

# Microbial Community Diversity in Water and Sediment of an Eutrophic Lake during Harmful Algal Bloom Using MiSeq Illumina Technology

Jiangqi Qu<sup>1</sup>, Qingjing Zhang<sup>1+</sup>, Nan Zhang<sup>1</sup>, Laixin Shen<sup>2</sup> and Peibin Liu<sup>2</sup>

<sup>1</sup>Department of Fisheries Ecology and Environmental, Beijing Key Laboratory of fishery Biotechnology, Beijing Fisheries Research Institute, Beijing, 100068, China

<sup>2</sup>Beijing institute of water, Beijing, 100048, China

**Abstract.** Microbial communities of water and sediment during cyanobacterial bloom in an eutrophic lake were studied for the first time by Miseq sequencing of 16S rDNA gene fragments; Biodiversity of microbial communities and the differences in their composition was revealed. *Sphingobacteria*, *Cyanobacteria*, *Phycisphaerae*, *Chlorobia* and *Alphaproteobacteria* were mostly distributed in water samples, whereas *Betaproteobacteria*, *Anaerolineae*, *Gammaproteobacteria* and *Deltaproteobacteria* were mainly observed in sediment samples. Biodiversity index values indicated that microbial diversity in the sediment sample was a slight higher than in the water sample, and there were 278 OTUs overlapped. Present findings may indicate that cyanobacterial blooms can regulate the microbial community structure and diversity.

**Keywords:** Microbial community, harmful algal bloom, Miseq sequencing

## 1. Introduction

High-throughput sequencing platforms have made it possible to recover DNA sequence data directly from environmental samples [1]-[3]. In recent years, the MiSeq Illumina platforms have been applied to human and model organism genomic whole-genome resequencing due to its high capacity [4], [5]. Previous studies have shown that the MiSeq Illumina technology was very useful for the investigation of microbial community composition and biodiversity in the environment [6], [7].

Harmful algal bloom dominated by *Microcystis* spp. in lakes has become a serious environmental problem in China, which do harm to both livestock and human health [8], [9]. It commonly results from the accelerated eutrophication of lake have increased the occurrence and intensity of harmful algal blooms. As an essential part of aquatic ecosystem, microorganism was considered to be closely involved in the nutrient cycling and organic matter decomposition [10], [11]. So far, only little information is available on the diversity of microbial community in water and sediment of an eutrophic lake during the course of harmful algal blooms [12], [13].

In this study, the microbial community characteristic was investigated in a typical shallow hypertrophic lake. This research aims to determine the composition of microbial community during harmful algal blooms by Miseq Illumina platform and improve understanding how cyanobacterial blooms affected the diversity and population structure of microorganism in an eutrophic lake.

## 2. Materials and Methods

### 2.1. Study Area and Sediment Sampling

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<sup>+</sup> Corresponding author. Tel.: + 86-10-67588901; fax: +86-10-67588901.  
E-mail address: qingjingzhang2007@aliyun.com.

Lake Taoranting (39°87'N, 116°38'E) is located at the southwestern corner of Beijing's historic Xicheng District), where eutrophication and harmful algal blooms have been an intractable problem since 2000s. Water and sediment samples were collected from three representatives sites and then mixed to homogeneity to generate one composite sample and stored in -80 °C for further analysis. Samples are used for physical-chemical analysis have carried to laboratory immediately and measured by the standard method [14].

## 2.2. DNA Extraction, PCR Amplification and Pyrosequencing

DNA isolation according to the protocol reported previously [15]. PCR amplification and pyrosequencing were carried out as described by Fan *et al* (2014) [16].

## 2.3. Bioinformation Analysis

The OTU(operational taxonomic unit), Rarefaction analysis, ACE (abundance-based coverage estimator) and Chao1 estimator were all calculated in mothur by Shanghai Majorbio Bio-pharm Technology C., Ltd.

## 3. Results

### 3.1. Environmental Conditions and Cyanobacterial Composition

The environmental conditions of Sediment and water samples were investigated in Lake Taoranting (Table I).The concentrations of TN, TP, NH<sub>4</sub><sup>+</sup>-N and PO<sub>4</sub>-P were 6.5 ± 1.2, 0.14 ± 0.0, 0.07 ± 0.0 and 0.05 ± 0.0 mg/L, respectively. Bacterial abundance was 4.5 × 10<sup>3</sup> CFU/ml in water column. In sediment sample, the concentrations of TN and TP were 3.2 ± 0.7 and 0.42 ± 0.2 mg/g, respectively.The cyanobacterial community was dominated by *Microcystis* spp. (>60%) during harmful algal bloom. Within *Microcystis* spp. six species, such as *Microcystis flos-aquae* (22%), *Microcystis aeruginosa* (35%), *Microcystis pseudo filamentosa* (12%), *Microcystis incerta* (6%), *Microcystis westenbergii* (15%) and *Microcystis marginata*(10%), were observed by the microscope.

Table I: Environmental conditions of Sediment and water samples

Sample	TEMP °C	DO mgmg/L	TN	TP mg/L	NH <sub>4</sub> mg/L	PO <sub>4</sub> -P mg/L
Sediment	25.3±0.2	-	3.2±0.7 mg/g	0.42±0.2 mg/g	-	-
Water	28.8±0.1	3.3±0.3	6.5±1.2 mg/L	0.14±0.0 mg/L	0.07±0.0 mg/L	0.05±0.0 mg/L

### 3.2. Richness and Biodiversity

The microbial community structure in water and sediment samples were analyzed using MiSeq Illumina platforms of the V1-V3 region of the 16S rDNA gene. The number of sequence reads obtained from each sample was 21810 and 31111, respectively. We used the 97% similarity to calculate richness and biodiversity, which estimates based on the indices of Chao1, Ace, Shannon and Simpson (Table II). The estimated richness and biodiversity during the course of harmful algal blooms showed a slight high diversity in the sediment, but no significant differences ( $p>0.05$ ) between water and sediment samples.

Table II: Richness and biodiversity indices relative to each sample

Sample	Reads	0.97				
		OTU	Ace	Chao1	Shannon	Simpson
Sediment	21810	735	774	776	5.69	0.0076
water	31111	555	663	657	4.35	0.0327

### 3.3. Comparison of Microbial Communities

At class level, there were five dominate classes (abundance >10%) among the total 34 classes exist in water sample (Fig. 1), namely, *Sphingobacteriia*, *Cyanobacteria*, *Phycisphaerae*, *Chlorobia* and *Alphaproteobacteria*. From the Fig. 2, it was obvious that *Betaproteobacteria* were the most in sediment sample, followed by OTUs belonging to *Anaerolineae*, *Gammaproteobacteria* and *Deltaproteobacteria*. We also found that the structures of microbial communities in water and sediment samples were quite differently

according to heatmap diagram (Fig. 3), and there were 278 OTUs overlapped between the water and sediment samples and the sediment sample (Fig. 4).

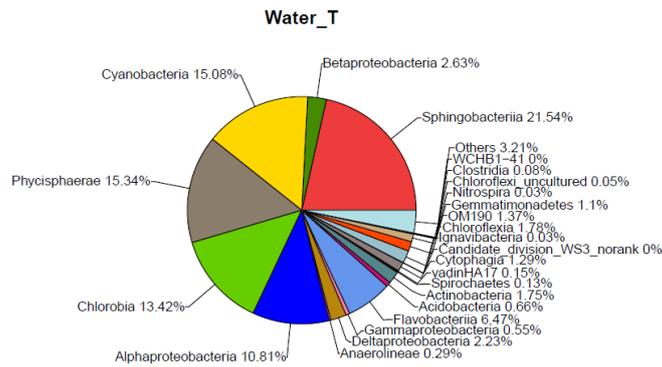


Fig. 1: Relative abundance of microbial community in water sample

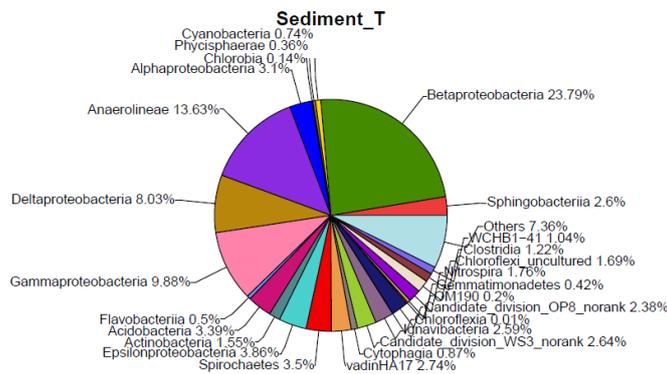


Fig. 2: Relative abundance of microbial community in sediment sample

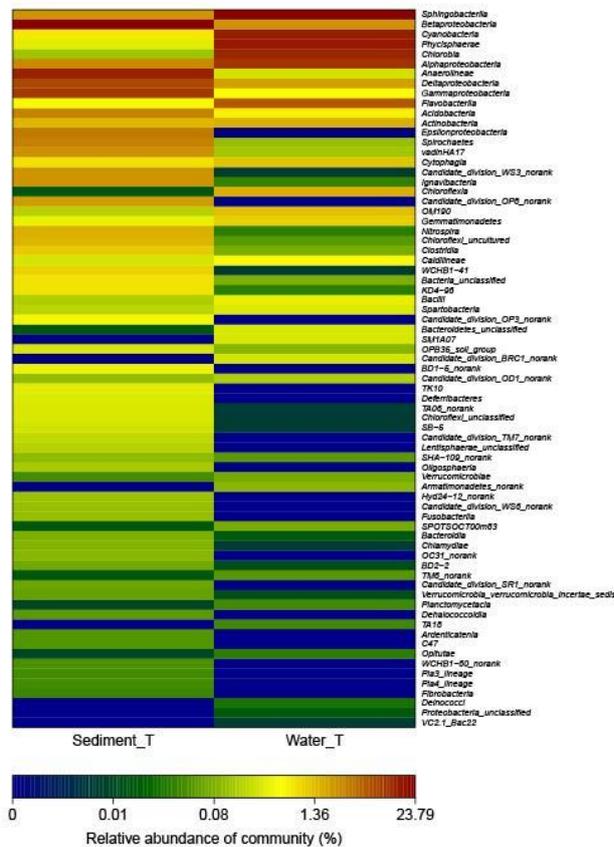


Fig. 3: Heatmap diagram in water and sediment samples

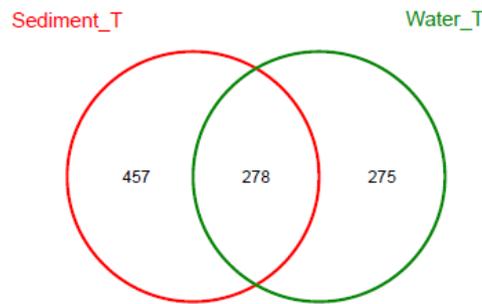


Fig. 4: Venn diagram showing the number of shared OTU between water and sediment samples

## 4. Discussion

The ability of microbial community to adapt to harmful algal blooms is regarded as a critical topic of research in recent years [17]. We used the MiSeq Illumina platform to characterize the effect of toxic phytoplankton blooms on the microbial community of an eutrophic lake. Our result showed that cyanobacterial blooms have heavily affected microbial community structure and diversity.

In the water column, *Cyanobacteria*, *Sphingobacteriia* and *Phycisphaerae* were the most frequently detected OTUs. Our 16S rDNA gene Miseq sequencing data identified numerous *Cyanobacteria*, while Microscopic examination, observations mainly confirmed the sequencing results. The Miseq sequencing analysis clearly indicated that the importance of *Sphingobacteriia* during blue-green blooms, and they are able to degrade not only nitrogen, phosphorus and complex organic macromolecules such as phenoxybenzoic acid, cypermethrin and PCBs, but also microcystin cells [18]-[21]. Previous studies reported that a *Sphingomonas* sp. species belonging to *Sphingobacteriia* was capable of degrading microcystin toxins (Microcystin-LR, RR, YR, LW and LF) [22], [23]. This might be one of the reason why a high abundance of *Sphingobacteriia* in bacterial community during the decomposition of cyanobacterial blooms. *Phycisphaerae* belongs to *Planctomycetacia* phylum which is some common population with cyanobacterial blooms in hypertropication water. Our findings are consistent with Alexander Eiler (2004) reported, which suggests five bacterial divisions, including *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Planctomycetes* and *Fibrobacteres* were closely to toxins algal blooms [24].

*Betaproteobacteria* and *Anaerolineae*, coupled with a bloom event, were predominant in sediment samples in the current study. This finding is not consistent with previous studies of Lake Xuanwu that reported that sequences affiliated to *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia* and *Nitrospira* [25], but similar to Lake Taihu [26]. *Betaproteobacteria* widely exist in freshwater ecosystems, where they are the most numerically dominant class and clone type [27]. The abundance of *Anaerolineae*, *Gammaproteobacteria* and *Deltaproteobacteria*. in sediment sample indicates the anaerobic nature of the sediment. It is suggested that the structure and function of microorganisms are closely with regional environment, and plays an important role in material circulation and energy flow of the eutrophic water sediment.

There are quite differences in microbial communities between water and sediment samples. *Sphingobacteriia*, *Cyanobacteria*, *Phycisphaerae*, *Chlorobia* and *Alphaproteobacteria* were mostly distributed in water samples, whereas *Betaproteobacteria*, *Anaerolineae*, *Gammaproteobacteria* and *Deltaproteobacteria* are mainly observed in sediment samples. Our results have shown that the richness and biodiversity of sediment had a slightly high diversity, but no significant differences ( $p > 0.05$ ) between with water samples. It is suggested that cyanobacterial blooms affect not only the plankton community structure, but also the microbial community structure in both water and sediment. The analysis of the relationship between microbial community structure and physical-chemical parameters may reflect changes in the blue-green blooms environment [28], [29]. In the present study, correlations between environmental factors and microbial communities were analyzed, showing most bacterial assembles were negative with TP. This result anticipated that TP has directly or indirectly affected the microbial community structure of this region.

## 5. Conclusions

In the present study, we investigated the microbial community diversity in an eutrophic lake using Miseq sequencing technology for the first time. The results of this study provide comprehensive information about the biodiversity and the structure of bacterial assemblages were the difference between water and sediment during cyanobacterial blooms. The biodiversity indices of Chao1, Ace, Shannon and Simpson indicate the higher diversity in sediment samples, and similar bacterial communities exist in the regional environment. *Cyanobacteria* and *Sphingobacteriia* were predominated in water sample suggested that blue-green algal bloom heavily affected microbial community structure and diversity.

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