

## Diversity and distribution of bacteria and archaea in Tuosu Lake in Qaidam Basin

Weijun Song\*, Chunyan Sun

Chemical Engineering Institute, Qinghai University, Xining 810016, Qinghai, China

\*Correspondence to: WJSong@qhu.edu.cn

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**Abstract:** Microbes in plateau lakes are important participants of material circulation and energy flow in plateau ecosystems. Knowledge of the microbiota, such as bacteria and archaea, community distribution and diversity in plateau lakes is the basis to understand the species succession, adaptation, maintenance and metabolic mechanisms of specific environmental microbial ecosystems. This work aimed to reveal the diversity and succession of the microbiota in Tuosu Lake to provide a biological basis for the exploration and development of microbial resources in the plateau lakes. The distribution and diversity of microflora in Tuosu Lake, hypoxia, high altitude, alkaline, closed plateau lake with fresh water supply, was investigated. The total DNA was extracted from six samples with different salinity from different geographical locations of Tuosu Lake. The 16S rRNA gene of bacteria and archaea were determined by using high-throughput sequencing-based on an Illumina Miseq sequencing platform. The microbiota in Tuosu Lake has a high diversity and complexity and there are a large number of unclassified microbial species. The bacterial communities in Tuosu Lake are dominated by *Proteobacteria* (44.3%) and *Actbacteria* (17.2%). Among them,  $\beta$ -*Proteobacteria* is the dominant genus in the low-salt sample, while  $\gamma$ -*Proteobacteria* is more advantageous in the samples with higher salinity. The archaeal communities are dominated by *Euryarchaeota* (50%) and *Woesearchaeota* \_DHVEG-6 (42.6%). The vast majority of the methanogenic archaea in Tuosu Lake samples belong to *Methanomicrobia*, and the methanogens in low-salinity samples are significantly more abundant than those in high-salt samples. Diversity and distribution appear to be highly influenced by water salinity and pH.

**Key words:** Tuosu Lake; High-throughput sequencing; Microbial diversity.

### Introduction

It is important to study the diversity and distribution of bacteria (1-5). Plateau lakes constitute an important part of the plateau terrestrial ecosystem and play an important role in maintaining the stable cycle of the plateau ecosystem. The study of micro-organisms in the lakes of the Plateau is of great significance to reveal the response of micro-organisms to climatic and environmental changes in the context of global warming. The Qaidam Basin in Qinghai Province in China is dotted with salt lakes with various sizes, forming a special plateau environment with high cold, high altitude, high salinity, high alkali, and anoxia. According to the current research reports (6, 7), the vast majority of the plateau environmental microorganisms that have been isolated are undiscovered.

Tuosu Lake is located at the eastern end of the Qaidam Basin in the Qinghai Tibet Plateau, with an average altitude of 2800 meters. The oxygen content in the air is only 50-60% of that in the plains. The climate is arid, with little rain and wind, and the surrounding area of the lake is a typical Gobi Desert climate (8). Tuosu Lake is a high-alkaline saline lake, which can be used as a special plateau environment. This environment can breed different species of environmental microbes that are different from the general environment. In addition to being inaccessible here, the lake is less affected by human activities and is basically in the original state,

which provides a "non-polluting" natural materials for scientific research. Moreover, microbes themselves can also serve as extremely important biological resources. There are many types of microbial metabolism in the special environment of the plateau, and it also occupies a very important position in the stable cycle of the ecosystem. Therefore, it is of great significance to carry out the study of microorganisms on the ecological cycle of the plateau, and their adaptation mechanism in the plateau environment.

In recent years, research on microbial diversity and adaptation mechanisms in special environments has received increasing attention, and the research has also made certain progress. In particular, with the rapid development of molecular biology, people's understanding of special environmental microbes has been gradually deepened. High-throughput sequencing technology is the most commonly used next-generation sequencing technology, which can simultaneously sequence millions of DNA molecules. It has unique advantages in analyzing the structure of microbes and can reveal the complexity and diversity of microbes in situ environment (9-12), so as to be widely concerned and adopted by researchers.

This study aimed to study the microflora in the high altitude, low atmospheric pressure and high salinity alkaline environment represented by Tuosu Lake and constructing a bacterial and archaeal 16S rRNA gene library by high-throughput sequencing. The community

distribution and diversity of microflora in the plateau lakes were determined, which could provide a reference for subsequent studies on the material circulation and energy flow in the ecosystem of the plateau lakes, and the succession and metabolic maintenance of the ecosystem.

## Materials and Methods

### Sampling sites

Tuosu Lake is located in the east of the Qaidam Basin, 40 km away from Delingha, the capital city of Hercynian. The lake has an average elevation of 2,800 meters. The annual rainfall is 100 mm, and the annual radiation is 726.6 KJ/cm<sup>2</sup>. It belongs to the semi-desert continental climate. The summer climate is warm, with little rain. The average temperature in July is 16.7°C, the extreme maximum temperature is 33.1°C, and the annual average temperature is 3°C (8). The salt content of the water is 23.8 g/L, and the average pH of the lake is higher than 9.0 which is a typical high-altitude saline-alkaline lake. The main source of water in Tuosu Lake is Crook Lake in the northern. The two lakes are connected by a river, as shown in Figure 1. Around the lake, there are sparse reeds and a small amount of plankton. The rest of the area is the Gobi, with occasional sporadic white thorns.

The samples used in this study are mixtures of lake water and surface sediment collected in September 2016 in the Tuosu Lake. The salinity at the sampling sites gradually increases from northwest to southeast. The specific distribution and information of sampling sites are shown in Figure 1 and Table 1. The lake water temperature and pH were measured on-site. The mixtures of lake water and sediment were packed in sterile test tubes, sealed and stored in a car refrigerator at 4°C and transported to the laboratory for subsequent treatment. The total nitrogen (TN), total phosphorus (TP), and total organic carbon (TOC) in the waters of Tuosu Lake were measured by Pony Testing International Group, Shanghai, China. Total salinity (TS) was measured by the ATC handheld salinometer (Hebei Hongqi Instrument Co., Ltd., China). Lake pH was measured by Ray Magnetic PHBJ-260 (Shanghai Precision Scientific Instrument Co., Ltd., China).

### Total DNA extraction and PCR amplification of 16S rRNA gene

The collected lake water and sediment samples were mixed and moved into 50 mL sterile centrifuge tubes, centrifuged at 12000 rpm for 20 minutes, discarded the supernatant, resuspended the bottom pellet and transferred into a 2 ml sterile centrifuge tube, centrifuged at 12000 rpm for 20 minutes, and collected the pellet for total DNA extraction (13). The total DNA extraction was performed according to the AxyPrep™ Bacterial Genomic DNA Extraction Kit instructions. The extracted DNA was immediately frozen at -20°C for use (14).

During the PCR amplification, bacterial primers were: 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3); archaeal primers were Arch344F (5'-ACGGGGYGCAGCAG-GCGCGA-3') and Arch915R (5'-GTGCTCCCCCGC-CAATTCCT-3'). 3 replicates per sample. PCR amplification products of the same sample were mixed and detected by 2% agarose gel electrophoresis. PCR products were recovered by cutting the gel using AxyPrep DNA Gel Recovery Kit (AXYGEN), then Tris\_HCl elution and 2% agarose electrophoresis were performed.

### Sequencing and analysis

Purified and purified PCR products were subjected to high-throughput sequencing of the 16S rRNA gene (V3 to V5 region) (15). The sequencing platform was Illumina Miseq. The sequencing work was completed by Shanghai Majorbio Co., Ltd. The 16s bacterial and archaeal ribosome database used the silva database.



Figure 1. The space distribution map of sampling sites.

Table 1. Information description of different sampling sites at Tuosu Lake.

Sample	Water temperature (°C)	Altitude (m)	Latitude and longitude	SDWS	TN (mg/L)	TP (mg/L)	TOC (mg/L)	pH	TS (g/L)
T-a	16.5	2810	37°19'35"N 96°87'93"E	16cm	3.52	<0.01	11.0	8.87	3.12
T-b	17	2810	37°20'89"N 96°90'55"E	30cm	2.53	<0.01	20.3	9.07	19.2
T-c	20	2810	37°21'52"N 96°92'37"E	20cm	3.79	0.03	17.6	9.01	23.0
T-d	21	2809m	37°20'26"N 96°95'53"E	30cm	5.64	<0.01	15.6	9.11	21.4
T-e	23	2810	37°18'92"N 96°97'65"E	30cm	3.07	0.02	16.6	9.07	23.7
T-f	20°C	2810	37°15'93"N 97°00'24"E	30cm	2.84	<0.01	20.3	9.13	23.8

SDWS (Sediment depth to water surface), TN (Total nitrogen), TP (Total phosphorus), TOC (Total organic carbon) and TS (Total salinity). Relative humidity of air in all sample were 30%.

Using RDP classifier Bayesian algorithm, the taxonomy of 97% similar operational taxonomic units (OTUs) representative sequence was analyzed, and the community of each sample was analyzed at different classification levels: domain, kingdom, phylum, class, order, family, genus, and species.

**Results**

**Analysis of bacterial community**

As shown in Figure 2, there were 35 phyla, 77 classes, 140 orders, 267 families, 445 genera and 690 species of bacteria in 6 samples collected in this experiment. The number of OTUs was 1037. Of these, 10 phyla accounted for more than 1%. Proteobacteria accounted for the highest proportion (44.3%) in all microbes, followed by Actinobacteria (17.2%), Bacteroidetes (12.1%), Verrucomicrobia (9.81%), Firmicutes (9.30%), Chloroflexi (3.62%), Planctomycetes (1.39%), Deinococcus-Thermus (1.22%), Tenericutes (1%) and Cyanobacteria (1.0%).

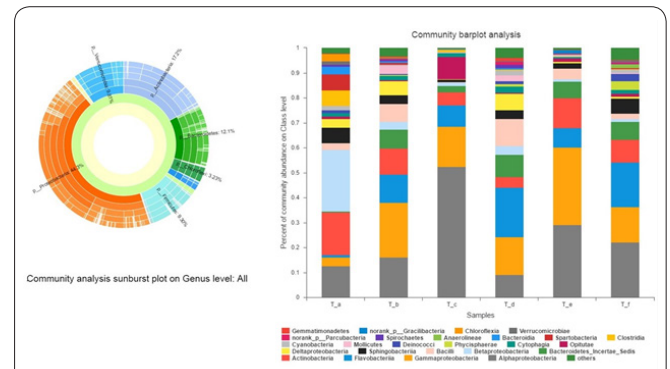
The histogram of microbial colonies under the class classification level showed that Proteobacteria is a predominant species in the Tuosu Lake. The  $\beta$ -Proteobacteria in the samples T-a of the river to the lake was 24.78%, followed by  $\alpha$ -Proteobacteria 12.66%,  $\gamma$ -Proteobacteria, 3.36%, and  $\delta$ -Proteobacteria 3.45%. In the other samples with relatively high salt content,  $\beta$ -Proteobacteria was significantly decreased to 3.16% in T-b, 1.39% in T-c, 3.53% in T-d, 1.11% in T-e and 1.28% in T-f, whereas the  $\gamma$ -Proteobacteria was significantly increased to 21.87% in T-b, 16.20% in T-c, 15.29% in T-d, 31.06% in T-e and 14.17% in T-f.  $\alpha$ -Proteobacteria was significantly increased in T-c.  $\delta$ -Proteobacteria was not significantly changed. In addition, Flavobacteria was significantly increased with lake salinity: 1.03% at T-a, 11.23% at T-b, 8.43% at T-c, 19.68% at T-d, 7.78% at T-e, and 17.73% at T-f.

Figure 3 is a cluster analysis of the bacteria on the genus level in Tuosu Lake. The T-f and T-e samples were similar, T-d and T-b were similar, and the sample with the lowest salinity in the T-a sample was quite different from other samples.

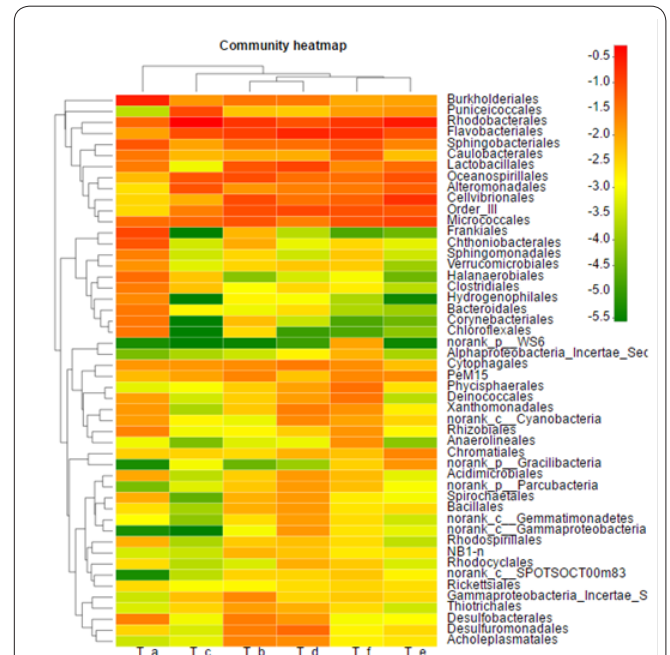
From the order classification level, the predominant bacteria in the sample T-a,  $\beta$  proteobacteria mainly belonged to Burkholderiales, which accounted for about 22.0% of the total abundance of the samples. In these Burkholderiales, the undefined species *Limnohabitans* are predominant and their abundance is about 8.86%. Among the other samples with relatively high salt content, abundances of Order\_III, Flavobacteriales, Alteromonadales, Cellvibrionales, Puniceococcales, and Oceanospirillales were significantly increased, compared with T-a.

**Effect of physical and chemical properties of lake water on bacterial community**

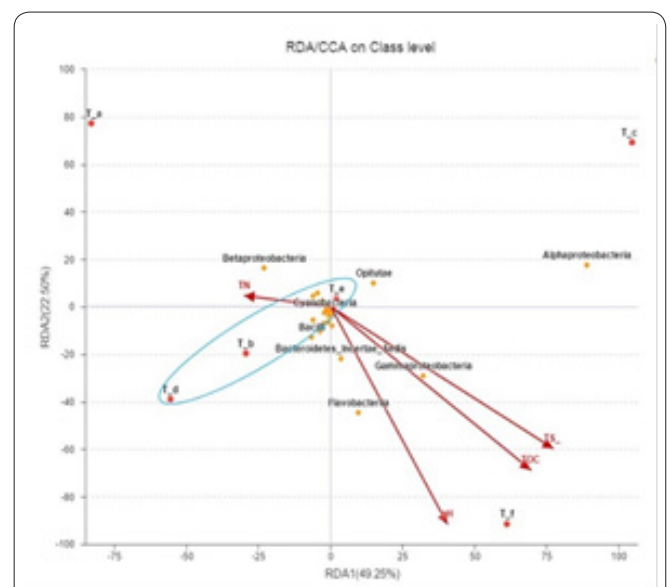
Figure 4 shows the corresponding CCA analysis among the sampling sites, bacterial communities and environmental factors based on the class level. The results showed that the distances between the T-b, T-d, and T-e sampling sites were closer than other sites, indicating that the bacterial communities at these sites were also similar. The sample points of T-f, T-c, and



**Figure 2.** Statistical analysis of bacterial relative abundance at class levels of Tuosu Lake.



**Figure 3.** Community heatmap at the genus level of bacterial composition in different sampling sets of Tuosu Lake.



**Figure 4.** Canonical correspondence analysis of species information in the Tuosu Lake at the class level. Note: Round dots (●): Different samples; rhombus (◆): Bacteria classes; Arrows: Environmental factors.

T-a were relatively far away from the other sites, indicating that the bacterial communities in these samples were quite different from other sites. In environmental

factors, total salinity (TS), total organic carbon (TOC), and pH had a relatively large influence on the bacterial community of the sample. The influence of total nitrogen (TN) in the sample is relatively weaker than other factors.  $\gamma$ -Proteobacteria, Bacteroidetes\_incertae\_sedis were positively correlated with TS, TOC, and pH.  $\beta$ -Proteobacteria was positively correlated with TN.

**Analysis of the archaeal community**

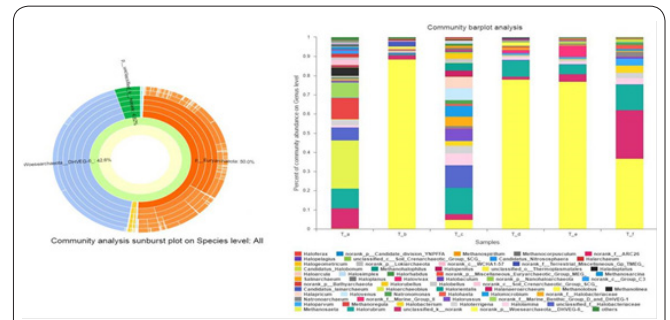
Based on the analysis of the alpha diversity of archaea at the genus level, it was found that the Chao range of the sample ranged from 43 to 85.33, the Shannon index ranged from 0.68 to 3.03, and the Simpson index ranged from 0.06 to 0.78. Among all samples, the T-c sample community had the highest diversity, the Shannon index value was 3.03, and the T-b sample community had the lowest diversity with a Shannon index value of 0.68. There were 13 species, 25 classes, 30 orders, 49 families, 99 genera, and 199 species of archaea. The number of OTUs was 1178.

Figure 5 shows the total classification map of Salt Lake archaea based on the level of phylum. The archaea mainly belonged to Woesearchaeota\_DHVEG-6 (42.60%) and Euryarchaeota (50.0%). In addition, Tuosu Lake also contained a certain amount of unclassified\_k\_norank (4.82%) and a small amount of Thaumarchaeota (1.43%). The histograms of archaeal based on genus level showed that Woesearchaeota\_DHVEG-6 was relatively high in the samples with relatively high salinity, while its content in T-a in the fluvial inlets was relatively low: T-a (0.39%), T-b (88.55%), T-c (4.87%), T-d (78.07%), T-e (76.97%), and T-f (36.73%). In addition, the abundant *Marine\_Benthic\_Group\_D\_and\_DHVEG-1* archaea in the T-a sample had a significant decrease in abundance in samples with relatively high salinity, and their content in each sample was T-a (8.03%), T-b (0.09%), T-c (0.00%), T-d (0.08%), T-e (0.14%), and T-f (0.10%), respectively.

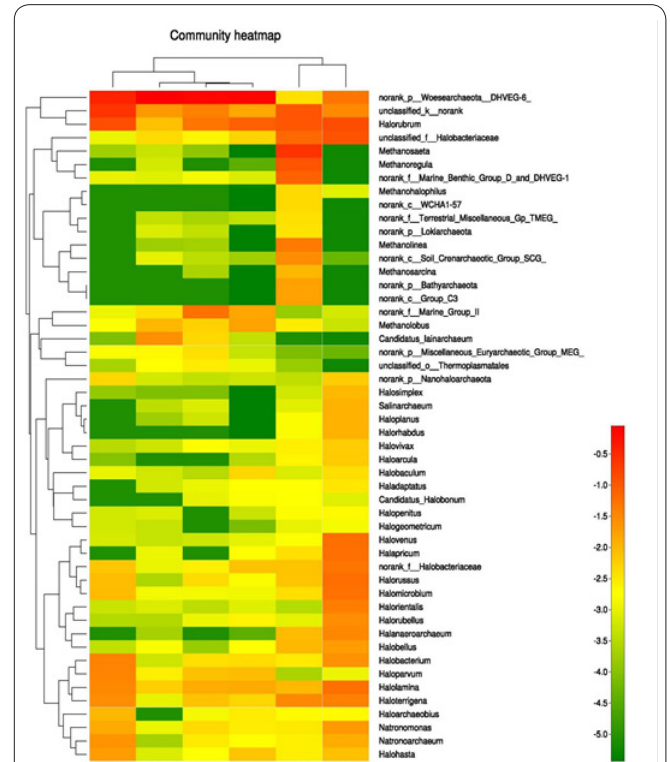
Figure 6 shows the heat map of the archaea based on the genus level. All the samples contained a certain number of methanogenic archaea closely related to the metabolism of methane. Among them, *Methanosaeta* (25.03%), the most abundant methanogenic bacterium in sample T-a, had the highest abundance. The abundance of *Methanoregula* was 11.06%. In addition, the sample also contained other mesophilic archaea with an abundance greater than 1%, including *Methanolinea* (4.51%), *Methanosarcina* (1.01%), *Methanohalophilus* (0.44%), and *Methanolobus* (0.27%). Except for *Methanolobus*, the contents of other methanogenic archaea in the samples with higher salinity were significantly decreased. In other words, the diversity of methanogenic archaea was significantly reduced with the increase of the salinity of samples. The phylogenetic structure of *Woesearchaeota\_DHVEG-6* is closed to *Halorubrum*.

**Effect of physical and chemical properties of lake water on archaeal community**

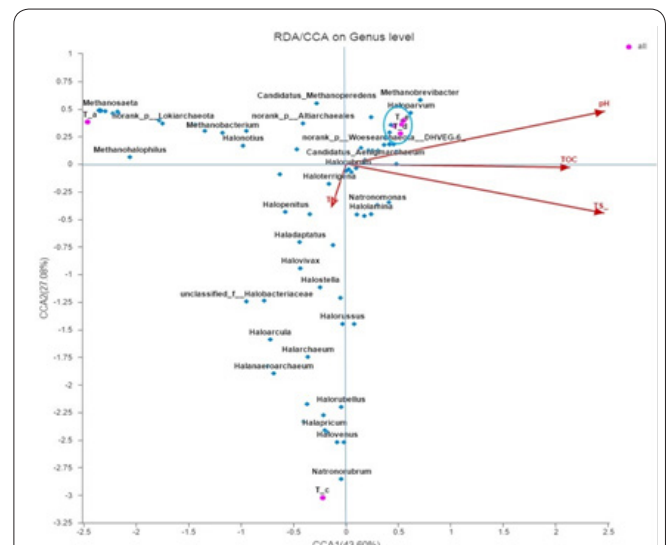
Figure 7 shows the corresponding CCA analysis among the sampling sites, archaea communities and environmental factors based on the class level. The results showed that the distances between the T-b, T-d, T-e and T-f sampling sites were closer than other sites, indicating that the archaeal communities at these sites were



**Figure 5.** Statistical analysis of archaeal relative abundance at genus levels of Tuosu Lake.



**Figure 6.** Community heatmap at the genus level of archaeal composition in different sampling sets of Tuosu Lake.



**Figure 7.** Canonical correspondence analysis of archaeal class information in the Tuosu Lake at class level Note: Round dots (●): Different samples; rhombus (◆): Archaeal classes; Arrows: Environmental factors.

archaeal communities in these samples were quite different from other sites. In environmental factors, total salinity (TS), total organic carbon (TOC), and pH had a relatively large influence on the archaeal community of the sample. The influence of total nitrogen (TN) in the sample is relatively weaker than other factors. *Norank\_p\_Woeseearchaeota\_DHVEG-6* was positively correlated with TS, TOC, and pH. *Methanosaeta* was negatively correlated with TN.

## Discussion

Climatic conditions affect the characteristics of organisms (16). The distribution of microbial species in lakes is related to their geographic location and climatic conditions but also varies depending on the chemical composition and concentration of lake water. The results of previous studies have shown that except for the bacteria species commonly found in lakes such as Proteobacteria, Actinobacteria, and Bacteroidetes, most of the bacterial organisms in freshwater lakes ( $TS \leq 0.5$  g/L) belong to the Cyanobacteria and Planctomycetes. The bacteria in saltwater lakes ( $0.5 \text{ g/L} < TS \leq 30 \text{ g/L}$ ) are mostly Verrucomicrobia and Firmicutes. There are abundant bacteria in Tuosu Lake, about 37.73% of them with undefined genus and species (combined the genus with threshold value less than 1%). The microflora is similar to that in other plateau lakes such as Qinghai Lake (pH=9.1, TS=18.1g/L) (10, 17), Xiaochaidan Lake (pH=7.9, TS=110.83 g/L) (11), Tea Card (pH such as =7.8, TS=330.8 g/L) (12, 17) and Dabson (pH=5.3, TS=470.18 g/L) (18). In Tuosu Lake, the bacterial community was mainly Proteobacteria (44.3%), followed by Actinobacteria (17.2%), Bacteroidetes (12.1%), Verrucomicrobia (9.81%), and Firmicutes (9.30%). The bacterial communities in Qinghai Lake were mainly Proteobacteria (59.46%), Firmicutes (13.97%), Bacteroidetes (12.59%) and Actinobacteria (6.31%). The bacterial community in the two lakes are very similar, which may be related to the similar pH values of the two lakes. Thus, the total salinity TS and pH of the lake are important factors affecting the bacterial community. The salinity of the environment can affect the physiological metabolism of the microorganisms, which in turn affects the classification of the microflora in the environment. Even in the same lake, differences in TS and pH between the microenvironment and the microenvironment at the sampling site will have a significant impact on the community, distribution, and abundance of the bacteria. The cluster analysis of the six samples used in this experiment can also confirm the differences in the composition and diversity of microbial communities caused by the water quality and environment of the sampling sites. The dominant species in Tuosu Lake such as  $\beta$ -Proteobacteria and  $\gamma$ -Proteobacteria are particularly evident. In sample T-a with lower salinity at the river entrance,  $\beta$ -Proteobacteria was the dominant bacteria. However, in other samples with higher salinity,  $\gamma$ -Proteobacteria was even more abundance.

This study found that there is a certain amount of Planctomycetes bacteria in Tuosu Lake, and its abundance in the sample is as high as 3.7% (T-f). This is slightly different from the previous results of Long Qifu et al. (19). As the dominant genus in the lake, the

sequencing results in this study showed that the predominant genus of the lake is *Luptanella* (12.04%), which is a red pigment-producing marine bacterium, followed by *Halioglobus* (4.95%) and *norank\_o\_order\_III* (3.75%), *Lactobacillus Lactococcus* (3.34%), *norank\_f\_Flavobacteriaceae* (2.80%), and undefined *Rhodobacter Rhodobacteraceae* (2.88%) and *Flavobacteriaceae* (2.79%), and *Pseudomonas Pseudoalteromonas* (2.64%). The results of Long Qifu et al. (19) showed the predominant genus *Halomonas* in the Tuosu Lake was extremely high (90.02%). In our study, the *Halomonas* abundance was relatively low (T-a, 0.47%). The possible causes of the bias in the sequencing results of the same lake sample are the sampling time and sampling sites. The sampling time of Long Qifu et al. was mid-June 2015, and the lake water temperature was 5.5-7.5°C. In this experiment, the sampling time was mid-September 2016, and the average lake temperature was 20°C. Li Guoqiang (8) studied the Planctomycetes bacteria in Qinghai Lake and found that the abundance of Planctomycetes was positively correlated with air temperature, water temperature, light intensity and pH. From June to August each year, the bacterial abundance of the phytoplankton Planctomycetes were significantly increased in Qinghai Lake. Therefore, it was suggested that the structure and abundance of the microflora in the Salt Lake in Tuosu Lake is affected by the seasonal temperature.

In addition, there is a certain amount of bacteria that can utilize photosynthesis to produce energy, such as *Bacteroidetes* (*Rhodobacteraceae* and *Flavobacteriaceae*), *Chloroflexi*, and *Cyanobacteria*. The reason for the existence of bacteria that can use photosynthetic energy to produce energy may be that the lake is located at the plateau Gobi. The content of nutrients needed for growth such as carbon, nitrogen and phosphorus in the lake water is low, but the light in the lake is abundant and strong. Irradiation provides the metabolic energy basis for the presence of these bacteria.

As an ancient living organism active in extreme environments, archaea have long attracted the attention of researchers. Hot springs, ocean bottoms and plateau lakes had been found the archaea (20). In general, the types of archaea in saltwater lakes are relatively single, mostly belonging to the Euryarchaeota. For example, the abundance of Euryarchaeota archaea in Huamachi, Shanxi is 99.49%, in Dianchi Lake, Shanxi is 99.99%, in Yu Ncheng, Shanxi is 99.89% (21), and in Xinjiang, Dombashita is 92.0% (22). In the research report on the diversity of archaea in the plateau lakes, the abundance of Euryarchaeota archaea in the Sakha Lake was 96.5% (12) or 100% (21, 23). Its abundance in Qinghai Lake is 98.25% (21). In this study, the abundance of Euryarchaeota archaea in Tuosu Lake was much lower than that in the above lakes. In addition, there was also a relatively high abundance of *Woras archaeota\_DHVEG.6* (42.60%) archaea and a certain amount of unclassified unclassified\_k\_norank (4.82%) taxa and a small amount of *Thaumarchaeota* (1.43%). Similar to Tuosu Lake, Liu Jing et al. (11) found that the Xiaochaidan Salt Lake also contains a large number of archaea species, *Woeseearchaeota\_DHVEG-6*, with an abundance of 80.84%. In this study, *Woeseearchaeota\_DHVEG-6* is the main archaea in Tuosu Lake, but its content in

the samples at the entrance to the lake is only 0.39%, but in the average abundance in other five sites reaches 54.6%. The results of cluster analysis also showed that *Woeseearchaeota\_DHVEG-6* has a close relative structure with *Halorubrum*. *Halorubrum* is a halophilic archaeon that tends to grow in high salt environments. Therefore, it can be speculated that the distribution of *Woeseearchaeota\_DHVEG-6* has a very close relationship with the environmental salinity. Although there are few reports on the classification of the bacteria, there are also reports that the bacteria are mainly found in marine sediments, microbial mats and deep-sea thermal springs, and their distribution is closely related to pH and altitude (24). Up to now, there have been no reports about the culture of *Woeseearchaeota\_DHVEG-6*. The metabolic pathways are not yet clear, so its adaptation to growth requires further research on the specific ecological and biological interaction mechanisms.

Methanogens are archaea that can convert anaerobic fermentation of inorganic or organic compounds into methane and carbon dioxide. Methanogens are important environmental microbes that play an important role in the carbon cycle in nature (25, 26). According to the results of this study, there are abundant types of methanogen-associated microflora in Tuosu Lake, and there are significant differences in the structure and abundance of the bacteria in the sample. Methanomicrobia is the main methanogenic bacteria in Tuosu Lake. A few Methanobacteria and Thermoplasma (*Methanomasiculus cuscus*) were also detected in some samples (27). In sample T-a at the entrance to the lake where the salt content is relatively low, the methanogenic archaea are the highest in both sample size and population abundance. There are a total of 17 methanogenic archaea in sample T-a, and their abundance accounts for about 42.93% of the total T-a archaea. The dominant methanogens in sample T-a were *Methanosaeta* (25.03%), followed by *Methanoregula* (11.06%), *Methaneinea* (4.51%) and *Methanosarcina* (1.01%). *Methanosaeta* is an acetic acid-producing methanogen. *Methanoregula* is a hydrogenotrophic ( $H_2/CO_2$ ) methanogen and cannot use formic acid, acetic acid, methanol, and acetic acid (28-31). The *Methaneinea* is a hydrogen ( $H_2/CO_2$ ) nutrient-producing methanogen, although *Methanosarcina* has a wide range of metabolic types, including the existing methylotrophs, acetic acid, and hydrogenotrophs, which can produce methane using methanol (amine), acetic acid, and  $H_2/CO_2$ , its abundance in sample Ta is much lower than other predominant methanogens. In the remaining five samples, the methanogens had a significant decline in both bacterial type and number, with a maximum abundance of only 1.76% (T-d), a maximum of 6 species (T-b), with the predominant *Methanlobus* (32). *Methanlobus* is a methylotrophic methanogen that can only use methylamine and methanol, and it prefers to live in hypoxic lakes and marine sediments. It was suggested that in areas with low salinity in the Tuosu, methane metabolism is dominated by hydrogen and acetic acid nutrition and that methane metabolism gradually changes to methylotroph with the increase of salinity in the lake. Our group's follow-up experiment provide evidence by using different carbon sources (acetic acid, ethanol, methanol, sawdust, petroleum, etc.) to detect the naerobic methane production. Only methanol and sawdust as

substrate have a significant amount of methane production in all samples, and no methane gas is produced in the remaining samples (Data not shown).

Using high-throughput sequencing methods to study the bacterial structure and diversity of six samples with different salinities in Tuosu Lake, it was found that 1) the bacteria in the lake are mainly Proteobacteria and Actinomycetes. The archaea are mainly Euryarchaeota and Woeseearchaeota\_DHVEG-6. 2) The water salinity (TS) and pH value are the key constraining factor for the structure and abundance of microbia in the Tuosu Lake. The changes in water temperature and microenvironment in the Tuosu Lake can lead to differences in the composition and diversity of the microbia. 3) The change of water salinity in the Tuosu Lake causes changes in the main types of methanogenic archaea and their metabolic patterns. The vast majority of the methanogenic archaea in Tuosu Lake samples belong to *Methanomicrobia*, and the methanogens in low-salinity samples are significantly more abundant than those in high-salt samples.

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### Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

### Conflict of Interest

The authors declared they have no conflict of interest.

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