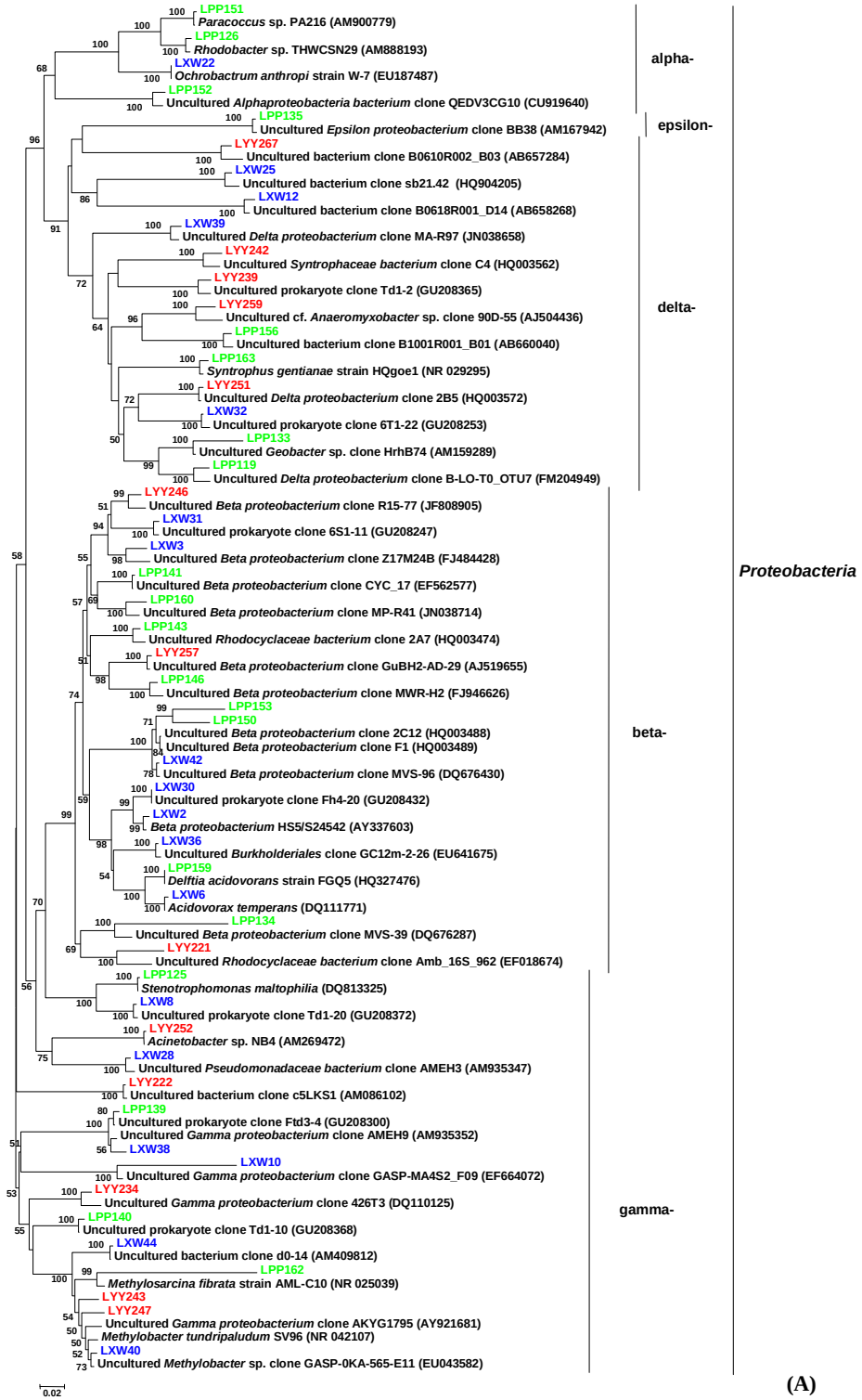

Figure 2A. Neighbor-joining phylegenetic tree for phylum *Proteobacteria* detected in this study. Numbers at nodes represent the percentages of bootstrap resamplings based on 1000 replicates; only the values higher than 50 are presented. Sequences obtained in this study were in color words, with blue, red and green labels for sequences recovered from sediment of Lake Xuanwu, Lake Yueya and Lake Pipa, respectively.

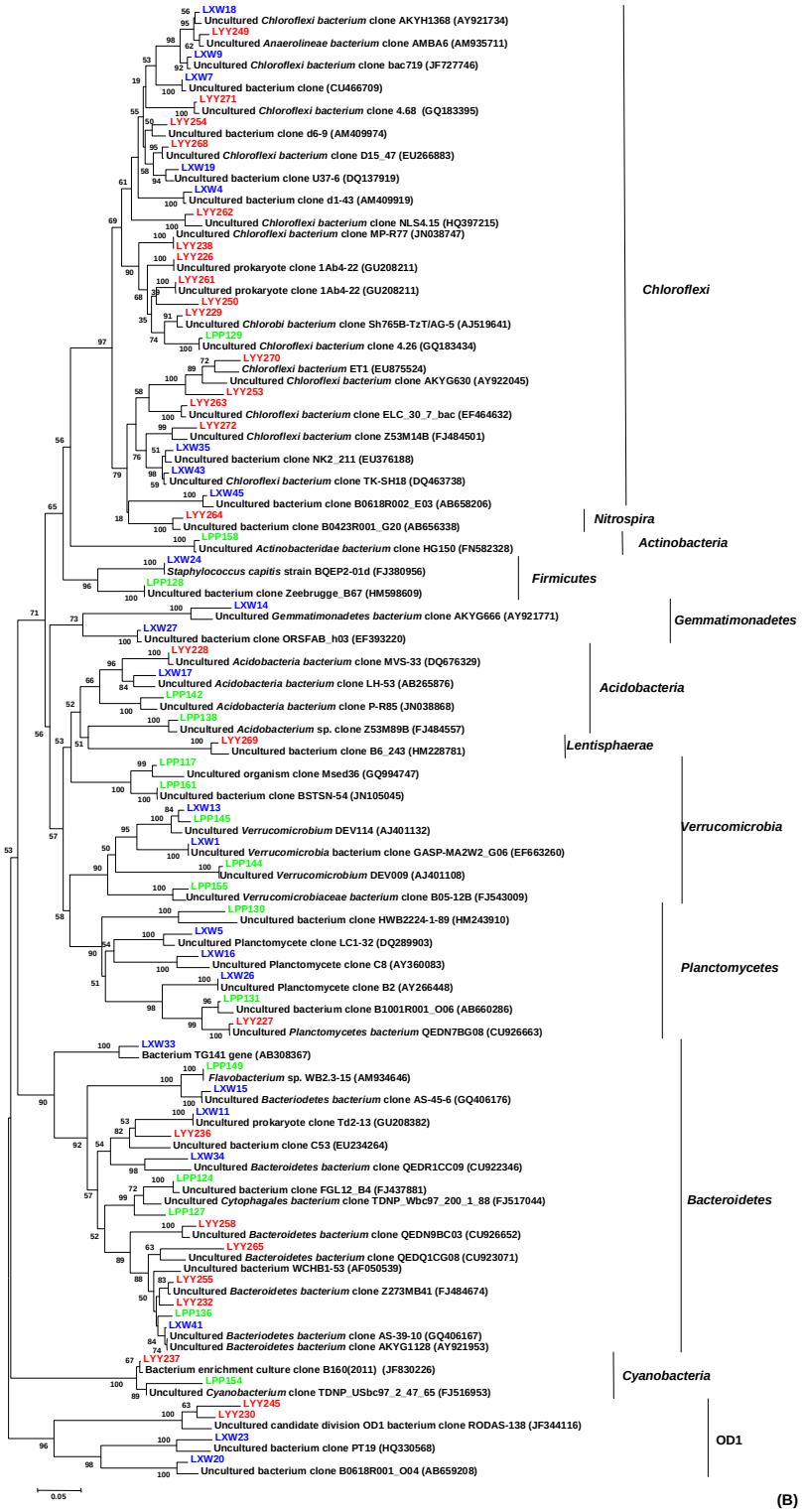

Figure 2B. Neighbor-joining phylegenetic tree for all other phyla detected in this study. Numbers at nodes represent the percentages of bootstrap resamplings based on 1000 replicates; only the values higher than 50 are presented. Sequences obtained in this study were in color words, with blue, red and green labels for sequences recovered from sediment of Lake Xuanwu, Lake Yueya and Lake Pipa, respectively.

Table 3. LIBSHUFF comparisons of the bacterial community of the three clone libraries.

Comparison (X vs Y)	*P*-value	Significantly different
Lake Xuanwu vs Lake Yueya		No
XY	0.5845	
YX	0.1201	
Lake Xuanwu vs Lake Pipa		No
XY	0.9262	
YX	0.8493	
Lake Yueya vs Lake Pipa		Yes
XY	0.8354	
YX	0.0036*	

Libraries were considered significantly different when the *P* value is less than 0.0083. (* indicates significant difference between the two clone libraries.)

Table 4. Phylogenetic affiliations of bacterial 16S rRNA sequences retrieved in clone libraries constructed from the sediment samples of three lakes.

Phylogenetic group	T-RFs (bp)									
	X1	**X2**	**X3**	**X4**	**X5**	**Y1**	**Y2**	**Y3**	**P1**	**P2**
Acidobacteria			370	370	370	370		370		
Bacteroidetes								103		
Chloroflexi	61,62	62	61,62	61	62	62	61,62	62,568	62	62
Firmicutes	230	230	230	230	230					
Gemmatimonadetes	221	221								
Proteobacteria (Beta-)										
Burkholderiales	214		214	214	214	214		214		
Proteobacteria (Delta-)										
Desulfuromonadales									81	
Desulfobacterales									81	
Syntrophobacterales				83*			83*			83*
Myxococcales						78				
Unclassified			98		98	359	98	98		
Proteobacteria (Gamma-)										
Methylococcales	87	87	87	87	87,212	87,212	87	87,212	87	87
Xanthomonadales					212	212		212		
Pseudomonadales	210			210	210	210	210			
Unclassified	214,86	86	214,86	214,86	214,86	214	86	214,86	86	86
Verrucomicrobia			93	83*	203	93,99*,203	83*,93	93,203		83*,93,99*,203
Unidentified	96	96	96		96	96,99*	96	96		96,99*

T-RFs with relative abundance of more than 4% are indicated in bold and T-RFs detected in more than one phylogenetic group are marked with an asterisk.

could be assigned to more than one phylogenetic group. T-RFs obtained from the sediment of Lake Xuanwu could be assigned to *Acidobacteria, Chloroflexi, Firmicutes, Gemmatimonadetes,Verrucomicrobia, Betaproteobacteria* (Burkholderiales) and *Gammaproteobacteria* (Methylococcales and Pseudomonadales) (Table 4). The T-RFs of 230 bp (assigned to *Firmicutes*) and 221 bp (assigned to *Gemmatimonadetes*) were detected only in Lake Xuanwu.

Meanwhile, the T-RFs of 86 (assigned to Unclassified *Gammaproteobacteria*) and 87 bp (assigned to *Methylococcales*) accounted for 4.5-10.3% and 3.8-17.1% of the total bacterial communities in sediment of Lake Xuanwu. In sediment of Lake Yueya, the T-RFs were mainly assigned to *Acidobacteria, Bacteroidetes, Chloroflexi, Betaproteobacteria* (Burkholderiales) and *Gammaproteobacteria* (Methylococcales, Xanthomonadales and Pseudomonadales). Specific

T-RFs of 103 bp (affiliated with *Bacteroidetes*) and 78 bp (affiliated with *Myxococcales*) were only found in Lake Yueya, while the other specific T-RFs were not represented by any of the clone sequences and therefore could not be assigned to any phylogenetic group. *Chloroflexi, Verrucomicrobia, Gammaproteobacteria* (*Methylococcales*) and *Deltaproteobacteria* (*Desulfuromonadales, Desulfobacterales* and *Syntrophobacterales*) were the dominant bacterial populations in sediment of Lake Pipa. The specific T-RF (81 bp) were affiliated with *Desulfuromonadales* and *Desulfobacterales.*

CCA analysis

To explain the relationship between the bacterial community composition and environmental factors, canonical correspondence analysis was carried out, and the results are shown in Figure 3. Arrows represent the environmental factors and the sampling stations are indicated by upward triangles. The first and second axes combined explained 40.4% of the species-environment relationships. Forward selection and Monte Carlo testing indicated that OM significantly ($P < 0.05$) accounted for the variability in the bacterial community composition.

DISCUSSION

The eutrophication status and the diversity of bacterial community in large shallow eutrophic lake such as, Lake Taihu in China have been well documented (Zeng et al., 2009). However, the bacterial communities which take part in the important nutrient cyclings in sediment of small urban lakes were overlooked for a long time. In the present study, the bacterial community compositions in three small urban lakes in Nanjing were compared based on two culture-independent methods, T-RFLP and cloning library. Additionally, multivariate analysis was carried out to explain the relationship between the sediment properties and microbial community structure.

The result of T-RFLP analysis indicated that there were several specific T-RFs representing the specific bacterial taxa in sediment of each lake. At the same time, the bacterial communities in different sampling stations within the same lake were also not the same, which would be attributed to the different sediment properties within the same lake. For example, the concentrations of TN, NH_4^+ and OM in sampling station X1 of Lake Xuanwu were significantly higher than other sampling stations of Lake Xuanwu (Table 1). LIBSHUFF analysis of the clone libraries constructing from three different lakes indicated that there was no significant difference between the bacterial community from Lake Xuanwu and the other two lakes, however, the difference between Lake Yueya and Lake Pipa was significant. Lake Xuanwu is the most important urban lake in Nanjing with the surface area of 3.7 km^2, which is significantly larger than that of Lake Pipa (2.66×10^4 m^2) and Lake Yueya (0.17 km^2). In this study, five sediment samples were collected from three different lake zones of Lake Xuanwu, which would also explain the diversity of clone library from this lake.

CCA analysis revealed that OM was a significant environmental variable which driving the bacterial community composition in sediment of the three lakes. The effect of OM on the bacterial community compositions has been reported previously in both water column and sediment (Li et al., 2011; Macalady et al., 2000; Zeng et al., 2009). Macalady et al. (2000) found that bacterial community structure was strongly related to sediment organic carbon content in a mercury-polluted lake. Autochthonous organic matters in the lake ecosystem mainly come from the phytoplankton and its exudates, which would affect the growth of macrophytes in the lake (Rooney-Varga et al., 2005). The growth status of macrophytes may have different effects on environmental conditions (that is, chemical composition and dissolved organic matter composition) (Zeng et al., 2012), which may affect the bacterial community compositions indirectly. Furthermore, a positive correlation was observed between the number of T-RFs and the OM concentrations in sediment, suggesting OM may promote the growth of heterotrophic bacteria.

Previous studies indicated that the soil bacterial communities were normally comprised of the nine major bacterial phyla: the *Proteobacteria* (mainly the *Alpha*, *Beta* and *Delta* subdivisions), *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Bacteroidetes*, *Firmicutes*, *Planctomycetes*, *Verrucomicrobia* and *Gemmatimonadetes* (Janssen, 2006). There are totally eleven bacterial phyla were observed in this study including, *Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, OD1, Planctomycete* and *Verrucomicrobia.* Among these bacterial phyla, one that should be paid more attention to is the *Chloroflexi*.

Previous studies indicated that *Chloroflexi* was one of the major bacteria group in deep subsurface sediments of the ocean floor (Huber et al., 2006). At the same time, it is also the cosmopolitan members in the activated sludge of various wastewater treatment systems (Björnsson et al., 2002). In the present study, *Chloroflexi* covers 26.0% of the total clone number of the library from Lake Yueya, which was significantly higher than those of the other two lakes. Most of the 13 *Chloroflexi* clones were defined as Anaerolineae and the others were affiliated with Caldilineae. Anaerolineae are the most abundant group in the *Chloroflexi*-specific 16S rRNA gene libraries of activated sludge (Juretschko et al., 2002). Caldilineae are known to prevent membrane fouling in wastewater treatment plant (Miura et al., 2007).

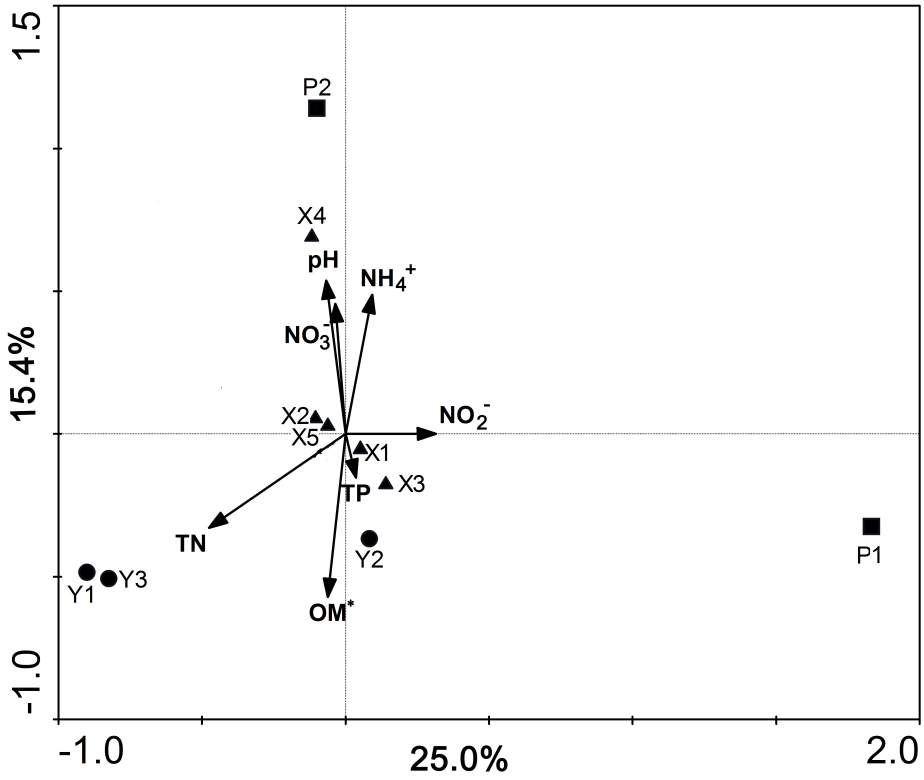

Figure 3. Canonical correspondence analysis (CCA) of the relationship between bacterial community compositions and environmental variables in the sediment samples of three lakes. Arrows indicated the environmental factors. (▲), (●) and (■) represent samples collected from Lake Xuanwu, Lake Yueya and Lake Pipa, respectively.

The *Chloroflexi* sequences obtained in this study was affiliated with previous clones isolated from hydrocarbon-contaminated soil (AM935711) (Militon et al., 2010), petroleum-contaminated saline-alkali soils (JF727746), tar-oil contaminated aquifer sediments (EU266883) (Winderl et al., 2008), wetland mesocosm (GQ183395) or soil (JN038747) and activated sludge (EU875524). Winderl et al. (2008) found that *Chloroflexi* was one of the most abundant groups in the polycyclic aromatic hydrocarbons (PAHs) contaminated soil zone, which further confirmed that the *Chloroflexi* was the dominated group in hydrocarbon-contaminated soil and may involved in the biodegradation process of hydrocarbon pollutants. In this study, the sediment samples collected from Lake Yueya exhibited higher concentrations of OM and TN, suggesting the sediment may be polluted by organic pollutants which partly explained the high abundance of *Chloroflexi* in this lake.

Further isolation of bacteria in this group and investigation of their eco-physiological characteristics would be helpful for revealing their exact role in the decomposition of organic matters in freshwater lakes.

In summary, this study shows the bacterial community compositions in surface sediment of three urban lakes. *Chloroflexi* were the most dominant bacterial group in the clone library from Lake Yueya, whereas *Betaproteobacteria* appeared to be dominated colonizers in sediment of Lake Pipa. Multivariate statistical analysis indicated that OM had significant effect on bacterial community structure in lake sediments. This study points to the interactions between bacterial community composition and sediment environmental characteristics, which could shed light on the roles of microorganisms involved in the biogeochemical cycling of nutrient elements in the lake ecosystem.

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