

SUMMER AND WINTER OF PROKARYOTIC COMMUNITY STRUCTURE AND THEIR RELATIONSHIP WITH ENVIRONMENTAL FACTORS IN XIANGXI RIVER AND SHENNONG CREEK IN CHINA

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Abstract. Few studies have reported about the summer and winter seasonal changes of prokaryotic community structure and their relationship with environmental factors in Xiangxi River and Shennong Creek in China. In this study, the 16S ribosome RNA (rRNA) gene cloning library was used to test the diversity of the aquatic prokaryotes along the Three Gorges Reservoir of the Yangtze River in China over two seasons in 2013. We analyzed the effects of summer and winter seasonal variation of the prokaryotic community composition and environmental factors. Diversity index analysis revealed that Shannon index, Chao1 index, Observed species index, and PD whole tree index of Xiangxi River were the highest in winter, and the four indices of Shennong Creek were the lowest in summer. Spatial analysis reported that the species diversity of Xiangxi River was significantly higher than that of Shennong Creek. Temporal analysis reported that the species diversity of Shennong Creek and Xiangxi River were higher in winter than in summer. The community classification analysis indicated that: at the phylum level, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* were the dominant bacteria, and their relative abundance accounted for 65.05% of the total community. At the class level, *Acidimicrobiia* had the highest relative abundance, accounting for 13.72% of all sequences, followed by *Betaproteobacteria* (13.12%) and *Alphaproteobacteria* (11.28%). The change of temperature was the main factor causing the summer and winter seasonal differences of prokaryotic biodiversity and community structure.

Keywords: 16S rRNA, Illumina MiSeq sequencing, biodiversity, Three Gorges Reservoir, spatiotemporal variation

Introduction

Prokaryotes are single-celled organisms that have no real morphological nuclei or mitochondria and play important role in the microbial community, mainly archaea and bacteria (Tanaka, 2009). It has become evident that, although prokaryotes generally lack membrane-bound organelles, they have a sophisticated intracellular organization (Rudner and Losick, 2010; Govindarajan and Amster-Choder, 2016; Surovtsev and Jacobs-Wagner, 2018). The ocean is the birthplace of prokaryotes, and its area covers about 70% of the world (Sun et al., 2010), because of the prokaryotic adaptability to a wide range of habitats and their high variability, which makes them spread all over the world. In aquatic ecosystems, prokaryotes play important roles in some processes, such as the circulation of biomass elements (Sorokin et al., 2014; Li and Qin, 2015), organic decomposition

(Gomez-Consarnau et al., 2012) and pollutant purification (Celik et al., 2008; Das and Chandran, 2011). In addition, prokaryotic plankton communities have complex effects and rapid response to changes in environmental conditions, hence the changes of their aggregate structure composition can serve as a good indicator of the environmental status of the water bodies (Massana and Logares, 2013). Prokaryotes play an important role in water quality monitoring, especially in lakes and reservoirs (De Wever et al., 2005). At present, great progress has been made in studies on the distribution of prokaryotes community in freshwater bodies (Li et al., 2017). However, there are few studies on the diversity of prokaryotes in reservoir ecosystems.

The Three Gorges Dam on the Yangtze River is the world's largest hydropower station. Reservoirs perform invaluable functions such as fresh water supply, flood control, and hydropower generation (Ouyang et al., 2021). After the first impoundment of the Three Gorges Reservoir in 2003, the hydrodynamic conditions and hydrological environment had changed remarkably (Yang et al., 2015), the eutrophication and algal blooms of some tributaries have attracted extensive attention at home and abroad (Ye and Cai, 2011; Zhang et al., 2016a). Xiangxi River and Shennong Creek are the first and second-largest tributaries of the Three Gorges Reservoir area, the ecological environment was directly related to the water quality of the Three Gorges Reservoir. After the storage of the Three Gorges Reservoir, Dinoflagellate and Diatom blooms have been a frequent occurrence in the Xiangxi River every spring (Fang et al., 2013). At different stages of operation, Shennong Creek has different forms of trunk flow backward, and often accompanied by cyanobacterial blooms (Wang et al., 2011). Large quantities of researches have certified that algal blooms in freshwater have led to serious environmental and societal problems (Rose et al., 2019), with long-term negative effects on water quality. Therefore, it is particularly important to study the structure of prokaryotic plankton communities in the Xiangxi River and Shennong Creek.

With the emergence of new molecular technologies, the field of microbial ecology has been promoted and developed, which makes the study of microorganisms in the freshwater environment more mature (Uyaguari-Diaz et al., 2016). In recent years, high-throughput sequencing technology (Lee et al., 2017) has developed rapidly. Based on the PCR amplification of the 16S rRNA gene of prokaryotes and high-throughput sequencing, their rapid and sensitive advantages have greatly promoted the depth and breadth of environmental microbial diversity research (van Vliet, 2010). In this study, Xiangxi River and Shennong Creek were selected as the research regions. The prokaryotic biodiversity and community composition were studied by 16S rRNA Gene-Illumina Miseq high-throughput sequencing and further biological information analysis. In addition, the correlation of environmental factors is of vital importance to reveal Xiangxi River and Shennong Creek prokaryotic plankton community structure and temporal and spatial evolution law of summer and winter, it provides a reference and scientific basis for the Three Gorges Reservoir area environmental quality monitoring.

Materials and methods

Sampling and determination of environment variables

We selected representative sampling of Xiangxi River and Shennong Creek, six sampling points were set up, each tributary three sampling points (*Fig. 1*). Water samples were collected from the Three Gorges Reservoir of the Yangtze River of China in August (summer) and December (winter) of 2013 (*Fig. 1*). At each sampling point, 3 bottles of

surface water (900 mL/bottle) are collected and then mixed. And Water samples were collected at a depth of 0.5 m beneath the water surface using a 5-L plexiglass sampler and were pre-filtered through 5 μm pore-size polycarbonate filters (Millipore) followed by filtration through GF/F filters (0.45 μm , Whatman) for molecular analyses. The samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

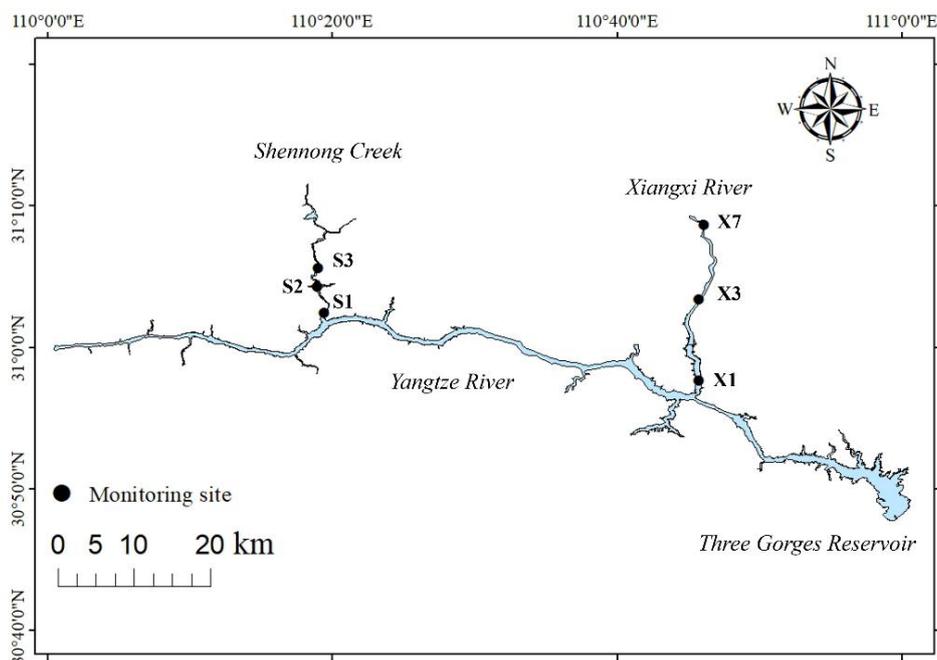


Figure 1. Locations of the monitoring sites in the Xiangxi River and Shennong Creek in Three Gorges Reservoir

The main physical and chemical indicators of water environment were tested through field test records and laboratory tests. The physical and chemical indexes of field tests include water temperature (WT), Turbidity (NTU), dissolved oxygen (DO), transparency (SD), pH, conductivity (SPC). The indicators of indoor water chemistry are total nitrogen (TN), total phosphorus (TP), orthophosphate (P- PO_4), nitrate (N- NO_3), ammonium (N- NH_4), chlorophyll a (Chl *a*) and chemical oxygen demand (COD). The physical and chemical data of the field were measured mainly through YSI (EXO3, USA), transparency disk (Secchi, Shanghai ShuoGuang Electronic Science and Technology Ltd. Co), and the determination method of water chemical indexes was mainly measured by the national standard method (Administration, 2002).

Ribonucleic acid (RNA) extraction, polymerase chain reaction (PCR) amplification and sequencing of 16S rDNA

Total community RNA was extracted from each core from each site. The extraction method used was a modification of that described by (Irastortza-Olaziregi and Amster-Choder, 2020; Dell'Orso et al., 2021). Amplification of the V4 region of the 16S rDNA from the samples, the following prokaryotes specific primers were used: 515F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Bates et al., 2011; Liao et al., 2015). PCR amplification and purification were based on

our laboratory's own methods. PCR was carried out under the following conditions: 35 cycles (denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, extension at 72 °C for 2 min) proceeded by 3 min denaturation at 94 °C and finally, an extension at 72 °C for 8 min. PCR products were quickly checked using 1% agarose gel electrophoresis and purified using the Gel Extraction Kit (Tiangen biotech China).

The purified amplicons were quantified with TBS-380 (Turner BioSystems Inc, USA). Paired-end (PE) sequencing of purified amplicons was carried out on an Illumina MiSeq PE250 platform (Majorbio Bio-Pharm Technology Ltd, Shanghai, China) (Hou et al., 2020) to perform batch analysis.

Statistical analyses

The raw data obtained by Illumina MiSeq sequencing platform were divided, primers were removed, Paired-end (PE) Reads were spliced, Tags' quality and length were filtered and intercepted and chimeras were removed to obtain the final effective tags. Then, Operational Taxonomic Units (OTUs) were formed by clustering all effective tags sequences (the default selected identity is 97%). Based on the homogenization OTU abundances table, use the QIIME software package of alpha_diversity. Py (http://qiime.org/scripts/alpha_diversity.html) script for four kinds of diversity indexes (Shannon, Chao1, Observed species, PD whole tree) calculation (Wang et al., 2012). Using the Bray-Curtis distance matrix as the method of clustering analysis, community structure was compared with Non-Metric Multi-Dimensional Scaling (NMDS). Analysis of similarities (ANOSIM) was based on Bray-Curtis distance measurements, which measured differences between sample groups in permutations. Both NMDS and ANOSIM were carried out using R package “vegan” (Warton et al., 2012). R (<https://www.processon.com/diagraming/5fd8629cf346fb07100fe483>) version 3.0.1 was used to draw relative abundance heat map of class level and cluster analysis between samples and species was also performed. First, detrended correspondence analysis (DCA) was used to detect the length of the environmental gradient. For the result of detrending correspondence analysis (DCA) was more than or equal to 4, Canonical correspondence analysis (CCA) was adopted (Lepš and Šmilauer, 2003). When conducting CCA analysis, the selected environmental factors were examined by the Monte Carlo permutation test (999 permutations) to ensure that the environmental factors had a good explanation for the microbe. When the results presented significant differences ($P < 0.05$), the relationship of environmental factors and prokaryotes community was analyzed using CCA in CANOCO 5 software (Borcard and Legendre, 2011).

Results

Spatiotemporal variation of environmental parameters

Eleven physical and chemical factors were listed in this study (*Table 1*). These factors didn't change much among all sampling sites, but they varied greatly in different seasons of summer and winter. The variation patterns of each factor between the Xiangxi River and Shennong Creek were basically similar. COD, P-PO₄, DO, pH, turbidity and N-NH₄ were highest in summer, and ORP and N-NO₃ were highest in winter and increased rapidly between summer and winter. However, the turbidity peaked in summer and dropped sharply in winter.

Table 1. Spatiotemporal variation of environment variables

	Summer						Winter					
	XX01	XX03	XX07	SNX01	SNX02	SNX03	XX01	XX03	XX07	SNX01	SNX02	SNX03
TP (mg/L)	0.08	0.06	0.26	0.04	0.04	0.02	0.12	0.14	0.10	0.10	0.080	0.10
TN (mg/L)	1.60	1.51	1.34	1.18	1.75	1.41	1.49	1.61	1.54	1.52	1.56	1.50
COD (mg/L)	2.59	2.39	3.69	2.40	2.72	2.25	1.34	1.30	1.50	1.34	1.34	1.46
P-PO ₄ (mg/L)	0.10	0.06	0.05	0.16	0.16	0.01	0.07	0.09	0.08	0.05	0.04	0.030
N-NH ₄ (mg/L)	0.02	0.04	0.07	0.08	0.04	0.05	0.00	0.00	0.04	0.02	0.02	0.02
N-NO ₃ (mg/L)	1.00	1.05	0.12	0.67	0.88	0.75	1.38	1.39	1.48	1.33	1.33	1.30
WT (°C)	29.60	29.60	29.50	30.90	30.00	30.90	18.10	18.10	17.70	17.50	18.20	17.60
DO (mg/L)	12.53	11.01	15.55	14.51	10.46	10.06	7.33	6.94	7.65	6.03	6.95	7.13
ORP (mV)	61.20	56.10	48.10	68.90	62.90	64.70	129.20	157.80	133.70	146.60	167.80	152.20
pH	9.31	9.23	9.75	9.49	9.34	9.43	8.56	8.71	8.58	8.63	8.60	8.67
Turbidity (NTU)	8.20	6.10	17.80	7.60	9.10	7.30	2.70	2.60	2.70	1.70	1.40	1.30

Analysis of alpha diversities

For the analyzed 12 samples in this study, a total of 602175 sequences (an average of 256 bp) were obtained after denoising and chimera detection, Then, a cut-off value of 97% sequence identity was performed and a total of 1691 OTUs were generated.

Alpha diversities of prokaryotes were high and varied greatly among the 12 samples, with Shannon index of 6.45 to 7.54, Chao 1 index of 595.60 to 824.80, Observed species index of 476 to 778 and PD whole tree index of 37.54 to 60.84 (Table 2). In the two different tributaries of Xiangxi River and Shennong Creek, the species diversity in winter was higher than that in summer. At the different stations of Xiangxi River and Shennong Creek, the differences were not significant in the same season ($P > 0.05$); but at the same site, the differences of most species diversity indices between different seasons were significant ($P < 0.05$). The community assemblages of diversity indices of all samples were congregated comparing α -diversity indices of prokaryotic communities among different groups. In the intergroup distribution map of the α -diversity indices, Shannon index (Fig. 2A), Chao1 index (Fig. 2B), Observed species index (Fig. 2C) and PD whole tree index (Fig. 2D) of XXwin were the highest, indicated that the abundance and evenness of species composition in Xiangxi River were highest in winter, and corresponding species indices had the highest group diversity. The four diversity indices of Shennong Creek in summer were the lowest, indicated that Shennong Creek had the lowest species diversity in summer. Differences in most species diversity indices were significant ($P < 0.05$). On the whole, the changes of the four indices were mostly similar. In space, the species diversity of Xiangxi River was significantly higher than that of Shennong Creek. In season, the species diversity of Shennong Creek and Xiangxi River was higher in winter than in summer.

Table 2. Indices of the alpha diversity of sampling

Seasons	Shannon	Chao 1	Observed species	PD whole tree
XX01sum	6.54	752.62	625	46.67
XX03sum	6.63	595.60	560	43.32
XX07sum	6.50	677.87	598	44.63
XX01win	7.45	824.89	742	57.23
XX03win	7.15	763.11	711	54.39
XX07win	6.57	749.56	664	51.87
SNX01sum	6.51	562.80	513	39.23
SNX02sum	6.91	712.44	636	47.23
SNX03sum	6.45	513.14	476	37.54
SNX01win	7.08	819.92	778	60.85
SNX02win	6.69	657.32	645	52.26
SNX03win	7.00	794.42	687	52.46

Note: XX01sum, XX03sum, XX07sum represent Xiangxi Creek 01, 03, 07 sites in summer, respectively; XX01win, XX03win, XX07win represent Xiangxi Creek 01, 03, 07 sites in winter, respectively; SNX01sum, SNX02sum, SNX03sum represent Shennong Creek 01, 02, 03 sites in summer, respectively; SNX01win, SNX02win, SNX03win represent Shennong Creek 01, 02, 03 sites in winter, respectively

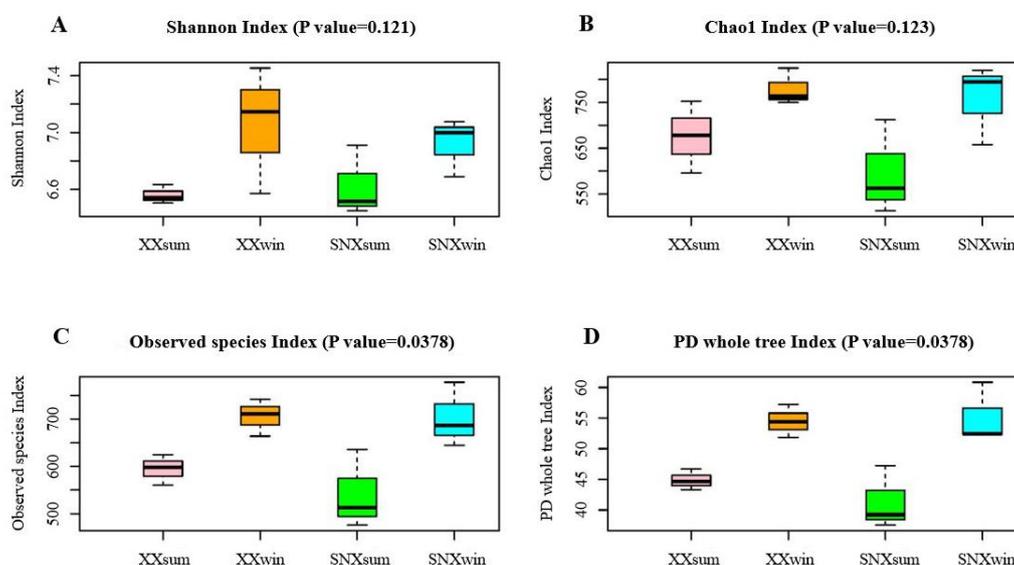


Figure 2. Alpha diversities of prokaryotes. XXsum represent Xiangxi Creek in summer; XXwin represent Xiangxi Creek in winter; SNXsum represent Shennong Creek in summer; SNXwin, represent Shennong Creek in winter

Distribution of prokaryotic communities

Based on the phylogenetic analyses, 34 taxa at the level of phylum were identified. These 34 phyla contain 71 classes, 111 orders, 206 families and 311 genera.

At the phylum level, we divided the community into two parts according to the season: summer (Fig. 3A) and winter (Fig. 3B). The results revealed that the prokaryotic communities of Xiangxi River and Shennong Creek in the Yangtze River basin were composed of 34 species. *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* were dominant species, accounting for more than 65% of total communities. *Planctomycetes*,

Verrucomicrobia, *Firmicutes*, *Cyanobacteria*, *Chloroflexi*, *Acidobacteria*, and *Saccharibacteri* were the subdominant populations of prokaryotes in the two major water areas, while the relative abundance of other phyla were relatively low. *Figure 3* provides an overview of the community composition of prokaryotes in two seasons. The dominant bacteria in summer were *Proteobacteria* (26.63%), *Actinobacteria* (24.23%), *Bacteroidetes* (19.37%) and *Cyanobacteria* (13.48%). In winter, *Proteobacteria* (39.25%) were dominant, followed by *Actinobacteria* (25.61%) and *Bacteroidetes* (9.30%), *Planctomycetes* (6.21%) and *Acidobacteria* (5.92%). Comparing the dominant phyla of the two seasons, it was found that the composition of prokaryotes in Xiangxi River and Shennongxi Creek in the Yangtze River basin was significantly related to the seasons, among which the number of *Proteobacteria* and *Bacteroidetes* varied greatly from summer to winter.

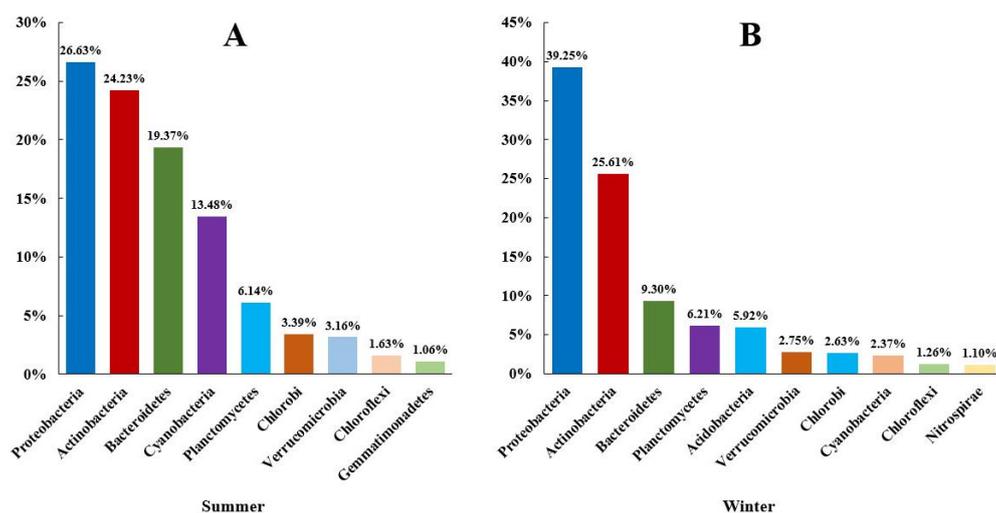


Figure 3. Prokaryotic communities of Xiangxi River and Shennong Creek were divided into two parts (3A and 3B) according to the season of summer and winter

Heat map of prokaryotes with a level relative abundance greater than 0.1% was selected. Throughout the whole profile, the prokaryotic communities of Xiangxi River and Shennong Creek presented a unique distribution pattern (*Fig. 4*). The clustering results based on the horizontal similarity of the OTUs community revealed that the prokaryotic communities exhibited obvious seasonal differences and station differences. The relative abundance heat map based on class level revealed that *Acidimicrobiia* had the highest relative abundance, accounting for 13.72% of all sequences, followed by *Betaproteobacteria* and *Alphaproteobacteria*, accounting for 13.12% and 11.28% of the total sequences respectively. *Armatimonadia* had the lowest relative abundance, accounting for only 0.11%. Further analysis founded that *Spartobacteria* and *Chloroflexia* tended to change with the seasons, and their relative abundance was higher in summer than in winter. In contrast, *Holophagae*, *Nitrospira* and *Jg30-KF-CM66* were higher in winter than in summer. Sequences assigned to *Cyanobacteria* accounted for 2.5% of the total number, which dominated in Xiangxi River in summer. The comparison between Xiangxi River and Shennong Creek revealed that the relative abundance of Shennong Creek was smaller than that of the Xiangxi River. The species composition of Xiangxi River was richer and the community structures were more complex.

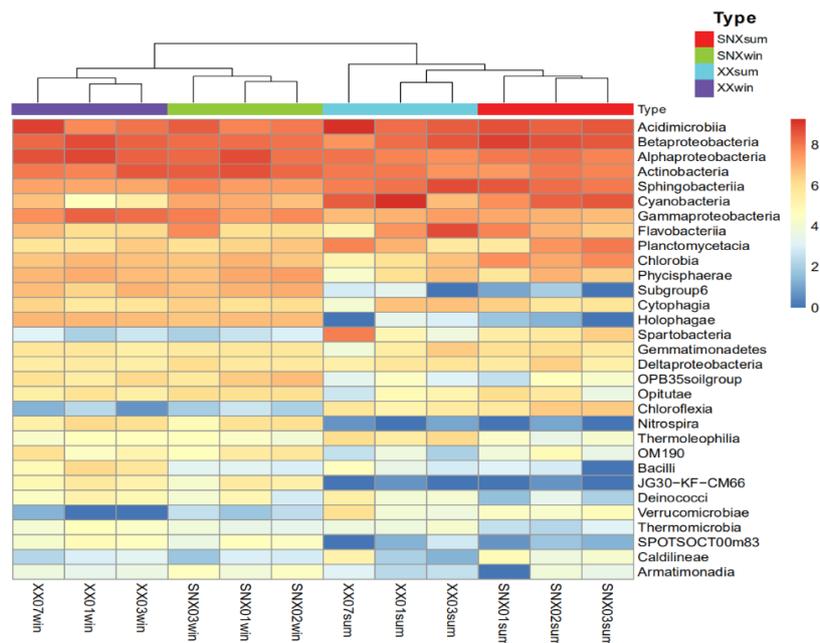


Figure 4. The community distribution and relative abundance of Prokaryotes. The operational taxonomic units (OTUs) were defined at 97% sequence similarity threshold. Tree chart of 12 samples from two large bodies of water in winter and summer, based on the similarity of Bray-curtis. The color-coding at the end of the branch is based on four groups of six sampling sites. The heat map shows the relative abundance of each sample at the class level. The class with a relative abundance greater than 0.5% is selected and combined with the phylogenetic data to achieve the sample clustering (longitudinal clustering) at the level of the class. The sequence data is standardized by scaling the range of each sample (0-8)

Variations of community structure and composition

A total of 1961 OTUs were generated and the shared OTUs of the different groups were further identified by the Venn diagram (Fig. 5). 345 OTUs were shared among the two seasons of Xiangxi River and Shennong Creek. Among them, Shennong Creek has the highest OTUs value in winter, and has the lowest OTUs value in summer. The shared OTUs of Shennong Creek and Xiangxi River in summer were 621, and 735 in winter. OTUs were higher in winter than in summer.

Based on the calculation of the Bray-Curtis distance matrix, the Non-metric multidimensional scaling (NMDS) analysis method was used to study the differences of prokaryotic community structures in Xiangxi River and Shennong Creek in summer and winter (Fig. 6). NMDS analysis revealed that the difference between XX03 summer and SNX01 summer is the largest, while that between SNX03 summer and SNX02 summer was the smallest. Basically, there were significant differences ($P < 0.05$) in the community structures of prokaryotes between Shennong Creek and Xiangxi River in summer and winter. The species similarity of Xiangxi River in winter is similar to that of Shennong River in winter. And Xiangxi River in summer is similar to that of Shennong River in summer. The species similarity in winter is higher than in summer.

Analysis of similarities (ANOSIM) revealed that there were significant differences ($P = 0.001$) between groups (Fig. 7). It indicted that differences between groups were greater than differences within groups. Among the groups, the similarities from small to

large are as follows: XXwin (Xiangxi River in winter) - SNXwin (Shennong River in winter) - SNXsum (Shennong River in summer) - XXsum (Xiangxi River in summer).

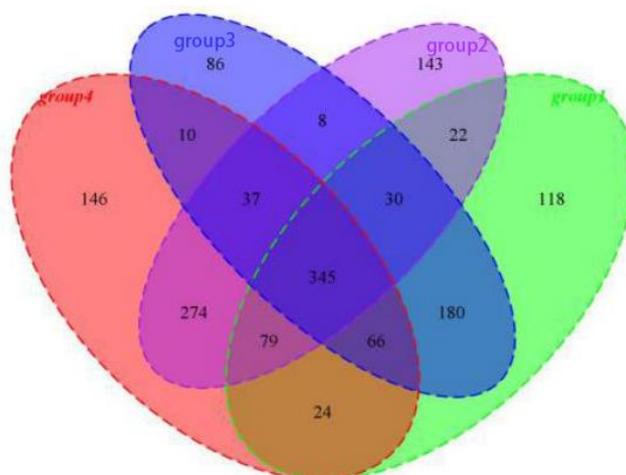


Figure 5. Unique and shared OTUs of prokaryotes in Xiangxi River and Shennong Creek. Group1 represents XXsum; Group2 represents XXwin; Group3 represents SNXsum; Group4 represent SNXwin

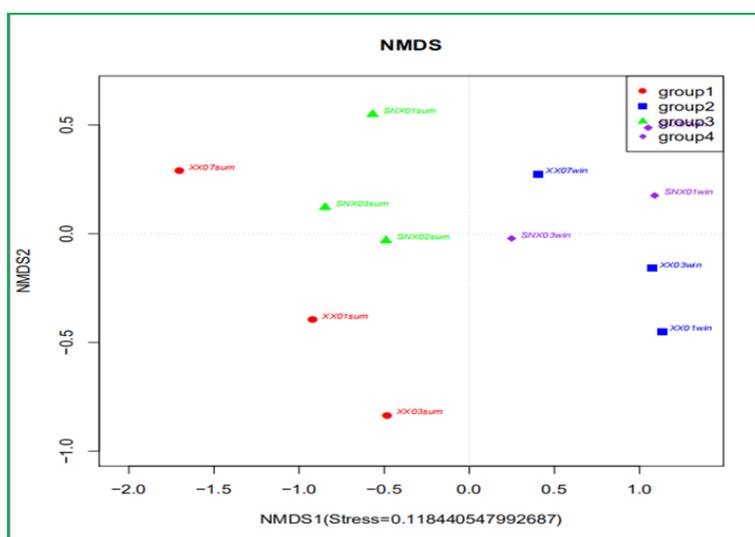


Figure 6. Non-metric multidimensional scaling (NMDS) ordination of the prokaryotic community based on Bray-Curtis distance. The points in the figure represent the samples, and the distance between the point and the point indicates the degree of difference. When Stress is less than 0.2, it indicates that NMDS analysis has certain reliability

Influences of species and physicochemical factors

Prokaryotes grow in water and were affected and restricted by a variety of physical and chemical factors in water, such as WT, ORP, N-NH₄, P-PO₄, DO and pH, which may affect the prokaryotic biodiversity. From the 11 environmental physicochemical factors, 4 influential factors ($P < 0.05$) were selected for CCA analysis. Based on CCA (Fig. 8), the bacterial composition was significantly correlated with temperature ($P = 0.06$), pH

($P=0.08$), ORP ($P=0.018$) and $N-NH_4$ ($P=0.05$). The relationships between the 8 dominant bacteria groups (relative abundance greater than 2%) and environmental factors were as follows: *Bacteroidetes* were significantly positively correlated with WT; *Chloroflexi* was significantly positively correlated with pH, $N-NH_4$. Cyanobacteria were positively correlated with pH, $N-NH_4$, WT, and negatively correlated with ORP; *Firmicutes*, *Actinobacteria*, *Proteobacteria* and Planctomycetes all showed a positive correlation with ORP.

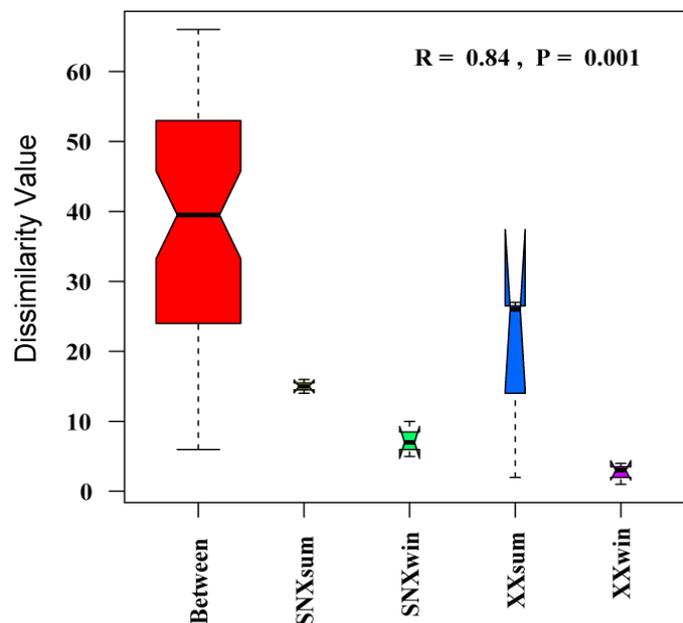


Figure 7. Analysis of similarities (ANOSIM) of between and within groups in Xiangxi River and Shennong Creek in summer and winter

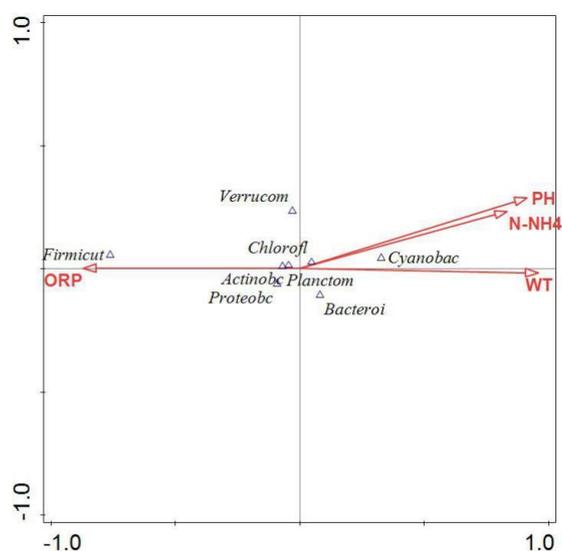


Figure 8. Canonical correspondence analysis (CCA) of the prokaryotic community-environmental factors relationships. Firmicut: Firmicutes; Verrucom: Verrucomicrobia; Chlorofl: Chloroflexi; Actinobc: Actinobacteria; Planctom: Planctomycetes; Proteobc: Proteobacteria; Bacteroi: Bacteroidetes; Cyanobac: Cyanobacteria

Discussion

Seasonal variation of dominant bacteria and corresponding ecology in summer and winter

Prokaryotic diversity and community composition of Xiangxi River and Shennong Creek in summer and winter were analyzed by using the 16S rRNA gene cloning library. Although this method could not reflect all prokaryotic groups in the sample, it can reflect the main dominant bacterial groups in the sample. At the phylum level, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Cyanobacteria*, *Planctomycetes* and *Acidobacteria* were common bacteria in summer and winter, it indicates that the two-bay has formed a stable prokaryotic community. The composition is similar to that of other dominant groups of freshwater habitats (Simonato et al., 2010). The study has found that *Cyanobacteria*, *Actinobacteria*, *Verrucomicrobia* and *Proteobacteria* dominated in two shallow and small natural lakes (Ávila et al., 2016). The previous study has reported on the diversity of bacterial communities in Taihu Lake, the main bacterial communities in this lake included *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Planck oomycetes*, *Verrucomicrobia* and *Chlorobi* (Tang et al., 2017). All these studies have confirmed that *Proteobacteria* and *Actinobacteria* were typical freshwater phyla and have relatively high abundance distributions in different freshwater habitats. In this survey, the dominant groups in the Xiangxi River and Shennong Creek were *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Cyanobacteria*. The distribution of dominant bacteria in two seasons was different, among them, the dominant bacteria were *Bacteroidetes* and *Cyanobacteria* in summer and *Proteobacteria* in winter. Significant seasonal changes of the dominant bacteria may be attributed to the high summer temperature, which is conducive to the growth of *Bacteroidetes* and *Cyanobacteria*. In addition, the seasonal variation of bacteria may be related to the content of nutrients in the water, which will lead to the growth of some bacteria being promoted or inhibited (Dai et al., 2016). Our study found that the number of *Proteobacteria* increased gradually as the season transited from summer to winter and the *Bacteroidetes* decreased. *Proteobacteria* is usually an important indicator of water eutrophication, to some extent, the water quality of Xiangxi River and Shennong Creek has changed. Recently, several studies have been carried out on different marine ecosystems, these studies have reported the main flora of the bacterioplankton community in the South China Sea or its sediments. In the Southern Ocean, Antarctica, the main flora of the Bacterioplankton community were *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, and *Firmicutes* (Singh et al., 2015). In the northern region of the South China Sea, *Proteobacteria*, *Firmicutes*, *Planctomycetes*, *Actinobacteria* and *Chloroflexi* were most diverse (Zhu et al., 2013). Thus, it implies that the composition of prokaryotes in marine and freshwater environments is a little similar, but the difference between dominant bacteria in each group is more significant ($P < 0.05$).

Effects of summer and winter seasonal variation on prokaryotic biodiversity and community structure

Alpha diversities are an important indicator of species diversity. The analysis results of 16S rDNA sequencing on prokaryotic communities in Xiangxi River and Shennong Creek showed that the diversity index was different in summer and winter, and the diversity of prokaryotic communities was higher in winter than in summer. Our findings may be related to the optimum growth temperature for most prokaryotes. A study on the seasonal variation of bacterioplankton community structure in Mochou Lake and Zixia

Lake in Nanjing, and found that the season is an important factor affecting the phytoplankton community structure, and the seasonal changes lead to changes in the water temperature, pH and other environmental factors (Cao et al., 2016). The water temperature of Xiangxi River and Shennong Creek in winter is about 18°C, which is favorable for the growth of prokaryotes. However, the water temperature in summer is 29 - 31°C, which may limit the growth of prokaryotes. Some studies on the gulf and lakes showed that the main driving force of the change of bacterial community structure was the evolution of environmental factors such as temperature caused by seasonal changes (Wu et al., 2007; Bucci et al., 2014). Therefore, the water temperature is an important factor influencing the seasonal difference of prokaryotic biodiversity.

Clustering results at the phyla level indicated that the composition of prokaryotic communities in Xiangxi River and Shennong Creek was related to the summer and winter different seasons ($P < 0.05$), but had little correlation with the sites ($P > 0.05$). This was similar to the research that the time change of the microbial community in Taihu Lake was significantly greater than that in space (Li et al., 2015). Other studies have found that seasonal changes have a more significant impact on the community structure (Tsuchiya et al., 2011; Chen et al., 2013), which may be the common characteristics of eutrophic lakes. These results provide important information for the study of bacterial community composition and spatiotemporal changes in other freshwater lakes. At the same time, the study of seasonal changes of phytoplankton community structure in lakes is of great significance for understanding the relationship between prokaryotic community and environmental factors.

Correlation between the community structure of prokaryotes and environmental factors

Previous studies suggested that the bacterial community was significantly impacted by environmental factors, the contribution of different environment variables to the change of planktonic bacteria community was different (Song et al., 2012; Zhang et al., 2016b). In the present study, the CCA analysis showed that bacterial communities were significantly correlated with temperature, followed by pH, ORP and the concentrations of N-NH₄. These factors were important environmental factors influencing the community composition of prokaryotes in Xiangxi River and Shennong Creek. Some studies have reported that the variation of bacterial community structure was related to water temperature in lakes and streams (Sakami, 2008; Adams et al., 2010). As a result, it was found that the water bodies of the Xiangxi River and Shennong Creek were alkaline in summer and winter. The average pH value of 6 stations was 8.6 in winter and 9.4 in summer, while the proportion of Cyanobacteria amount was 2.3679% and 13.4813%, respectively. The quantity of Cyanobacteria was more abundant in summer. However, other research found that Cyanobacteria was more abundant in the lake bodies with lower alkalinity (Ballot, 2004). The difference may be related to physical environmental factors. Water characteristics, especially water temperature, might play a significant impact on the abundance of Cyanobacteria (Wang et al., 2008). In our study, the water temperature was significantly higher in summer than in winter, which might result in the Cyanobacteria population increasing.

Conclusions

Prokaryotic communities in Xiangxi River and Shennong Creek were related to the summer and winter different seasons, but had little correlation with the sites. The number of *Proteobacteria* increased gradually as the season transited from summer to winter and the *Bacteroidetes* decreased. Our research found that the composition of prokaryotes in marine and freshwater environments is a little similar, but the difference between dominant bacteria in each group is more significant ($P < 0.05$). This may be related to the optimum growth temperature for the growth of prokaryotes.

In future research, we can combine more molecular biology techniques such as qPCR, single molecule real-time sequencing with HPLC-CHEMTAX technology to study prokaryotic communities in Xiangxi River and Shennong Creek. And future research we can combine four seasons and interannual variability to study succession law of prokaryotic plankton community structure in Xiangxi River and Shennong Creek.

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