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Full Length Article

Bacterial Diversity of Surface Sediment at Lake Dian in Summer and Winter Seasons

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Abstract

Sediment bacteria are the main exerciser of nutrient metabolism in most lake ecosystems and play a key role in the process of material circulation and energy transfer. The bacterial diversity is also an important indicator of the stability of the water ecosystems. In order to understand the composition and seasonal variation characteristics of bacterial community in eutrophic lake sediments, we analyzed sediments of Lake Dian by applying the sec-generation sequencing technology to sequence the V4 region of 16S rDNA of Lake Dian sediments in summer and winter. The data were interpreted to comparatively map the bacterial diversity and nature in both summer and winter seasons. Alpha diversity analysis showed that Shannon index and Chao1 index of samples collected in summer were significantly higher than those collected in winter, indicating higher diversity and richness of bacterial communities in summer sediments similar to the abundance rarefaction curve. The PCA principal component analysis further demonstrated that seasonal changes resulted in significant distribution change of bacterial communities. Sediment bacteria in summer mainly belonged to the Proteobacteria, followed by Planctomycetes, Chloroflexi and Bacteroidetes phyla. Major sediment bacteria in winter were mainly grouped in Planctomycetes phylum. In summer, the average relative abundance of sediment bacteria belonging to the Planctomycetes phylum was significantly lower than that of winter, whereas other 9 phyla were significantly higher than that of winter season. The species and abundance of sediment dominant bacterial flora showed significant seasonal variations. With the decreased classification level, the abundance of rare bacteria in the sediments of summer samples were increased. At each classification level, the abundance of rare bacteria in sediments of summer samples was significantly higher than that of winter samples, indicating that the low-abundance species in sediments in summer was significantly higher than that in winter. The sediment in summer has higher diversity and richness of bacterial community. The species and richness of sediment dominant flora showed significant seasonal differences. © 2020 Friends Science Publishers

Keywords: Diversity; Lake dian; Microorganism; Season; Sediment

Introduction

Lake Dian is a famous plateau-lake located in the Southern suburb of Kunming city of Yunnan province of China. It enjoys a water area of 306 km^2 with total average water storage of 1.57 billion M^3 (Fan *et al.* 2017). Ever since middle and later periods of the 1970s to 1980s, especially in the 1990s, water eutrophication in Lake Dian became worse. Although environmental degradation of the Lake Dian obtained certain degree of ease after 20 years of pollution control, the pollution levels in Lake Dian has not been completely eliminated. At present, water quality of the Lake is still lower than VV class, belonging to inferior V-class water and cannot meet the standards of utilizable water (Sun *et al.* 2017). Lake Eutrophication is an important environmental problem in the field of water resources in China. Sediment is the source and collection of water substance and an important source of lake nutrients. It carries an important impact on water body Eutrophication (Yang *et al.* 2018). Sediment bacteria are the main exerciser of nutrient metabolism in most lake ecosystems (Gibbons *et al.* 2014), and play a key role in the process of material circulation and energy transfer (Ibekwe *et al.* 2016). They are also one of the important indicators to determine the stability of water ecosystems (Conn *et al.* 2015).

Haglund *et al.* (2002) have found that the metabolic capacity of bacteria in lake sediments is much higher than that of bacteria plankton in water. Bacteria can influence the

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distribution and transformation of nutrient elements in sediments through assimilation and alienation (Johnson *et al.* 2014). The study on the structure and composition of bacterial community in lake sediments is of great significance to understand the cycling process of nutrient elements in the lake ecosystem. Secly, the study on the seasonal change of bacterial community structure in sediments is helpful to understand the feedback effect of bacteria on the environment (Yang *et al.* 2016). Several studies have mapped the microbial diversity in water; Jiang *et al.* (2016) have identified that the OTU number of bacteria community in sediments of Poyanghu Lake is correlated with heavy metal elements such as copper and lead to different degrees. Bier *et al.* (2015) have found that the composition of bacterial communities in river sediments changes significantly due to mine drainage, and the community diversity is highly related to the concentrations of cadmium, manganese, zinc and nickel. Li *et al.* (2013) have reported that the diversity of microbial community structure in water is significantly different in horizontal and vertical directions, and that the microbial community structure is significantly correlated to environmental factors such as water depth, pH value *etc*. Over the past few decades, studies have conducted on Lake Dian mainly focused on the control of non-point source pollution, ecology and biogeochemical cycle of biogenic elements (Zou *et al.* 2017). However, understanding on the response mode of microorganisms caused by seasonal changes within the unique sediment ecosystem remained to be explored.

In this study, the sediments of Lake Dian were obtained and the diversity of bacteria in the sediments in both summer and winter seasons were analyzed and compared using the sec-generation sequencing technology. The generated data provided novel insights in the composition and seasonal characteristics of bacteria community in the eutrophic lake sediments.

Materials and Methods

Sample collection

Summer sediment samples (DD1.1, DD1.2 and DD1.3) and winter sediment samples (DX1.1, DX1.2 and DX1.3) were collected from Lake Dian during May at the turn of spring and summer 2018, and during November at the turn of autumn and winter 2018, respectively. The longitude coordinates of the sampling location was E102°41 '8" and latitude coordinates was N24°55' 59". Surface sediments (0–10 cm) were collected using grab-type sediment sampler. Repeated random sampling was carried out in summer and winter for 3 times with intervals of 10 months. The collected sediment samples were quickly sealed with sealing bags and stored into a low-temperature freezer and shipped to the analyzing laboratories.

DNA extraction and PCR amplification

Soil DNA Kit (D5625-01) from Omega Ltd. was used to extract genomic DNA from each sample and was quality controlled at gel electrophoresis (0.8% agarose). The PCR was performed using specific primers 515F (5 '-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') targeting the barcode region of the 16S rDNA-V4 (Hugerth *et al.* 2014). The reaction mixture contained 10 μL of 5×Taq Buffer, 1 μL of dNTP (10 m*M*), 1 U of KOD-Plus-Neo high-fidelity DNA polymerase and 1 μL upstream and downstream primers (10 μM). The mixture was supplemented with DNA template (50 ng) and the reaction was brought up to 50 μL using sterilized water. Reaction conditions included: pre-denaturation at 94°C for 2 min; denaturation at 94°C for 30 s, renaturation at 60°C for 30 s, extension at 72°C for 30s; 35 cycles, extension at 72°C for 5 min; 10°C heat preservation. After the completion, a total of 3 μL PCR products were run on 2% agarose gel electrophoresis. The PCR products were recovered by AxyPrepDNA gel recovery kit and were sequenced by the Beijing Novogene Biological Information Technology Co., Ltd.

Quality control and analysis of off-line data

The data were divided into different datasets according to the label of sample collection. Barcode sequence and PCR amplification primer sequence were trimmed to utilize the gene sequences only for downstream analysis. FLASH software (Caporaso *et al.* 2011) was used to determine splicing events. The filtered sequences obtained by Qiime software (Hess *et al.* 2011) were compared with known sequences in Golddatabase. Afterwards, UCHIME software (Edgar *et al.* 2011) was used to remove chimeric sequences and obtain effective sequences. Uparse software (Edgar, 2013) was used to classify the operational classification units at 97% similarity levels. The representative sequences were annotated by RDP classifier software (Wang *et al.* 2007) and species annotation was performed using GreenGene database (Desantis *et al.* 2006). The Mothur software was used to estimate the dilution curve and calculate coverage. The calculation methods for Chao1 and Shannon indices were adapted from Caporaso *et al.* (2011). Differences between samples were shown by PCA principal component analysis using Canoco software. Fastunifrac software was used to analyze the distance matrix between samples, and the UPGMA clustering tree was constructed based on Weighted Unifrac distance.

Data statistics

SPSS v18.0 statistical analysis software was used to conduct variance analysis and Duncan's new multiple range test.

Results

Sequencing quality assessment

The effective sequence ratio and parameter Q30 were analyzed (Table 1). The effective sequence proportion of each sample was over 84%, and the Q30 value was over 96%. These parameters indicate that there is no unqualified condition such as poor database construction or contamination by other factors in the process and the sequencing quality meets the requirements of subsequent analysis. The average sequence length was 254 bp, which was consistent with the length of the 16S r dna-v4 region sequence (Table 1).

Sequencing depth assessment

The rationality of sequencing depth and richness of species in samples were analyzed through the dilution curve (Fig. 1). With the increase in sequence number, the dilution curve of each sample tends to be flat, indicating that the amount of sequencing data is reasonable and covers all bacterial species in the sample. The span of the 6 samples on the vertical axis, namely, the classification richness was DD1.2, DD1.1, DD1.3, DX1.3, DX1.2 and DX1.1 from large to small, indicating that the species richness of the sediments in summer was significantly higher than that in winter (Fig. 1).

Alpha diversity analysis

The valid sequences (Taxon Tags) can be classified as operational taxonomic units (OTU) (Fig. 2). The OTU clustering was unavailable only with a few sequences (Unclassified Tags and Unique Tags). The number of OTU in each sample sequence (DX1.1, DX1.2, DX1.3 and DD1.1, DD1.2, DD1.3) was 1223, 1192, 963 and 405, 420, 460, respectively. It is evident that a prominent difference in the number of OTU which were measured in sediments of summer and winter seasons. The OUT number for summer sediments was almost 2 or 3 times that of winter sediment. Alpha diversity analysis showed that the Shannon index and Chao1 index of summer samples were significantly higher than those of winter samples, indicating that summer sediments carried higher diversity and richness of bacterial communities, which was consistent with the results of richness dilution curve (Table 2).

Beta diversity analysis

The Weighted Unifrac distance matrix was used for UPGMA clustering analysis, and the clustering results were integrated and presented with the relative richness of species in each sample at the phylum level (Fig. 3). It can be seen that the summer sediment and winter sediment samples were clustered into two large branches with a relatively long distance, indicating relatively low species similarity between summer and winter samples. The PCA principal component analysis (Fig. 4) showed that the principal component 1 (PC1) and principal component 2 (PC2) contributed with 55.08 and 24.85% for sample difference (79.93% in total), being the main source of the difference. It is also clearly depicted from the analysis of principal component that samples DX1.1, DX1.2 and DX1.3 were all located in the negative region of PC2 and PC1, and samples DX1.1, DX1.2 and DX1.3 were all located in the negative region of PC2 and the positive region of PC1. This further indicated that seasonal changes result in the significant change in bacterial community distribution.

Species annotation analysis

A total of 89.28% of the sequences were annotated to Kingdom, 88.76% to Phylum, 88.46% to Class, 85.68% to Order, 79.04% to Family, 71.03% to Genus, and only 0.40% to Species levels (Fig. 5).

Species richness analysis

According to the annotation analysis results, the relative richness of the species with top 10 richness at the phylum, class and genus levels was selected for variance analysis. As for the classification level of phylum (Table 3), in reference to the average relative richness. Proteobacteria was the main type of sediment bacteria in summer sediments with an average relative richness of 53.28%, followed by Planctomycetes, Chloroflexi and Bacteroidetes with an average relative richness of 10.51~7.53%. The average relative richness of remaining phylum was less than 3.00%. Bacteria in winter sediment mainly belonged to the Planctomycetes phylum with an average relative richness of 95.71%. Average relative richness of the other phylum is less than 3.00%. These figures indicate that average relative richness of bacteria belonging to Proteobacteria phylum (the summer's most advantage bacterium groups) was greatly reduced in winter, becoming the non-advantage bacterium group. However, the average relative richness of the planctomycetes phylum bacteria were increased in summer significantly and becomes the most advantage bacterium group. These observations highlight that the most advantaged bacteria groups in winter sediment and summer sediment were entirely different, presenting obvious seasonal differences. The results of variance analysis showed that the average relative richness of Planctomycetes phylum in summer was significantly lower than that in winter, while that of the other 9 phyla was significantly higher than that observed in winter.

Table 1: Sequence statistics and quality analysis

Sample name	Raw PE	Effective tags	AvgLen (bp)	Q30 (%)	Effective Sequence Ratio
DD1.1	31,114	27079	253	96.24	87.03
DD1.2	24,945	21176	254	96.06	84.89
DD1.3	13,628	11894	254	96.27	87.28
DX1.1	40,755	35455	254	96.52	87.00
DX1.2	40,247	35001	254	96.52	86.97
DX1.3	44,995	38580	254	96.44	85.74

Note: Raw PE represents the number of PE reads of the original offline; Effective Tags refer to the number of Tags sequences that are finally used for subsequent analysis after filtering the chimera. AvgLen means the average length of Effective Tags; Q30 refers to the base mass value (percentage) in Effective Tags with base mass greater than 30 (sequencing error rate less than 0.1%). The Effective Sequence Ratio represents the percentage between the number of Effective Tags and the number of Raw PE

Table 2: Alpha diversity index

Sample name	Shannon index	Chao1 index
DD1.1	7.86	1201.77
DD1.2	8.16	1308.19
DD1.3	8.01	1998.51
DX1.1	2.21	330.59
DX1.2	2.11	345.42
DX1.3	2.15	443.25

On the classification level of “class” and in the aspect of average relative richness, the most dominant flora in summer sediments was Betaproteo bacteria class with an average relative richness of 25.48%, followed by Gammaproteo, Deltaproteo, Brocadiae and Anaerolineae classes, with an average relative richness of 12.57~7.73% (Table 4). The average relative richness of other five phyla was less than 5.0%. Bacteria in sediments collected in winter were mainly belonging to Brocadiae class with an average relative richness of 95.66%, and the average relative richness of remaining 9 phyla was less than 1.00%. These results highlight that the dominant flora in sediments collected in winter was dominated by a single species. Secly, the average relative richness of Betaproteo bacteria, which was the most dominant bacteria group in summer sediment, was greatly reduced and became the non-dominant bacteria group in winter. On the other hands, the average relative abundance of Brocadiae bacteria, the sec-dominant bacteria group in summer sediment, was greatly increased and became the most dominant bacteria group in winter sediment. According to the results of variance analysis, average relative richness of the Brocadiae class in summer sediment was significantly lower than that in winter sediment, however, the average relative richness of other 9 classes was significantly higher than that observed in winter sediment.

On the classification level of Genus and in the aspect of average relative richness, the most dominant flora in summer sediments was *Dechloromonas* with an average relative richness of 10.78%, followed by *Candidatus* Brocadia with an average relative abundance of 8.22% (Table 5). The average relative richness of the remaining 8 phyla was less than 3.00%. Bacteria in winter sediment were mainly composes of *Candidatus* Brocadia genus with an average relative richness of 92.96%, and the average relative richness of remaining 9 phyla was less than 0.2%. These results indicate that some species account for very tiny proportion in the whole bacterial community.

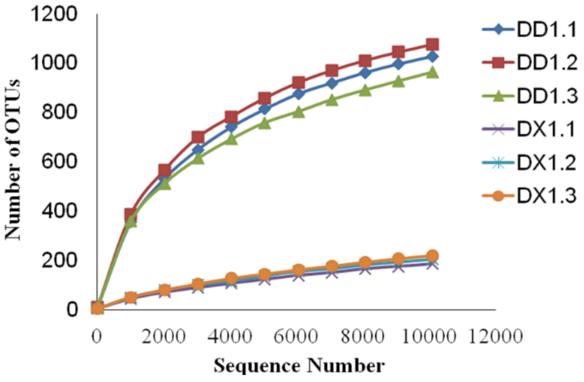

Fig. 1: Dilution curve of bacteria OTUs abundance

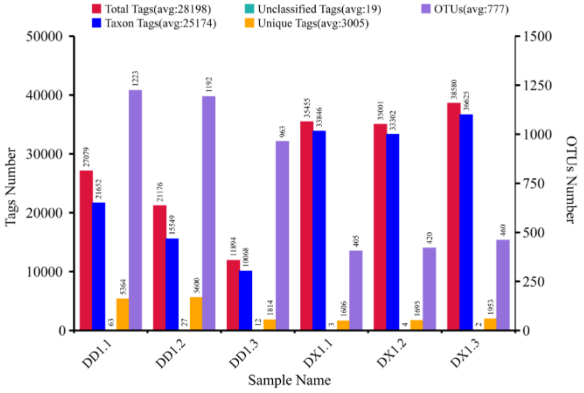

Fig. 2: Statistics of sample tag number and OUT number

The average relative richness of *Dechloromonas* genus, which was the most dominant bacterial group in summer sediment, was greatly reduced and became the non-dominant bacterial group in winter. The average relative abundance of *Candidatus* Brocadia bacteria, the sec-dominant bacteria group in summer sediment, was greatly increased and appeared to be the most dominant bacteria group in winter sediment. It is plausible to conclude from the result of the variance analysis that average relative richness of *Candidatus* Brocadia in summer sediment was significantly lower than that of winter sediment, and there was no significant difference between the average relative richness of *Neisseria* and *Clostridium* in summer and winter sediment. The average relative richness of the remaining 7 genera of summer sediment was significantly higher than that was observed in winter sediment.

Discussion

Lake sediments are an important part of lake ecosystem and sediment microorganisms play an important role in the geo-biological-chemical circulation of the entire lake ecosystem (Taylor and Cunliffe 2015). It is of great significance to understand the response mode of sediment microorganisms to seasonal changes in order to maintain the stability of lake ecosystem. High-throughput sequencing technology can reveal the genetic diversity of microbial communities at the gene level. Currently, reports of using this technology in the study of microorganisms in lake water and sediment are increasing and highlighting the importance of this domain (Sun *et al.* 2014).

In this study, the MiSeq sequencing platform of Illumina was used to study the composition of bacterial communities of surface sediments of Lake Dian in both summer and winter seasons. The results showed that bacteria of Lake Dian sediment with top 10 richness belonged to the Planctomycetes, Proteobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Acidobacteria, Firmicutes, Verrucomicrobia, Spirochaetes and OP3. These results co-relate with observations noticed at China's major freshwater lakes such as Taihu Lake and Poyanghu lake (Jin *et al.* 2017; Xue *et al.* 2017, 2018). All together, these studies show that sediment bacterial communities play an important role in the degradation of organic pollutants, carbon cycle and geo-biological-chemical circulation of the aquatic ecosystems (Cheng *et al.* 2014). A recent study indicated that Proteobacteria were usually the main prokaryotes in the water environment (Ke *et al.* 2015), including phototrophic, chemoautotrophic and chemoheterotrophic bacteria, which are generally distributed in the water environment.

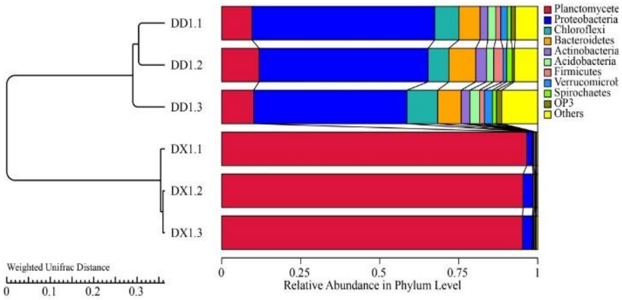

Fig. 3: Cluster analysis of bacteria relative abundance in phylum level

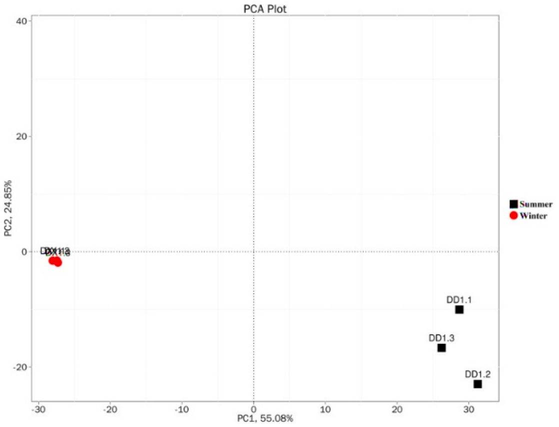

Fig. 4: PCA results of bacterial community

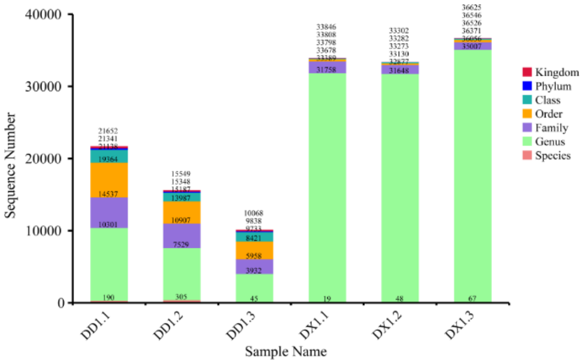

Fig. 5: Number of sequences at each classification level

Proteobacteria were the main bacteria in the summer sediment of Lake Dian, whereas in winter season, the average relative richness of Proteobacteria genus was greatly reduced, making it a non-dominant bacteria group. It is speculated that this might be related to damp and rainy climate in summer, and dry and less rain climate in winter. The amount of water flowing into the lake in summer is larger than that in winter, which brings more nutrients into Lake Dian and thus leading to an increase of organic matter content in this region. Appropriate nutritional conditions make it favorable for rapid multiplication of Proteobacteria, which became the most dominant bacteria group. It has been reported that Proteobacteria plays an important role in the degradation of organic matter. A variety of nitrogen-fixing bacteria have been found in this phylum (Xue *et al.* 2018). In addition, it was found that the average relative richness of Proteobacteria, which was the most dominant bacteria group in summer, has significantly decreased levels in winter and became the non-dominant bacteria group. The average relative richness of Planctomycetes, the secary dominant bacteria group, was significantly increased and became the most dominant bacteria group. This may be related to the low dissolved oxygen in surface sediments in winter. Temperature of sediments in winter is low and the metabolism of aquatic plants is weakened.

Table 3: The relative abundance of bacteria in phylum level

Season	Planctomycetes	Proteobacteria	Chloroflexi	Bacteroidetes	Actinobacteria	Acidobacteria	Firmicutes	Verrucomicrobia	Spirochaetes	OP3	Others
Summer	10.51 ± 1.20 b	53.28 ± 4.67 a	7.99 ± 1.62 a	7.53 ± 0.92 a	2.86 ± 0.49 a	2.62 ± 0.40 a	2.03 ± 0.79 a	1.93 ± 0.70 a	1.41 ± 0.30 a	1.21 ± 0.52 a	8.64 ± 2.37 a
Winter	95.71 ± 0.74 a	2.68 ± 0.78 b	0.17 ± 0.05 b	0.36 ± 0.08 b	0.36 ± 0.10 b	0.11 ± 0.02 b	0.03 ± 0.00 b	0.09 ± 0.02 b	0.05 ± 0.01 b	0.02 ± 0.01 b	0.42 ± 0.08 b

Note: Different lowercase letters indicate significant difference at alpha =0.05 level, same below

Table 4: The relative abundance of bacteria in class level

Season	Brocadiae	Betaproteobacteria	Gammaproteobacteria	Deltaproteobacteria	Anaerolineae	Bacteroidia	Alphaproteobacteria	Actinobacteria	Sphingobacteriia	Verruco-5	Others
Summer	9.07 ± 1.88 b	25.48 ± 4.37 a	12.57 ± 0.77 a	10.83 ± 1.35 a	7.73 ± 1.64 a	4.85 ± 0.78 a	4.31 ± 0.72 a	2.36 ± 0.52 a	1.45 ± 0.24 a	1.18 ± 0.50 a	20.16 ± 3.98 a
Winter	95.66 ± 0.75 a	0.66 ± 0.24 b	0.95 ± 0.34 b	0.37 ± 0.03 b	0.14 ± 0.05 b	0.10 ± 0.02 b	0.69 ± 0.17 b	0.33 ± 0.10 b	0.18 ± 0.06 b	0.06 ± 0.01 b	0.86 ± 0.12 b

Table 5: The relative abundance of bacteria in genus level

Season	*Candidatus* Brocadia	*Dechloromonas*	*Thiobacillus*	*Neisseria*	*Crenothrix*	*Sulfuritalea*	*Dok59*	*Geobacter*	*Desulfobacterium*	*Clostridium*	Others
Summer	8.22 ± 1.70 b	10.78 ± 2.68 a	2.87 ± 0.69 a	0.92 ± 1.43 a	1.53 ± 0.46 a	1.01 ± 0.44 a	0.93 ± 0.49 a	0.90 ± 0.45 a	1.10 ± 0.12 a	0.66 ± 0.47 a	71.08 ± 4.21 a
Winter	92.96 ± 0.60 a	0.10 ± 0.05 b	0.04 ± 0.01 b	0.00 ± 0.00 a	0.05 ± 0.00 b	0.01 ± 0.00 b	0.01 ± 0.00 b	0.02 ± 0.01 b	0.04 ± 0.01 b	0.01 ± 0.00 a	6.75 ± 0.68 b

The substance exchange between plant roots and sediments is reduced, and the amount of water running into the lake drops sharply. As a result, the interaction between water and surface sediments is reduced resulting in decreased dissolved oxygen. This is favorable for Planctomycetes propagation in the lake. The selective propagation of Planctomycetes inhibits the growth of Proteobacteria. Planctomycetes is mainly facultative anaerobic bacteria, while the others are strictly anaerobic autotrophic bacteria (Marinho *et al.* 2017). Study finds that some of the floating molds can perform anaerobic ammonia oxidation reaction under anaerobic conditions (Yao *et al.* 2017). It can be inferred that there may be anammox activities in the surface sediments of Lake Dian especially in winter. Chloroflexi is a photoautotrophic bacterium responsible for degradation of organic pollutants. The eutrophic conditions of Lake Dian provide this type of bacterium an obvious survival advantage (Jiang *et al.* 2018). Secly, this group is the main group in the sediments with low hydrate but abundant organic matter. The frequency of finding such groups in the sediments without hydrate is much higher than that in the sediments with hydrate. The presence or absence of this group can be used as an important indicator to determine whether the sediments contain hydrate or not (Wasmund *et al.* 2015).

Our results demonstrate that more and more sequences and OTU cannot be classified into already known taxon with the reduction of classification level. At the genus level, nearly 28.97% of the bacteria have not been classified, which means that there are still many unknown bacteria species to be discovered in the sediments of Lake Dian. It also indicates that the detection capability of the MiSeq sequencing platform developed by Illumina decreases with the decrease of classification level. This is related to the short MiSeq sequencing reading. Since MiSeq sequencing cannot sequence all the 9 variable regions of 16S rDNA, only 1–3 variable regions are usually selected as sequencing regions. In this study, only one variable region (V4 region) of 16S rDNA was selected for sequencing. The average length of the sequence was about 254 bp. Because of the short reading length, the resolution of species annotation for gene-level sequences was subsequently low. With the decrease of the classification level, the richness of rare bacteria (Others group) in the sediments in summer and winter increased, and the richness of bacteria in summer was significantly higher than that observed in winter, indicating that the species of low-richness group in the sediments in summer was significantly higher than that noticed in winter.

Conclusion

The sediment in summer had higher diversity and richness of bacterial community than in winter season. The bacteria of summer sediments mainly belonged to the Proteobacteria phylum, followed by Planctomycetes, Chloroflexi and Bacteroidetes phyla. The bacteria of winter sediments mainly belonged to the Planctomycetes phylum. In summer, the average relative richness of Planctomycetes phylum was significantly lower than that of winter, and that of remaining 9 phyla was significantly higher than that noticed in winter. The species and richness of sediment dominant flora showed significant seasonal differences.

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