



Community composition and spatial variation of bacteria in the sediments of a eutrophic fresh water urban lake, East Lake, Wuhan, China

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Abstract: [**Objective**] Sediment bacteria are the important biological factors for remediating of eutrophic environments. To enrich our understanding of the bacteria communities in eutrophic urban lake sediments for better environment protection and pollution control in urban lake eco-systems, we resolved the composition of bacteria communities and their spatial variation in the sediments of a middle-size eutrophic urban lake, East Lake. [**Methods**] We used 16S rRNA gene RFLP and sequencing methods to generate the phylogeny information of the bacteria community, used principal coordinates analysis (PCoA) and canonical correspondence analysis (CCA) methods to resolve the relationship between East Lake and other lakes, and the relationship between environmental factors and the bacteria communities. [**Results**] Sediments inhabited 13 phyla and 2 unclassified clusters. PCoA further revealed that the bacteria communities in three sub-lakes of East Lake sediments were closely related to the communities in similar eutrophic lake environments, and divergent from the hypereutrophic sub-lake Miao Lake, which was also found to inhabit a relative abundant amount of *Thermogymnomonas*-type archaea. CCA further revealed that the distribution of bacteria was closely correlated with the carbon, nitrogen and phosphate contents in the sediments. [**Conclusion**] The environment factors regulated the bacteria community composition and distribution. The results of this study providereference to the research, protection and pollution control on urban lake eco-systems.

Keywords: eutrophic lake, community composition, spatial variation, principal coordinates analysis (PCoA), canonical correspondence analysis (CCA)

Urban lakes are the important members of urban eco-system. They are generally the main undertakers of rainfall, sewage, industrial and agriculture waste^[1-2]. They are also the ecological regulators in sustaining the stability of urban ecological

environment, and contribute to the mitigation of urban climate for high quality of life. During the past 40 years, due to the rapid industrialization and urbanization, urban lakes are suffering serious eutrophication and deterioration^[3-4].

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To remediate the eutrophic lakes, a variety of methods including constructed wetland, active sludge, setting up the ecological floating bed, etc. have been developed^[5-7]. Regardless of the engineering measures, the ultimate purpose of these methods was to speed up the decomposition and remineralization of organic matters by improving the catabolism of the microorganisms. Selectively inoculating the bacteria agency into the lakes could significantly improve the efficiency of the bioremediation of the polluted and eutrophic waterbody. In most cases, the bacteria communities inhabited in the indigenous environments will be valuable resources for inoculums selection^[8-9]. Therefore, it's necessary to enrich our understanding on the composition of the bacteria communities in urban lake, and provide reference to the bacteria inoculumsselection and bioremediation of this ecosystem.

In recent years, the environmental factors, bacteria and phytoplankton communities composition in the environments such as the eutrophic lakes^[2,10], anoxic sediment of a sub-saline shallow lake^[11], rivers water and sediments^[12-14], and the crude oil polluted sediments^[15], etc. have been gradually resolved. But we are still lack of systematic research on the bacteria communities in urban sewage caused eutrophic lake sediments.

East Lake, which locates in the southeast district of Wuhan city, China, is a medium-sized shallow freshwater lake. Since the late of 1970s, due to the injection of sewage, industrial waster water and aquaculture, this lake quickly deteriorated into a eutrophic lake. Currently, with the recovery of environmental protection consciousness of local people and government, ecological remediation and restoration of East Lake have been put onto the

agenda. In this study, bacteria communities in the sediments of this eutropic shallow water lake have been resolved by methods of 16S rRNA gene clone libraries PCR-RFLP analysis and sequencing. We further comparatively analyzed the bacteria community from different ecological environments by principal coordinates analysis (PCoA), and explored the relationship between multiple environment variables and bacteria composition by canonical correspondence analysis (CCA). This study could enrich our understanding of the bacteria community composition, and provide reference to the research, protection and pollution control in urban lake environments.

1 Materials and Methods

1.1 Sample collection and environmental analysis

The sampling site East Lake (114°23'E, 30°22'N) locates in the southeast urban district of Wuhan, China (Figure 1). This lake has a 187 km² watershed area and consists of six sub-lakes. The water sources of this lake partially coming from the adjacent Yangtze River and the rainfall. A few of domestic sewage outfalls randomly distributed along lake. Superficial sediment samples were collected by using Perterson Grab from four sub-lakes Guozheng Lake (GZL), Tangling Lake (TLL), Tuan Lake (TL), Miao Lake (ML) of the East Lake in September 16-17, 2009, and stored in sterile plastic bags and then kept at 4 °C for DNA extraction, environmental and nutrients parameters checking and stored at -80 °C. Three to five samples were collected from each sub-lake (Figure 1). The details on the environmental parameters determination were described by Yang et al.^[16].

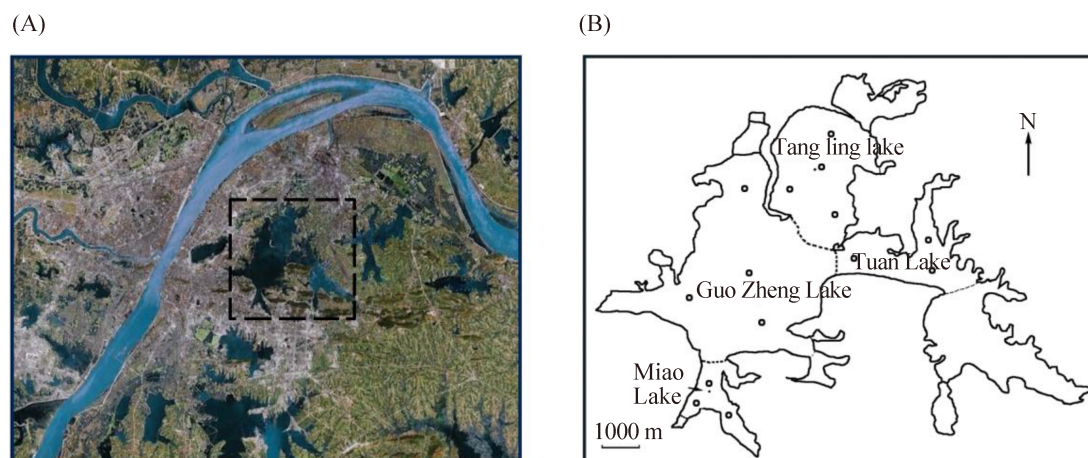


Figure 1. The sampling sites in East Lake, Wuhan, China. A: view of the East Lake and the adjacent areas. B: East Lake and its four sub-lakes. Hollow dots indicates the sampling sites.

1.2 Sediment DNA extraction, 16S rRNA gene amplification and clone library construction

DNA was extracted from 0.5 g sediments using a Mo Bio soil DNA isolation kit (Mo Bio Laboratories, CA, United States). For every sample, three replicates were conducted. The quality and quantity of DNA were checked with a Nanodrop device (Nanodrop, DE, United States). Primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 1492R (5'-GGYTACCTTGTTACGACTT-3') were used to amplify the 16S rRNA gene fragments in a Biometra thermal cycler (Göttingen, Germany). The PCR program was the initial denaturation at 94 °C for 5 min; 28 cycles of 94 °C for 50 s, 54 °C for 50 s, and 72 °C for 50 s; and a final extension of 6 min at 72 °C. PCR products from three reactions were pooled together and cloned into pMD18-T simple vector (TaKaRa, Dalian, China). Positive colonies were randomly picked and cultured in 96-well plates containing LB broth (100 g/mL ampiciline) to construct 16S rRNA gene clone library. Two clone libraries were constructed for each site. The plasmid DNA (about 192 clones) were extracted by N96

Plasmid Kit (Tiangen Biotech, Beijing, China) and then kept at -80 °C for use.

1.3 RFLP analysis and sequencing of 16S rRNA gene clone library

The 16S rRNA gene in every clone was re-amplified by using the above PCR condition. Restriction endonucleases *Hha* I and *Msp* I were used to digest an aliquot of 10 µL PCR products. The DNA fragments were separated electrophoretically on a 15% polyacrylamide gel at 110 mV for approximately 1 h. After ethidium bromide staining, the gels were photographed under UV illumination and the fingerprints of every clone were recorded.

Representative clones of every type of RFLP profile were selected for 16S rRNA gene sequencing by an ABI 3730 automatic sequencer (Applied BioSystems, Foster City, CA) with M13F and M13R primers. The 16S rRNA gene sequences obtained from this study were deposited in GenBank with the accession numbers KP266383–KP266524.

1.4 Clustering, diversity and phylogeny analyses

The 16S rRNA gene RFLP fingerprints of every clone generated from *Hha* I and *Msp* I were

combined and then converted into a two-dimensional binary matrix through a binary scoring system (1 for the presence of a band and 0 for the absence). Based on this binary matrix, the similarity between every 16S rRNA gene clone was evaluated by a simple matching (SM) coefficient. A dendrogram was constructed from the distance matrix by the means of UPGMA algorithm using the NTSYS software package (Applied Biostatistic, Setauket, NY). The rarefaction curves were also generated based on the RFLP patterns of the clone libraries.

The 16S rRNA gene sequences of clones were aligned and the phylogenetic analysis were carried out using the PHYLIP package (<http://www.phylip.com/>). To compare the communities from different environment, the community similarity among clones was determined by using weighted UniFrac environmental clustering and principal coordinates analyses (PCoA)^[17]. The reference sequences from the environments such as Honghu Lake (HM243763-HM243962), acid-impacted lake water (EF520350-EF520549), hexachlorobenzene degrading river sediment (FJ810668-FJ810797), anoxic sediment of a sub-saline shallow lake (HQ003464-HQ003658), and peat swamp forest soil (GQ402546-GQ402745) have extracted from GenBank with the accession number. The correlation between the bacterial communities and environmental parameters was evaluated by canonical correspondence analysis (CCA) using the software Canoco (version 4.5, Microcomputer Power, USA)^[18].

2 Results and Analysis

2.1 Environmental parameters

The environmental parameters in the four sub-

lakes, Guozheng Lake (GZL), Tangling Lake (TLL), Tuan Lake (TL) and Miao Lake (ML) of East Lake, Wuhan city were analyzed and reported by Yang et al.^[16] previously. As reflected by high concentration of chlorophyll-a (Chl-a), nitrogen, phosphate and organic carbon in bottom water and sediments, East Lake was a typical domestic sewage polluted eutrophic lake. Among these four sites, Miao Lake contains the highest content of Chl-a (162.5 g/L), chemical oxygen demand (COD=330 mg/L), NH₄-N (1.26 mg/L), total nitrogen (TN=21.79 mg/g), total phosphate (TP=41.5 mg/g) and total organic carbon (TOC=79.6 mg/g) and the lowest value of TOC/TN, reflecting this sub-lake is a hypertrophic sub-lake polluted by domestic sewage. Tuan Lake was the least eutrophic, with the lowest content of Chl-a, carbon and nitrogen nutrient.

2.2 RFLP clustering of the 16S rRNA gene clones

The RFLP fingerprints of every colony were generated by digesting the amplified fragment of 16S rRNA gene by endonuclease *Hha* I and *Msp* I. These fingerprints were then transformed into a two-dimensional binary matrix to generate the dendrogram of every sub-lake. A detailed view of the dendrogram of each sub-lake revealed East Lake inhabited very abundant diversity of bacteria. Bacteria cluster of Guo Zheng Lake were clustered into six groups, GZL-I, to GZL-VI, and the clones of Miao Lake were divided into ML-I to ML-V groups, the clones of Tuan Lake were divided into TL-I to TL-VI, and the clones from Tang Ling Lake were clustered into five cluster TLL-I, TLL-II, TLL-III, TLL-IV and TLL-V at the level of 40% similarity.

A complete dendrogram contains four sub-lakes were generated from the combined RFLP patterns using UPGMA algorithm (Figure 2). As shown by

the dendrogram, all clones were roughly divided into nine clusters. Cluster I, IV and V were the three major groups. They accounted for 29%, 27% and 14% of the whole community, respectively. The remaining cluster II, III, VI, VII, VIII and IX totally accounted for 30% of the whole community. Cluster I consisted of clones from four sub-lakes, Tuan Lake (25%), Tangling Lake (30%), Guozheng Lake (26%) and Miao Lake (19%). Cluster II contains clones from four sub-lakes, with 86% clones from Guozheng Lake, Tangling Lake and Tuan Lake, and

14% clones from Miao Lake. Similar phenomenon was found in cluster III. Most of the clones in this cluster come from Tang Ling Lake, Tuan Lake, and Guozheng Lake, and just about 18% of the clones from Miao Lake (Figure 2). Cluster IV, V and VII consisted of the clones from every sub-lakes, and Cluster VI was mainly consisted with clone coming from Miao Lake and Tangling Lake. Cluster VIII was mainly consisted of the clones from Guozheng Lake, and cluster IX was mainly distributed in Miao Lake and Tangling Lake (Figure 2).

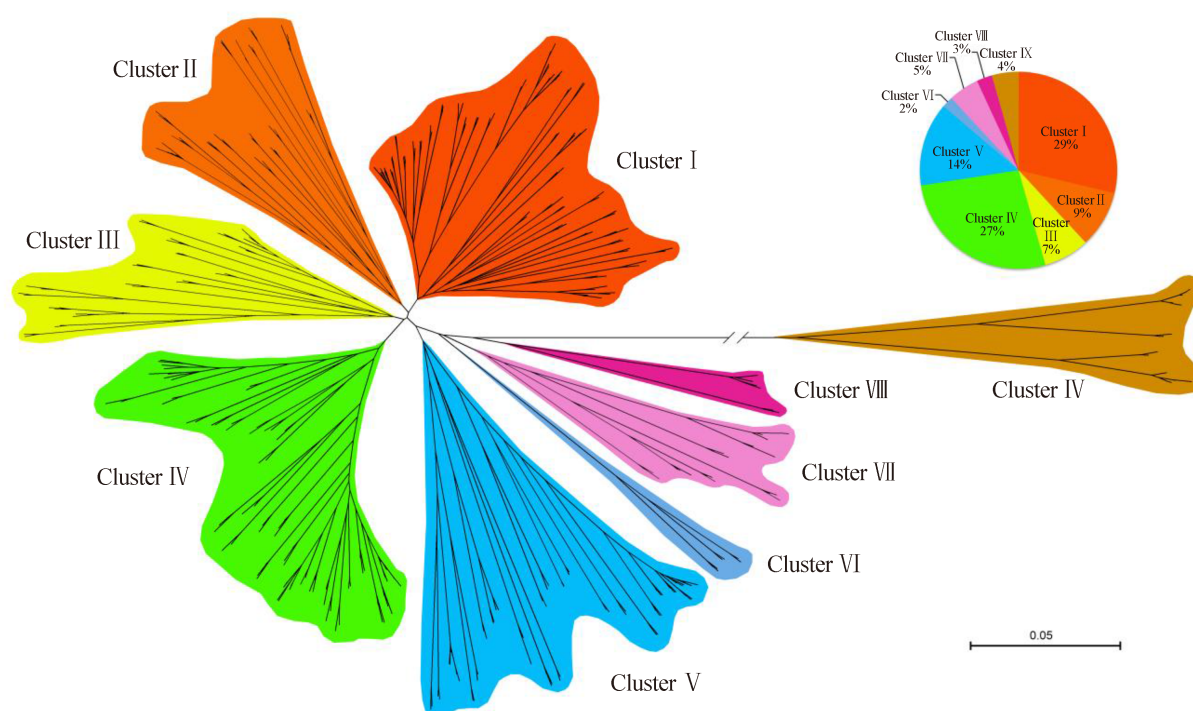


Figure 2. Dendrogram generated from the 16S rRNA gene RFLP fingerprints of all the libraries from four sub-lakes by UPGMA method. Pie charts showing the composition of every cluster in the whole community.

2.3 Phylogeny and community classification

The 16S rRNA genes of the representative clones from every site were sequenced and a phylogeny tree was constructed based on the evolutionary distance between the genes (Figure 3). A dendrogram showing the phylogenetic relationship between

clones and the reference sequences revealed that sequences in East Lake sediment were related to the reference sequences originated from a broad range of environments such as middle sediment from shallow fresh water lake, landfill cover soil^[19], anoxic sediment of a sub-saline shallow lake^[11], peat swamp

forest soil^[20], and crude oil polluted sediments^[15], etc.

About 13 phyla (sub-phyla) have been detected in the communities in East Lake sediments. Among them, Proteobacteria was the dominant group and

accounted for 51% of the whole community. Next to Proteobacteria, phylum Verrucomicrobia (8%), Acidobacteria (8%), Firmicutes (5%) and Bacteroidetes (4%) were also account for

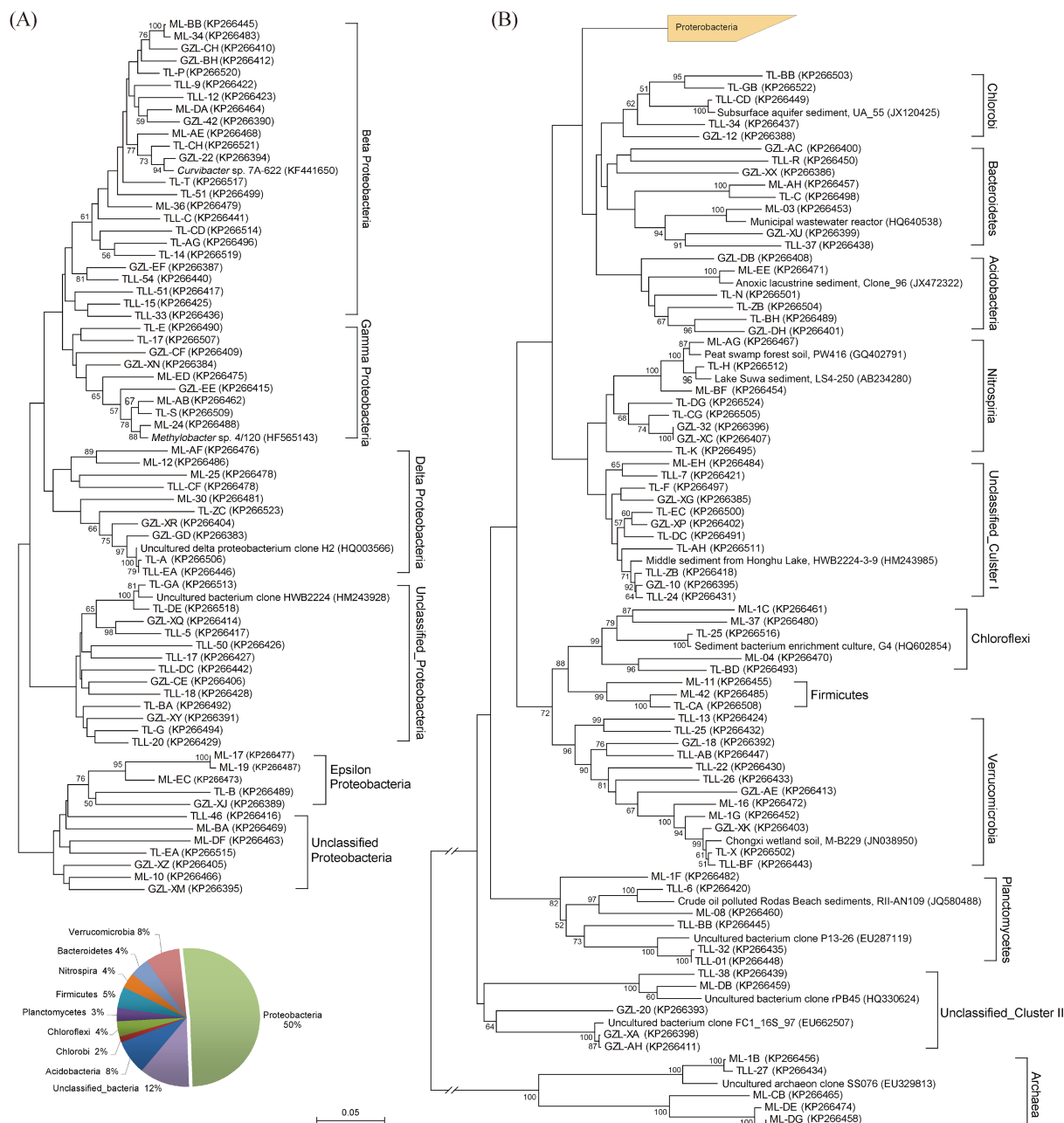


Figure 3. Phylogeny relationships of the representative clones from four sub-lakes basing on the 16S rRNA gene sequence. Bootstrap values (n=1000) greater than 50% are indicated at nodes. Scale bar represents a number of nucleotide substitutions per site. A: dendrogram of Proteobacteria phylum; B: dendrogram generated from whole clones.

phylum groups, clones from Miao Lake were generally belonging to Subdivision 3, and clones from Tangling Lake, Guozheng Lake and Tuan Lake were belonging to *Prostheobacter*, *Opitutus* and subdivision 5 (Figure 4-B).

2.4 Comparison of communities with difference ecological sources

We comparatively analyzed the similarity between communities in East Lake sediment and those from Honghu Lake sediment, acid-impacted lake water^[21], hexachlorobenzene degrading river sediment^[22], anoxic sediment of a sub-saline shallow lake^[11], and peat swamp forest soil^[20] by weighted PCoA analysis (Figure 5).

As shown in Figure 5-A, East Lake sediment communities were closely related to the communities derived from the Honghu Lake sediment (113°07'–114°05'E, 29°39'–30°02'N), a freshwater lake in the middle reaches of the Yangtze River, which was also suffering the eutrophication due to excessive aquaculture and human activities. East Lake communities were distantly divergent from the

communities from the environments such as the acid-impacted lake water, hexachlorobenzene degrading river sediment, anoxic sediment of a sub-saline shallow lake, and peat swamp forest soil. The communities from the eutrophic sub-lake Miao Lake were divergent from the other sub-lakes in East Lake and Honghu Lake and clustered alone. This founding was further proved by Jackknife environmental clustering analysis (Figure 5-B). As revealed by Figure 5-B, Miao Lake was divergent from other three sub-lakes and Honghu Lake. All these communities from East Lake and Honghu Lake were distinctly divergent from other environments such as acid-impacted lake water and sub-saline shallow lake sediment (Figure 5-B).

2.5 Environmental effect on the community classification of bacteria

CCA analysis was conducted to examine the bacterial communities in every sub-lake in response to the environmental variables (Figure 6). As showed by Figure 6, sub-lake Tuan Lake, Tang Lin Lake and Guozheng Lake with relatively lower nutrient content

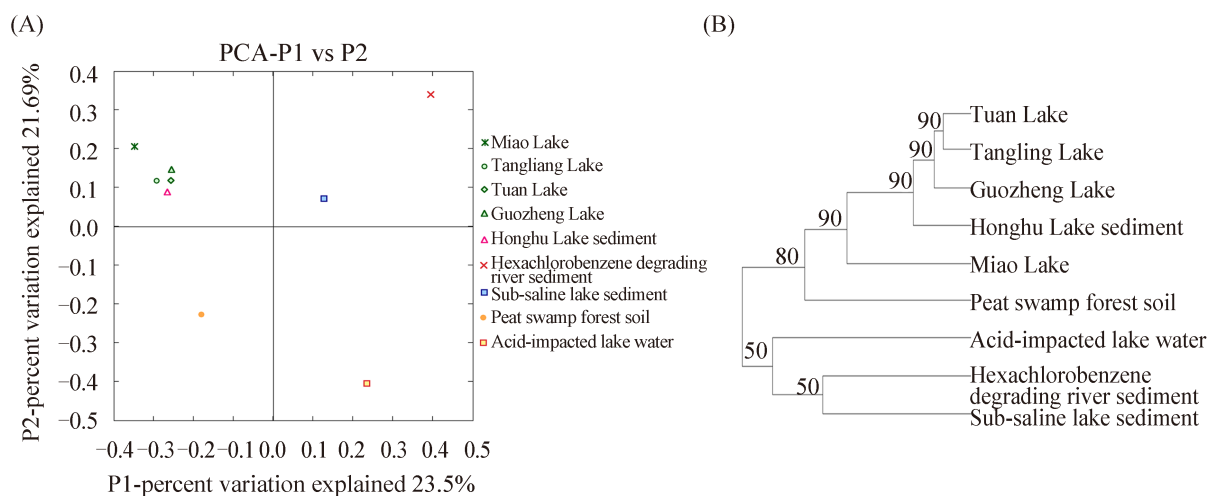


Figure 5. PCoA (A) and Jackknife (B) analysis of the similarity of bacteria communities in East Lake sediments and the references from different geographic locations based on weighted UniFrac analysis. The plot was drawn basing on the first two principal coordinate axes (CCA1 and CCA2).

distributed in the negative region in CCA1, while the hypereutrophic Miao Lake characterized with high concentration of $\text{NH}_4\text{-N}$, TN, TOC, SRP and Chl-a sites in the positive region. The spatial distribution of the bacteria clones classified at phylum level revealed that clones belong to Epsilonproteobacteria, Chloroflexi, Firmicutes, Nitrospira, Planctomycetes and Verrucomicrobia were closely connected with high concentration of carbon, nitrogen and

phosphate. Clones belong to Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Acidobacteria and Chlorobi were related to the lighter eutrophic environments such as Guozheng Lake and Tangling Lake, which have a higher TOC/TN and pH. Clones of Bacteroidetes and Gammaproteobacteria did not show the obvious preference on the environment and almost evenly distributed in every sub-lakes.

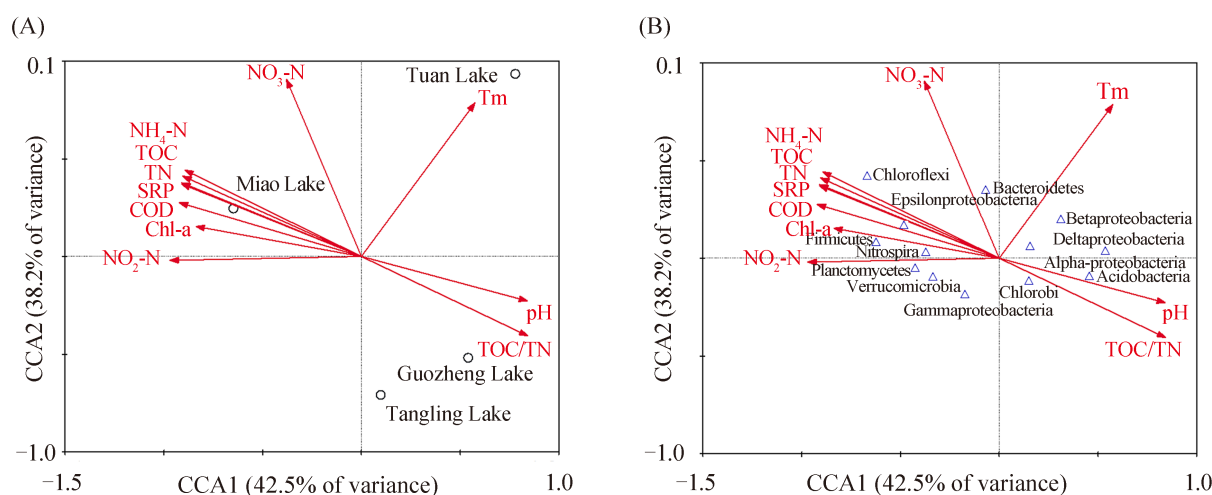


Figure 6. CCA analysis of the relationship between bacteria community composition and environments parameters in East Lake. Correlation between environmental variables is represented by the length and angle of arrows. A: correlationship between the sampling sites and the nutrients parameters; B: correlationship between East Lake bacteria communities classified in phylum level and the nutrients parameters.

3 Discussion

The East Lake, which has been an exurban lake with the water body regularly exchanged with the neighboring Yangzer River (Figure 1), has become an urban lake due to the rapid urbanization since the late of 1970s, and now is suffering eutrophication and deterioration^[16,23]. This lake was geographically divided into several sub-lakes (Figure 1). Among these sub-lakes, Miao Lake was the heaviest eutrophic sub-lake^[16]. This sub-lake was close to the residential communities, and in fact was a catchment of the sewage from the local areas. Moreover, this

lake has a narrow and shallow water area, and has less water exchange with outside sub-lakes. These factors made Miao Lake becoming a hypereutrophic sub-lakes, with the content of $\text{NH}_4\text{-N}$, TN, TP and TOC in lake sediment was far higher (~20-fold) than other mild or moderate eutrophic sub-lakes, shallow Mediterranean lake ecosystem^[1,16], mangrove sediments^[24], Qinhuai River sediments^[2], and the sub-saline shallow lake sediment^[11].

In this study, methods of the 16S rRNA gene RFLP and sequencing were used to evaluate the diversity and the community compositions of East Lake sediments. Presently, new methods basing on

the next-generation sequencing technique such as pyrosequencing and illumina have becoming popular on resolving the diversity and communities of microorganisms in the environmental samples^[25]. The 16S rRNA gene clone libraries RFLP analysis used in this study also appears a priori faster, cheaper and accessible to environmental sampling analysis. Compared with the traditional methods such as DGGE, which purely based on the finger prints of environmental genes, the clone library has supplied much abundant information. The environmental gene clone libraries RFLP analysis method also has its advantages on the traditional time-consuming bacteria isolation. Moreover, the following 16S rRNA gene sequencing on the representative clones supplied sequence data for phylogeny analysis and comparatively analysis among communities from different environments^[26].

In this study, about 13 phyla (sub-phyla) and 2 unclassified clusters have been detected among the East Lake bacteria communities. Apparently, the number of the identified bacteria groups in East Lake sediments was more than those from the environments such as the crude oil polluted sediments^[15], hexachlorobenzene degrading river sediment^[22], and anoxic sediment of a sub-saline shallow lake^[11], but close to the communities from the rivers water^[14], peat swamp forest soil^[20], and landfill cover soil^[19]. Although we did not systematically compare the diversity index among these environments, according to the numbers of the identified bacteria groups, it seems that the eutrophication of urban lake did not weaken the diversity of bacteria community. We speculated that the community composition might be affected by the nutrient sources. Single source of nutrients such as

crude oil^[15] and hexachlorobenzene^[22] might just enrich the specific group of bacteria, and weaken the diversity of the communities. But for the eutrophic lakes, the sources of pollutants were much diverse and the components were also very complicates, thus supplied necessary nutrients for various type of bacteria. Moreover, sediments are generally the carries of the microorganisms from the aerobic surface water, facultative aerobic middle layer water and the anaerobic bottom water. Thus it possibly inhabited more types than the environments such as the water body^[14] and land soils^[19–20].

The spatial variation among the communities from the sub-lakes was observed in this study. *Dechloromonas*, *Methylobacter*, *Sulfuricurvum*, and *Desulfatirhadium*, which participated in the metabolism of the sulfate, more abundantly existed in Miao Lake. As detected by CCA analysis, strains from Alphaproteobacteria, Betaproteobacteria, and Deltaproteobacteria were less related to high nutrient concentration (Figure 6). So they almost evenly distributed in every sub-lakes. While genus from Epsilonproteobacteria such as *Sulfuricurvum* were related to the high concentration of nutrients, and enriched in the heavy eutropic environment. Phyla Firmicutes, Nitrospira, Planctomycetes and Chloroflexi were also related to high nutrient environments, but phyla Acidobacteria and Chlorobi were negatively related.

According to the comparison between the East Lake sediment community and those from the related environments, PCoA analysis revealed that most clones from the three sub-lakes Tuan Lake, Tangling Lake and Guozheng Lake had their closest match with the clones originated from the Honghu Lake sediment, and divergent from the environments such

as the acid-impacted lake water, hexachlorobenzene degrading river sediment, anoxic sediment of a sub-saline shallow lake, and peat swamp forest soil. Honghu Lake (E 113°07'–114°05', N 29°39'–30°02') is a large fresh water lake sits in the middle reaches of Yangtze River. Due to the excessive aquaculture and human activity, this lake is suffering eutrophication. The nutrient environment of this Honghu Lake sediment was similar with three sub-lake of East Lake may explain the similarity of bacteria communities between them.

We also noticed that although our main aim was to detect the bacteria community composition, certain amount of archaea (Euryarchaeota and a group of unclassified archaea) in East Lake sediment were detected by using this 515F/1492R primer pair. Especial in the Miao Lake community, the archaea (mainly *Thermogymnomonas*) account for 8% of total community. *Thermogymnomonas* were first founded in a solfataric field in Ohwaku-dani, Hakone, Japan^[27]. It is possible that excess organic acids produced by the degrading of organic pollutant from sewage supplied a optimal niche for the inhabitation of *Thermogymnomonas*-type archaea, which might gives further testimony that *Thermogymnomonas*-type archaea have more broad ecological adaptability.

In summary, there were thirteen phyla and two unclassified clusters have been detected in eutrophic East Lake sediments. Although Proteobacteria was the dominant group in this eutrophic urban lake sediment, the distribution of bacteria were positively related to the content of $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, TN, TP and TOC, and changed among different sub-lakes. Bacteria communities inhabited in the middle eutrophic sub-lakes were divergent from the communities from hypereutrophic Miao Lake, and

those from peat swamp forest soil, hexachlorobenzene degrading river sediment, and anoxic sediment of a sub-saline shallow lake. This finding suggests similar environmental conditions inhabited similar bacteria community despite geographic distances, and testified that the environmental elements regulate the bacteria community composition and distribution. This study could enrich our understanding of the bacteria community composition in eutrophic urban lake, and provide reference to the research, protection and pollution control on urban lake eco-system.

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富营养化城市湖泊武汉东湖沉积物微生物群体结构及空间变化研究

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摘要: 【目的】城市湖泊沉积物中的微生物群体是富营养环境生物修复的重要因素。本研究拟对中型富营养化湖泊——武汉东湖沉积物细菌群体结构及空间变化规律进行研究。加深对富营养湖泊微生物群体结构的理解, 并为城市湖泊的保护和污染控制提供参考。【方法】采用16S rRNA基因RFLP分析和序列分析技术获得细菌群体系统发育信息。采用PCoA法分析武汉东湖沉积物细菌群体内以及与其它湖泊沉积物细菌群体间的相似性。采用CCA法分析环境因素对细菌群体的影响。【结果】武汉东湖沉积物细菌群体包含13个门和2个未知种群。PCoA分析进一步表明来自东湖3个子湖的细菌群体与其它地域富营养化湖泊的微生物种群结构相似, 并区别于重度富营养化子湖庙湖的细菌群体。在庙湖中, 我们发现了 *Thermogymnomonas* 类古菌的含量显著偏高。CCA分析表明细菌的分布与沉积物中碳、氮和磷元素的含量显著相关。【结论】本研究进一步确证了环境因素对细菌群体组成和分布的调节作用, 加深了对富营养水体微生物群体结构的理解, 并为城市湖泊的保护和污染控制提供了参考。

关键词: 富营养湖泊, 群体结构, 空间变化, PCoA, CCA

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