

Original Research

Bacterial Community Analysis of Two Neighboring Freshwater Lakes Originating from One Lake

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Received: 20 January 2020

Accepted: 16 March 2020

Abstract

Bacterial community compositions of two neighboring freshwater lakes (i.e. Inner Sand Lake and Sand Lake in Wuhan, China) originating from one lake were studied by triplicate sampling based on Illumina Miseq sequencing. The pollutant concentrations in Sand Lake were twice as in Inner Sand Lake generally. *Proteobacteria* mainly containing *Betaproteobacteria* and *Alphaproteobacteria* were most abundant in the two lakes. The most dramatic differences at phylum level were that Inner Sand Lake had a higher proportion of *Bacteroidetes* while Sand Lake had a higher proportion of *Cyanobacteria*. The eutrophic Sand Lake had more taxa as for *Alpinimonas*, *Flavobacterium*, *Lautropia*, *Pelomonas*, *Pseudomonas*, *Sphingorhabdus*, *Candidatus Aquirestis* and *Vogesella*. On the contrary, the oligotrophic Inner Sand Lake had more taxa as for *Aeromonas*, *Bradyrhizobium*, *Fluviicola*, *Limnohabitans*, *Luteolibacter*, *Polynucleobacter*, *Pseudarcicella* and *Sediminibacterium*. Correlation network analysis revealed that *Pseudarcicella*, *Sediminibacterium*, *Luteolibacter*, *Aeromonas* in fresh lakes were potential bacterial indicators of good-quality lakes. Conversely, *Flavobacterium*, *Pseudomonas* and *Candidatus Aquirestis* seemed to be bacterial indicators of bad-quality lakes. Results obtained from this study could gain more knowledge on freshwater lake ecosystems from the bacterial aspect.

Keywords: freshwater lakes, bacterial community, *Pseudarcicella*, eutrophication

Introduction

Water quality is crucial for freshwater lakes but urbanization usually has a negative impact on water quality [1]. The discharge of wastewater or treated

wastewater can somehow change the lake ecosystem gradually by reducing water quality and the microbial abundance [2]. Currently, there have been more than two-thirds of urban lakes are suffering from different levels of pollution and eutrophication in Wuhan, China [3].

Research on the bacterial community compositions is beneficial for keeping good water quality as well as preventing algae blooming [4, 5] of the lakes.

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Bacteria contribute to the fundamental energy flow and biogeochemical cycling of lake ecosystems [6], which can be control agents against toxic cyanobacterial blooms [7] by interacting with specific phytoplankton [6]. Moreover, bacteria even have the capacity to move towards inorganic nutrients [8]. Thus, the strong adaptability and sensitive environmental perception allow the bacteria to play an indispensable role in lake ecosystems.

In this study, two neighboring freshwater lakes, namely Sand Lake and Inner Sand Lake, were studied in terms with the bacterial community and water quality. The two lakes were separated from one lake since the construction of Canton-Hankow Railway in 1907 of the Qing Dynasty in China. Currently, Sand Lake and Inner Sand Lake covers an area of 3.197 and 0.134 square kilometers, respectively, which were separated by Youyi Ave. The smaller Inner Sand Lake has

Table 1. Main chemical indexes of Inner Sand Lake (JD07) and Sand Lake (JD08).

Sample ID	JD07 Mean/SD	JD08 Mean/SD
PH	6.95/0.02	7.04/0.02
F ⁻ (mg/L)	0.022/0.03	0.025/0.03
Cl ⁻ (mg/L)	0.725/0.18	0.874/0.21
COD (mg/L)	14.08/1.03	28.34/3.06
NO ₃ ⁻ -N (mg/L)	0.036/0.05	0.059/0.08
NH ₄ ⁺ -N (mg/L)	0.422/0.10	0.884/0.13
TN (mg/L)	0.616/0.14	1.607/0.24
PO ₄ ³⁻ -P (mg/L)	0.072/0.05	0.101/0.04
TP (mg/L)	0.081/0.05	0.141/0.07
SO ₄ ²⁻ (mg/L)	1.255/0.20	1.477/0.28

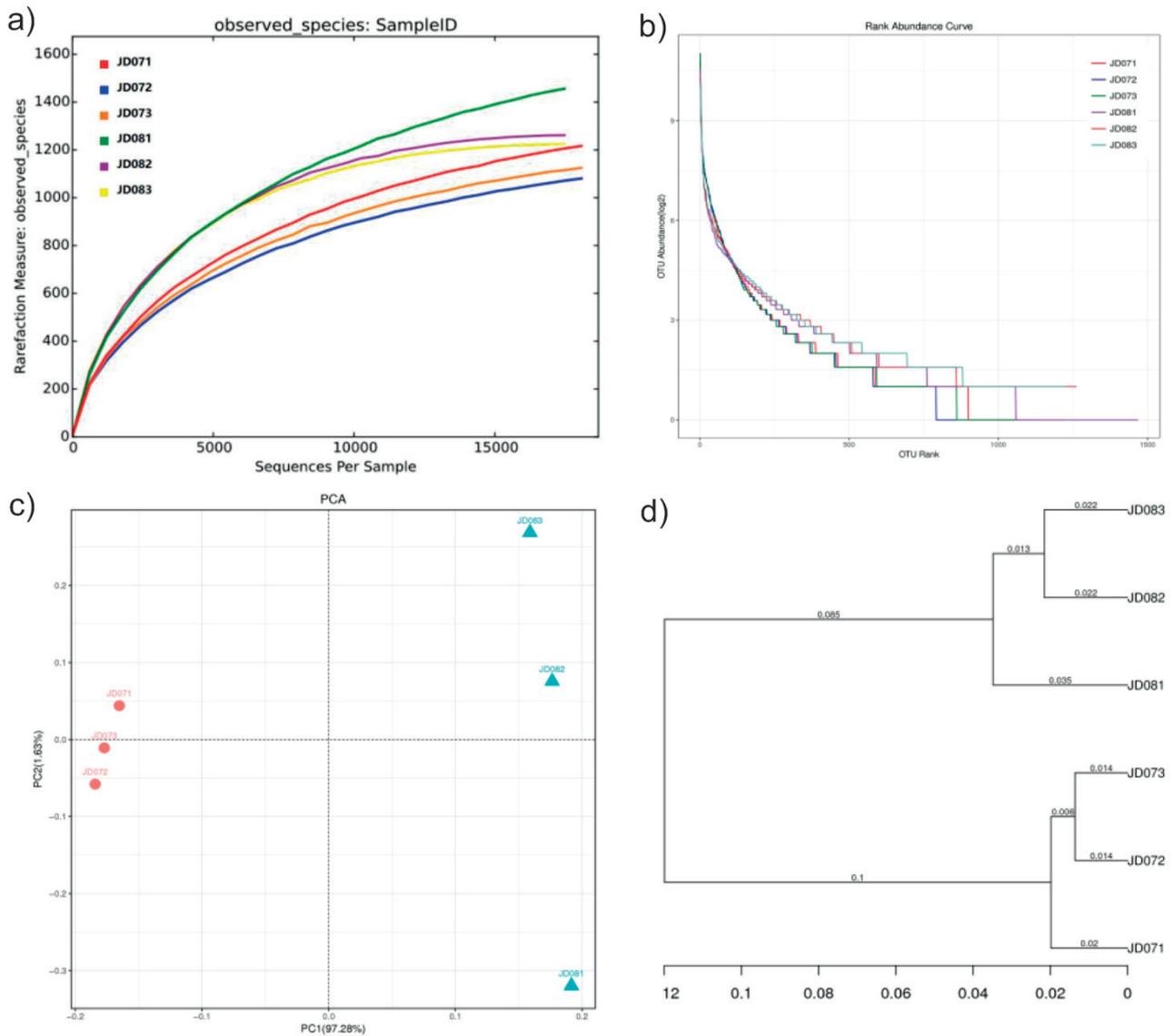


Fig. 1a) Rarefaction curves of operational taxonomic units; b) rank-abundance curves; c) Principal component analysis (PCA) showing the beta diversity among the samples; d) UPGMA clustering analysis graph based on weighted UniFrac distance matrix.

Table 2. The α -diversity indices of the bacterial communities.

	Chao1	ACE	Simpson	Shannon
JD071	1381.55	1488.09	0.9767	7.44
JD072	1281.03	1329.09	0.9742	7.28
JD073	1257.02	1328.49	0.9757	7.37
JD081	1748.42	1827.43	0.9788	7.83
JD082	1262.00	1262.00	0.9824	7.99
JD083	1225.00	1225.00	0.9845	8.09

a better water quality due to the artificial remediation since 2013 by sewage interception, sediment clearing, hydrophyte, etc. The study aims to disclose the specific bacterial community compositions in the two lakes, to investigate the bacterial similarities and differences of the two lakes, and to associate the bacterial community with water quality of the two lakes. This study could gain more knowledge on freshwater lake ecosystems from the bacterial aspect.

Experimental

Study Sites and Sampling

The specific locations of the two sampling points were JD07 (30°33'40.9"N, 114°18'53.2"E) and JD08 (30°33'27.3"N, 114°19'24.3"E) for Inner Sand Lake and Sand Lake, respectively. Triplicated water samples (500 mL) were retrieved from each sampling site at the water depth of about 5 cm below the water surface. The samples were collected at 1-2 p.m. on December 22, 2017, the Winter Solstice Day. The average water temperatures of JD07 and JD08 were 14.5°C and 15.0°C, respectively.

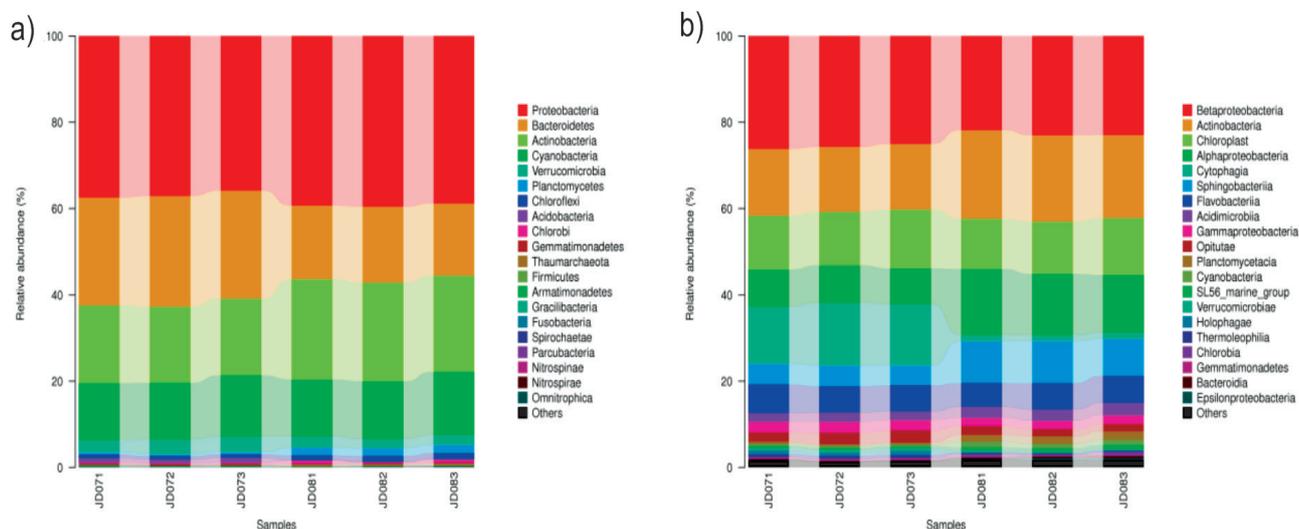


Fig. 2. Taxonomic classification of bacterial 16S rRNA gene reads at phylum level a) and class level b).

Measurement of Water Biochemical Parameters

The general biochemical parameters of collected water samples were analyzed and tested in 2 h. About 100 mL raw water of each sample was filtered through a 25 mm diameter, 0.2 μ m filter membrane (Osmonics, Livermore, California, USA) and the filters were stored respectively at -80°C . The pH values were measured using a pH meter (OHAUS, ST3100). The Standard Methods [9] were used to determine ammonium nitrogen ($\text{NH}_4^+\text{-N}$), total nitrogen (TN), phosphate ($\text{PO}_4^{3-}\text{-P}$), total phosphate (TP), nitrate nitrogen ($\text{NO}_3^-\text{-N}$), and chemical oxygen demand (COD) of the water samples. Concentrations of F^- , Cl^- and SO_4^{2-} were tested by ion chromatograph (DIONEX-ICS-600, USA) [10]. The mean values of the biochemical parameters of JD07 and JD08 were determined based on JD071/JD072/JD073 and JD081/JD082/JD083, respectively.

DNA Extraction, PCR Amplification and Miseq Sequencing

The E.Z.N.A. soil DNA isolation kit (OMEGA Biotek Inc., Norcross, GA, USA) was applied to extract the total genome DNA [11], verified by 0.8% agarose gel electrophoresis. Fragment of the V4-V5 region of bacterial 16S rRNA genes were amplified by using primers 515F and 907R. The procedures of PCR amplification and Miseq sequencing were conducted as described [2]. 16S rRNA gene sequences are available at NCBI Sequence Read Archive (accession numbers SRR8225311 for JD071, SRR8245887 for JD072, SRR8245884 for JD073, SRR8245885 for JD081, SRR8245886 for JD082, SRR8245975 for JD083).

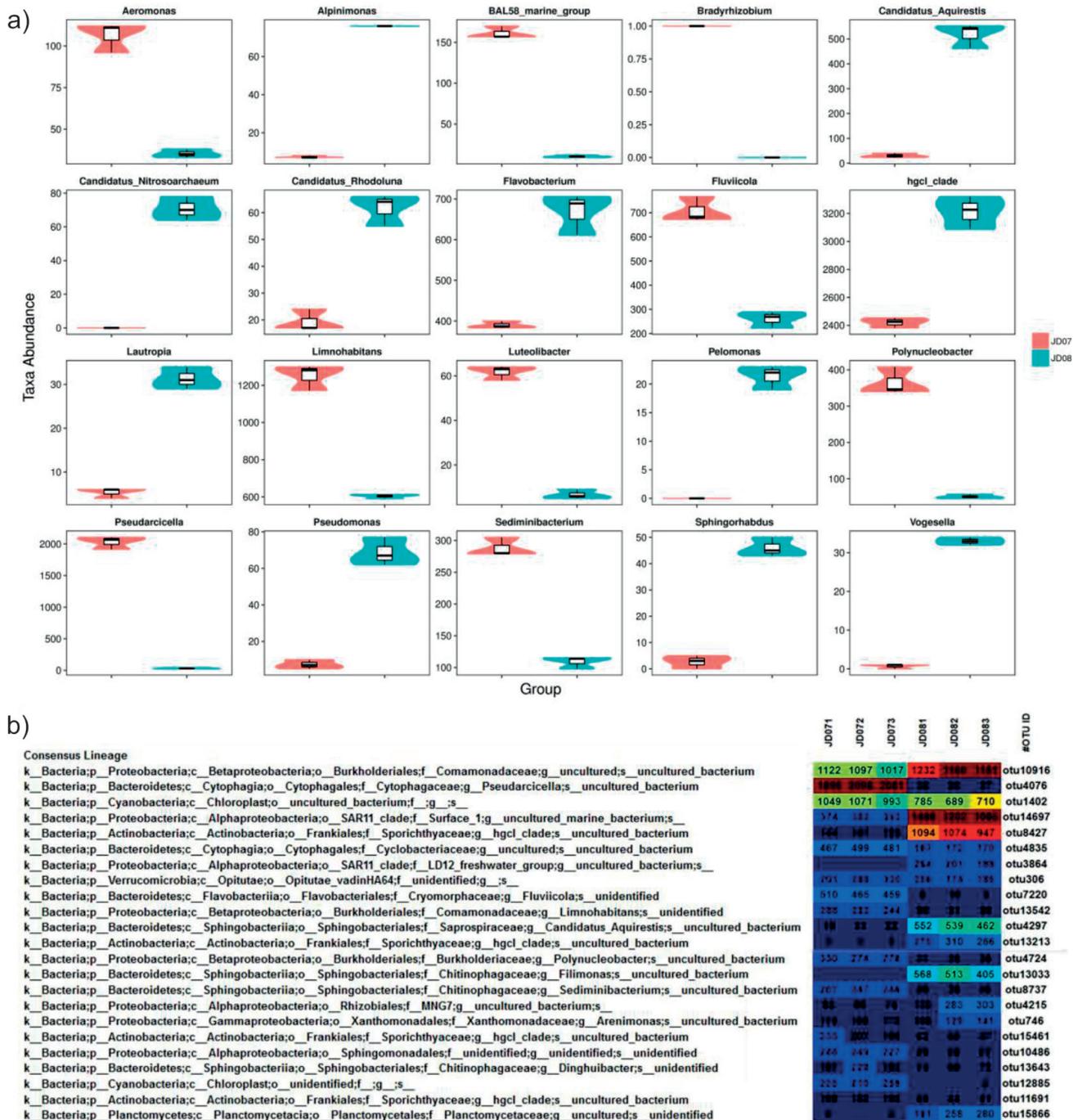


Fig. 3. a) Top 20 taxa with the most significant difference between Inner Sand Lake and Sand Lake by Metastats. b) Network inference based on the interrelationship among bacterial community.

Data Analysis

The QIIME (v1.8.0) pipeline and R software were used to analyze the sequencing data. UPGMA clustering analysis of weighed UniFrac distance matrix was carried out. Taxa abundances at the genus level were compared among samples by Metastats and visualized as violin plots. The Spearman's rank correlation coefficients were calculated and the network inference was constructed ($\rho > 0.6$, $P < 0.01$) [10]. Samples were compared at genus level by Mothur based

Metastats (<http://metastats.cbcb.umd.edu/>) ($p < 0.05$) [12]. The OTU hot map was constructed by using QIIME [13].

Results and Discussion

Water Properties

As shown in Table 1, Inner Sand Lake had a much better water quality than Sand Lake as indicated by

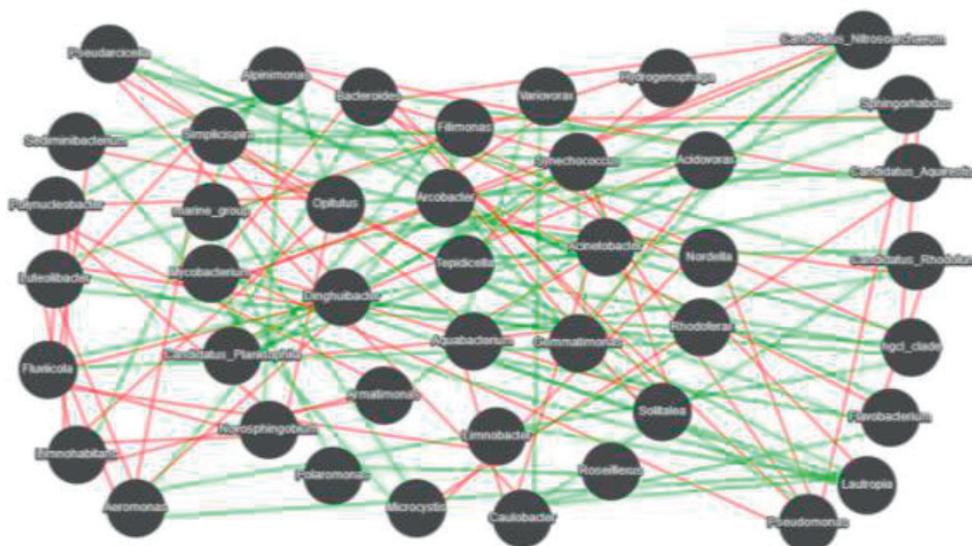


Fig. 4. Network inference based on the interrelationship among top bacterial species.

the biochemical parameters. In general, COD, nitrogen and phosphorus in Inner Sand Lake were half the values of those in Sand Lake. TN/TP ratios of Inner Sand Lake and Sand Lake were 7.6 and 11.4, respectively. Excessive nitrogen and phosphorus could cause nuisance growth of algae and other aquatic plants [14, 15], while both the dissolved carbon and nutrition were determining factors for the lake ecosystem [16, 17]. Among the factors, phosphorus was believed to be the key element for eutrophication [18]. The nitrogen in Inner Sand Lake was deficient according to N-deficient growth of TN: TP < 9 as reported [19]. Contrarily, the TN/TP ratio of 11.4 was favorable for phytoplankton growth [20] in Sand Lake. Moreover, Inner Sand Lake had lower concentrations of typical anions (F^- , Cl^- and SO_4^{2-}) than Sand Lake ($p < 0.05$), suggesting that Inner Sand Lake had a smaller salinity.

Abundance and Diversity of Bacterial Communities

A total of 307,755 high-quality 16S rRNA sequence reads were yielded for the six samples. Rarefaction analysis showed that the diversity of bacterial community was well captured [2] at the depth of sequencing used in this study (Fig. 1a). The length of the curve revealed that the sequencing depth of JD08 was slightly deeper than JD07. The evenness of microbial communities of JD08 was slightly higher than JD07 (Fig. 1b).

It could be inferred that the richness (Chao 1 and ACE) and biodiversity (Simpson and Shannon) showed no obvious differences of the two lakes as shown in Table 2. Fig. 1c) indicated that the distance of JD07 between the three repeating samples was closer than that of JD08, indicating that the similarity of microbial community structure between the three replicates in

Inner sand Lake (JD07) was higher than Sand Lake (JD08). The Weighted UniFrac analysis (Fig. 1d) indicated that samples of JD072 and JD073 were the most similar as the shortest branching length between the two samples [21].

Bacterial Community Structure

The Miseq sequencing results revealed that the dominating phyla were highly similar in the duplications of JD07 and JD08 (Fig. 2a). Similar to plateau freshwater lakes [22], the highest abundant phylum was found to be *Proteobacteria* in Inner Sand Lake (36.8%) and Sand Lake (39.2%). The most dramatic differences were that Inner Sand Lake had a higher proportion of *Bacteroidetes* while Sand Lake had a higher proportion of *Cyanobacteria*. *Cyanobacteria* blooming were always related to eutrophication of lakes [23]. The results also suggested that *Cyanobacteria* might inhibit the growth of *Bacteroidetes* [2].

At the class level, it could be found that the phylum *Proteobacteria* mainly consisted of *Betaproteobacteria* (24.1%) and *Alphaproteobacteria* (11.3%), followed by *Gammaproteobacteria* (2.2%) (Fig. 2b), which was different from those of plateau freshwater lakes [22]. A higher proportion of *Alphaproteobacteria* (14.2%) of Sand Lake could be found, which was reported to be dominant in eutrophic lake [24]. The phylum *Bacteroidetes* contains four classes of *Bacteroidia*, *Flavobacteriia*, *Sphingobacteriia* and *Cytophagia*. The taxa of *Cytophagia* in Inner Sand Lake were abundant (14.0%), which was accorded with oligotrophic lake [25]. Oppositely, the taxa of *Sphingobacteriia* in Sand Lake were dominant (9.4%), whose growth was related to the freshly leached dissolved organic carbon [26]. Hence, it could be speculated that the *Cytophagia* was favorable for lakes with good water quality and that

the *Sphingobacteriia* was associated with eutrophication of fresh lakes.

Significant Differences of Bacterial Communities of the Two Lakes

Significant differences of bacterial communities of the two lakes were further studied. The top 20 taxa with the most significant difference between Inner Sand Lake and Sand Lake were listed in Fig. 3a), which showed that the Sand Lake had more taxa as for *Alpinimonas*, *Flavobacterium*, *Lautropia*, *Pelomonas*, *Pseudomonas*, *Sphingorhabdus*, *Candidatus Aquirestis* and *vogesella*. For example, a diverse freshwater *Flavobacteria* community was reported in eutrophic lakes [27]. On the contrary, Inner Sand Lake had more taxa as for *Aeromonas*, *Bradyrhizobium*, *Fluviicola*, *Limnohabitans*, *Luteolibacter*, *Polynucleobacter*, *Pseudarcicella* and *Sediminibacterium*. Furthermore, the taxa over 600 OUTs presented in Fig. 3b) indicated the main taxa with significant abundant differences of the two lakes. It could be concluded that *Pseudarcicella* was the most significant indicator of a lake with good quality water. Indeed, *Pseudarcicella* was the representative genus of Lake Garda [28] and Three Gorges Reservoir [29] with good quality water.

Network Inference Based on the Interrelationship Among Bacterial Community

Correlation network analysis at genus level could reveal the interaction patterns of co-occurrence or co-exclusion among community members (Fig. 4). It indicated that *Sediminibacterium* favored the growth of *Fluviicola* and inhibited the growth of the potential eutrophic indicator of *Alpinimonas* and *Candidatus Aquirestis*. Moreover, *Sediminibacterium* could inhibit the growth of *Microcystis*. *Luteolibacter* had a possible collaboration with *Fluviicola* and *Aeromonas*. Combined Fig. 3 and Fig. 4, we could speculate that *Pseudarcicella*, *Sediminibacterium*, *Luteolibacter*, *Aeromonas* in fresh lakes might contribute to a good-quality lake. Conversely, *Flavobacterium*, *Pseudomonas* and *Candidatus Aquirestis* might contribute to the lake eutrophication.

Conclusions

This study reported the bacterial community compositions of two neighboring freshwater lakes at different trophic status originating from one lake in winter by triplicate sampling of surface water at two sampling sites. The similarities, differences and interrelationship of bacterial communities were characterized. It was found that the dominant aqueous *Pseudarcicella* was a potential indicator for lakes with good-quality water.

Acknowledgements

This research was financially supported by Open fund of Changjiang Academy of Sciences (CKWV2018490/KY), the Project of Key Laboratory of Healthy and Freshwater Aquaculture, Ministry of Agriculture (ZJK201810), the Science and Technology Research Project of Hubei Provincial Department of Education (Q20171107) and the Headmaster Funds of Xiamen University (20720170102).

Conflict of Interest

The authors declare no conflict of interest.

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