

RESPONSE OF MICROORGANISMS IN SURFACE SEDIMENTS TO TAIL-WATER DISCHARGE FROM SEWAGE TREATMENT PLANT IN PLATEAU CITY RIVERS DURING DRY SEASON

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Abstract. The response of sediment microbial communities of seven urban and rural rivers to wastewater from sewage treatment plants was analyzed by high-throughput sequencing technology in the upstream and downstream of main cities and towns in Nyingchi City, Tibet Autonomous Region. In the dry season, 14 sampling were collected from the upstream and downstream reaches of 7 towns, and the monitoring results of physical and chemical indices of water quality all reached the Class II standard of Environmental quality standards for surface water (GB 3838—2002, China) except Total Nitrogen; the diversity and richness of microbial community of the 14 sampling sites in Zayü River, Parlung Zangbo, Niyang River and the main stream of Yarlung Zangbo River were all lower than those in other regions, and there were significant differences between the main stream and tributaries of Yarlung Zangbo River; the dominant bacteria in surface sediment were Proteobacteria, Cyanobacteria, Actinobacteria, Bacteroidetes and Frimicutes, with a relative abundance of 77.87%-98.51%, the sum of the relative abundances of the top ten bacterial genera at the genus level was between 18.68 percent and 88.71 percent, its representativeness of collinearity analysis was very strong. The analysis of sediment microbial diversity and influencing factors showed that, dissolved oxygen (DO), the east longitude (E), total phosphorus (TP), ammonia nitrogen content index (NH₃-N) and total nitrogen (TN) had great influence. The functional proteins, dominant enzymes, and FAPROTAX function prediction also proved that the above indices influence different rivers in the plateau habitat as well. The domestic wastewater discharged from the sewage treatment plant had an impact on the microbial community diversity in the surface sediments at the sampling sites.

Keywords: *Nyingchi City, bacterial community, high-throughput sequencing technology, sewage treatment plant, Yarlung Zangbo River*

Introduction

As an important water source and component of the ecosystem, rivers provide water for agriculture, industry and other production and living needs. With the rapid development of social economy and the constant acceleration of urbanization process, the human interference mainly caused by external sources has increased significantly (Kamjunke et al., 2019). In particular, urban rivers, as the most complex part of the aquatic environment, are the most vulnerable to the impact of human activities. With the inflow of pollutants in the tail water of urban sewage treatment plants and surface runoff, the quality of river water is reduced and the ecological environment is destroyed, which further threatens the biological population of the river, ecosystem and human health, and weakens the self-purification capacity and ecological service function of the river (Luo et al., 2019a).

Microorganisms are sensitive to the changes from the external environment and are often used as indicator organisms to monitor and reflect water quality (Deng et al.,

2021). Studies have shown that the most important pollutant affecting urban water quality comes from the discharge of domestic wastewater (Zhao et al., 2016). After the occurrence of river pollution event, the microbial community will show corresponding changes to the changes of the environment. Therefore, in order to solve the pollution problem of urban rivers, the sewage treatment plant and industrial wastewater treatment facilities have been constructed to avoid the direct pollution of domestic sewage to the receiving water bodies, however the centralized discharge of the tail water from the sewage treatment plant will also have various differentiated impacts on the receiving water bodies. Therefore, under the premise of water environment treatment challenges, it is of great significance to systematically research the impact of tail water discharged from sewage treatment plant into urban water bodies, so as to improve the effect of tail water discharge on receiving water bodies (Shen and Jin, 2020).

The water flow carries biological and abiotic substances. The surface sediment of the river is an important part of the urban river ecosystem, which is the “source” and “sink” of various pollutants in the basin (Qin et al., 2021). Compared with the plankton bacterial community, the sediment bacterial community is more complex and the stable, sediment bacterial community is affected by human activities. Typically, the organic matter content in the river and lake sediments in the freshwater system is relatively high (Zhang et al., 2021). In addition, the land use patterns in different river basins lead to differences in the composition of land-based microorganisms entering the water body (Ruiz-González et al., 2015). the water environment is changed by the migration and transformation of biogenic elements and pollutants driven by land-area microorganisms. There are safety risks in water ecological environment and drinking water (Wang et al., 2022). For example, the water body containing a large amount of nutrient salt enters the natural water body (Fan et al., 2021), causing changes in the overlying water and the biogenic elements of the sediment in the receiving water body, so that the freshwater planktonic and sediment bacterial communities are changed accordingly. This in turn affects the health and community structure of other aquatic organisms. microbial immobilization and its combination with other methods to repair sediments. It has become a research hotspot in the field of environmental science and engineering (Peng et al., 2018).

Affected by high altitude, low temperature, low pressure and strong radiation, rivers on the Qinghai-Tibet Plateau are characterized by low metabolism, low nutrition, fragile environment and so on. Low pressure also leads to low partial pressure of oxygen in water, which makes it difficult for river microorganisms to obtain oxygen. The diversity and complexity of biological community are significantly lower than that of plain rivers, which resulting extremely sensitive rivers on the plateau to environmental disturbance. Many rivers originating in the Himalayas and the Qinghai-Tibet Plateau have sediments and nutrients that are easy to deposit, often creating large, high-yield alluvial plains. Sediments are natural sediments in rivers, lakes and oceans, consisting of sand, silt, clay, organic compounds and inorganic minerals. Tibet is known as the China Water Tower, with a total water resource of about 448.2 billion m³. The Yarlung Zangbo River originates from the Jema Yangzong Glacier at the northern foot of the Himalayas. Its formation process occurs simultaneously with the uplift of the Qinghai-Tibet Plateau, with an average elevation of over 3000 m. It is the largest river in Tibet. The river has unique characteristics in terms of shape and sediment (Wang et al., 2015).

In the ecological environment of plateau rivers, sediment is not only the habitat of benthic organisms, but also the place where microorganisms are relatively concentrated in the ecological process of the river. The number of microorganisms is far higher than that

of overlying water, which is about 2~1000 times (Luo et al., 2019b), and the bacterial community in sediments of glacier-fed rivers contributes more than 75% to the bacterial diversity of the aquatic system (Zhang et al., 2021). Under the plateau and alpine environment, the microbial community of the plateau sewage treatment system is significantly different from that of non-plateau areas (Fang et al., 2020, Fang et al., 2019), and the treatment efficiency will also decrease significantly (Tian et al., 2007). After the tail water of the sewage treatment plant is discharged into the receiving water body, the bacteria related to human activities and the changed microbial community will be introduced, thereby changing the water quality and microbial community of the receiving water body (de Santana et al., 2022, Hu et al., 2024), which not only damages the ecological function and service of the microbial community in the river system, but also poses a threat to the public health of the water cycle (Wang et al., 2022). For example, the antibiotic resistance genes (ARGs), a new contaminant from the tail water of sewage treatment plants, will cause an estimated 10 million deaths worldwide by 2050 (Qin et al., 2020), and the increase urban impermeability will also cause the sewage to overflow in the rainy season and directly flow into the river to cause water pollution problems (Xue et al., 2015). In recent 40 years, China's water treatment industry has developed rapidly, but the connection between the discharge standards of sewage treatment plants and local conditions and environmental protection needs exploring its unique path (Qu et al., 2019). For example, in order to control eutrophication in the process of reducing external measures of sewage treatment plants are usually aimed at the removal of single nutrient, resulting in a substantial increase in tail water N/P relative to influent water, which may have a negative impact on the ecosystem after draining the river (Tong et al., 2022).

The Yarlung Zangbo River basin is located in the sensitive area of global climate change, presenting typical plateau climate characteristics of alpine and oxygen deficiency, glacier landform development, and groundwater is mainly supplied in winter. Under its special alpine environment, the diversity of bacteria is more easily affected by evapotranspiration, altitude and annual average temperature (Zhang et al., 2021). Meanwhile, with the development of the city and the development and utilization of water resources, the dam construction, sewage discharge and other human activities in the basin increase, inevitably affecting the microbial community structure and function in the river.

Therefore, it is essential to study the environment and ecology of plateau river by exploring the influence of environmental pressure source, focusing on the influence of tail water discharge from cities and towns on the structure and function of microbial community of receiving water body, further analyzing the influence of tail water and direct discharge point source on microbial community composition and dominant group of river ecosystem in plateau, and monitoring their changes. In general, the mechanism affecting microbial diversity and succession in plateau area is not clear, and urban rivers are still in the blank, especially the influence of tail water discharge from cities and towns on the composition of river microbiota needs to be explored urgently.

Materials and methods

Overview of the survey region

The survey region is located in the lower reaches of the Yarlung Zangbo River, and the administrative region belongs to Nyingchi City. It is located in the southeast of Tibet Autonomous Region, 26°52'~30°40' north latitude, 92°09'~98°47' east longitude, 646.7 km long from east to west, and 353.2 km wide from south to north, with an

average altitude of 3000 m. It has unique environmental factors such as low atmospheric pressure (67.24 kPa) (Zong et al., 2019; Li et al., 2021), low oxygen partial pressure (14.09 kPa) (Zhang et al., 2021), low water temperature (1~14°C) (Qin et al., 2021), and high ultraviolet radiation intensity (924~1036 W/m²) (Peng et al., 2018).

The Sampling sites were selected from the upstream and downstream of 7 towns with high population concentration and relatively complete sewage treatment system in Nyingchi City, with 14 sampling sites respectively located in Gongbujiangda County, Bayi District, Mainling County, Nang County, Lulang County, Bomê County and Zayü County of Nyingchi City. The sampling sites are distributed in the mainstream of the lower reaches of the Yarlung Zangbo River and its tributaries Parlung Zangbo River, Niyang River and Zayü River Basin. The formation process of the Yarlung Zangbo River and the uplift of the Qinghai-Tibet Plateau occur at the same time, and the river has developed a very strong step-pool system, which make the river unique characteristics in terms of shape and sediment (Wang and Zhang, 2012; Wang et al., 2015; Gracas et al., 2011).

The Yarlung Zangbo River originates from the ice and snow mountains on the northern slope of the midstream section of the Himalayas, with a length of 2057 km in China and a drainage area of 935,000 km²; Parlung Zangbo originates from the Azagongla Glacier, which is the tributary of the Yarlung Zangbo River with the largest water volume, with a total trunk length of 266 km, a drop of 3360 m, an average slope of 12.6%, and a drainage area of 28,631 km²; the Niyang River is a tributary of the Yarlung Zangbo River, which is only second to Parlung Zangbo, and is an important water source of the basin. Located in the west of Gongbujiangda County, which originates from the south foot of Nyenchen Tanglha Mountain. It flows into the Yarlung Zangbo River near Geze Village, Bayi District, Nyingchi City. The total length of the main stream is 286 km. Total fall 2080 m. The drainage area is 17,800 km². The Zayü River originates from the Dumla Mountains. The source elevation is 5485 m. The total length is 295 km. The drainage area is 17,827 km².

The sampling sites of Niyang River are U1, D1, U2 and D2 from top to bottom, U3, D3, U4, D4, U5, D5, Yarlung Zangbo River points U6 and D6, and Zayü River points U7 and D7 from top to bottom. The distribution of sampling sites is shown in *Figure 1*.

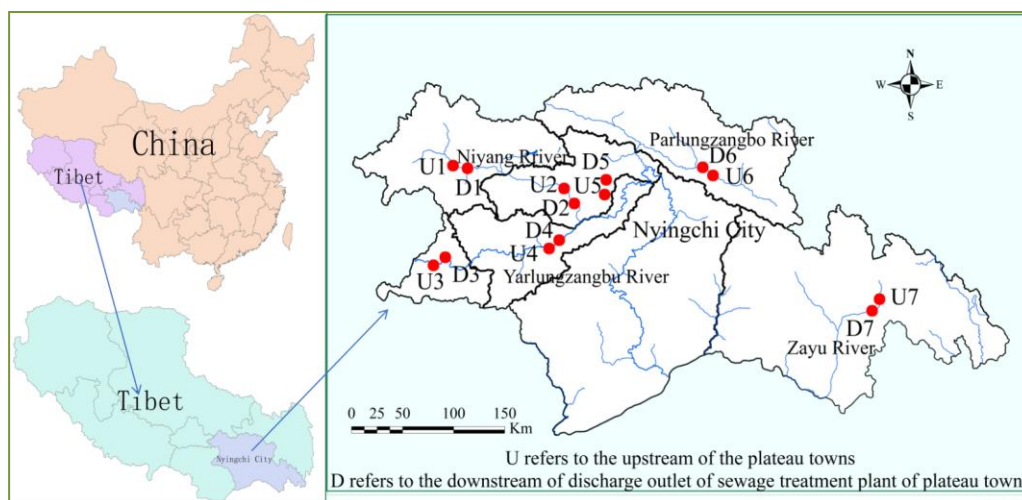


Figure 1. Location of the study area and sampling sites. U refers to the upstream of the plateau towns, and D refers to the downstream of discharge outlet of sewage treatment plant of plateau towns

As shown in *Figure 1*, U1 and D1 are located in Gongbujiangda County. Two sampling sites are taken from the upstream and downstream reaches of the Gongbujiangda County section of the transit river Niyang River. Gongbujiangda is located in the midstream and upstream reaches of the Niyang River, adjacent to Bomê and Bayi District in the east and Mainling County in the south. The county seat is 130 km away from Bayi District of Nyingchi City and 270 km away from Lhasa City. U2 and D2 are respectively located at the upstream and downstream of the Bayi section of the transit river Niyang River, at the eastern and southern foot of Nyenchen Tanglha on Qinghai-Tibet Plateau, where Yarlung Zangbo and Niyang River meet again, with an average altitude of 3000 m, 400 km away from Lhasa and 1700 km away from the southwest central city of Chengdu, with an area of 10238 km²; U3, D3 is located in Lang County with an average altitude of over 3700 m; U4 and D4 are located in Mainling County, in the southwest of Nyingchi City and the middle and lower reaches of the Yarlung Zangbo River, where the Yarlung Zangbo River traverses the whole territory from west to east, with a length of 1077 km, located between Nyenchen Tanglha Mountains and Himalayas, 72 km away from Bayi Town, Nyingchi City. The west is connected with Langxian County. It borders India in the south; U5, D5 is located in Lurang; U6, D6 is located in Bomê County. Located at the junction of the Nyenchen Tanglha Mountains and the Himalayas. It is connected to Gongbujiangda County of Nyingchi City in the west. Nanlian Zayü, Minto, Bayi District. The total area is 16,700 km². The east of the county seat is 1500 km away from Chengdu. 636 km to the west of Lhasa, 234 km from Bayi District. U7, D7 is located in Zayü County, belonging to the high mountain valley area where the Himalayas and Hengduan Mountains meet. It is 537 km away from Bayi Town and 934 km away from Lhasa City, with a total area of 31659 km².

Sample collection and determination of physical and chemical indexes

The sampling time was the last ten days of November 2023. At the sample collection site, Height (HEI) and longitude and latitude (LL) were measured by Magellan handheld GPD locator (Mobile Mapper10), flow rate (V) was measured by Doppler portable current meter (FLOW-ADC-10T), PH, temperature (TEMP), oxidation-reduction potential (ORP) and dissolved oxygen (DO) were measured by portable water quality analyzer (HACH HQ40d, USA). At the same time, water samples were collected, and TN, TP and NH₃-N were measured by the national standard method. The surface sediment is collected by a grab-type mud sampler, put into a collection bag, and is transported to the laboratory for low-temperature storage and stored in a -80 DEG C refrigerator to be tested. Physical and chemical indexes of overlying water quality of 14 samples are shown in *Table 1*.

Table 1. Water quality index of overlying water

Samples	N	E	V	TEMP	pH	DO	ORP	HEI	UV	TP	TN	NH3-N
	/	/	m/s	°C	/	mg/L	mv	m	mW/cm ²	mg/L	mg/L	mg/L
U1	29°53'6"	93°13'41"	1.16	5.5	8.72	10.97	162.8	3424.5	4.75	0.03	0.545	0.496
D1	29°53'6"	93°17'53"	1.22	5.6	8.83	11.62	142.8	3403.85	4.75	0.023	0.508	0.459
U2	29°42'44"	94°18'29"	0.59	8.3	8.85	10.83	137	2985.49	4.6	0.004	0.978	0.329
D2	29°35'7"	94°24'37"	1.02	7.9	8.81	10.18	106.3	2954.09	4.6	0.004	0.967	0.012
U3	29°2'47"	93°2'42"	4.78	8.9	8.67	10.95	158.6	3086.00	5.2	0.027	1.09	0.498

D3	29°2'53"	93°3'55"	3.57	8.8	8.82	11.06	144.9	3050.10	5.2	0.007	0.967	0.482
U4	29°12'7"	94°10'50"	1.00	7.1	8.63	11.29	148.5	2858.57	4.5	0.036	1.071	0.481
D4	29°13'57"	94°13'26"	0.57	7.4	8.07	10.82	65.5	2850.21	4.5	0.006	1.48	0.483
U5	29°42'1"	94°43'58"	1.67	5.9	9.20	11.81	88.2	3398.74	4.68	0.016	0.204	0.184
D5	29°45'8"	94°43'49"	1.2	6.1	9.94	11.45	74.5	3346.1	4.68	0.022	0.458	0.342
U6	29°50'8"	95°47'19"	1.97	8.3	8.73	12.98	98.4	2689.02	4.48	0.028	0.615	0.426
D6	29°52'47"	95°43'48"	1.37	8.5	8.67	13.68	94.3	2643.00	4.48	0.023	0.309	0.209
U7	28°41'17"	97°28'15"	1.28	5.5	8.34	13.58	103.8	2239.00	4.47	0.019	0.255	0.207
D7	28°38'42"	97°26'12"	0.68	5.6	8.29	13.45	144.9	2212.01	4.47	0.044	0.345	0.294

According to the water quality physical and chemical index analysis and surface water environmental quality standard analysis of 14 sampling sites, the concentration of TP is between 0.004 and 0.044, U2, D2, D3, D4, U5 and U7 belong to Class I standard ($TP \leq 0.02$), and other sampling sites belong to Class II standard ($TP \leq 0.1$). TN concentration is between 0.012 and 0.543; TN concentration of sampling sites of D5, U6, D6 and D7 reaches Class II standard of surface water ($0.2 < TN \leq 0.5$), TN concentration of sampling sites of U1, D1, U2, D2 and D3 reaches Class III standard of surface water ($0.5 < TN \leq 1.0$), TN concentration of sampling sites U3, U4 and D4 reaches Class IV standard of surface water ($1.0 < TN \leq 1.5$). The NH_3-N concentration of D2 reaches the surface water class I standard, The concentration of NH_3-N at other sampling sites all reached Class II standard.

The pH different sections are weakly alkaline, except for U5, D5 other sections have little difference. According to the analysis of physical and chemical indexes of water quality of 7 sampling sites and the analysis of surface water environmental quality standards, the average pH value of water quality is 8.76, which is close to the upper limit value of Class V standard for surface water 6~9, and the lowest value is 8.07, which indicates that the water quality of rivers in Nyingchi City is alkaline and needs to be monitored and controlled. The highest value is as high as 9.94, which may be related to the reaction of calcium salts in the riverbed with the carbonate ions in water to form calcium carbonate, which is the area where the degree of rock decay is high (Gindele et al., 2024, Wang et al., 2019).

Data processing method

16S rRNA gene assay was used to analyze surface sediment samples from 7 sampling sites. Statistical analysis was performed using SPSS24 software and Microsoft Excel spreadsheet. Using Analysis of variance (ANOVA) was used to analyze the difference between richness indicators of upstream and downstream sampling sites. The single-sample T-test was applied to show the dominant COG, enzymes, and functional groups of the sampling sites. In order to understand the functional characteristics of microorganisms and genes, the 14 samples obtained in this test study (at-80°C) were entrusted to Shanghai Majorbio, China (<http://www.majorbio.com>). The Sequencing experiments first required the extraction of sample DNA using the Fast DNA Spin Kit (O'Neill, 2014), and then use 1% agarose gel electrophoresis for detection. PCR amplification was then performed in the variable region (V3-V4) of the 16S rRNA gene using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') on a PCR instrument (ABI GeneAmp® model

9700). Three replicate PCR amplification products in the same sample were mixed and detected by 2% agarose gel electrophoresis. The PCR products were detected and quantified with QuantiFluor™-ST Blue Fluorescence Quantification System (Promega) with reference to the preliminary quantitative results of electrophoresis, and then mixed according to the sequencing requirements of each sample. The official linker sequence of Illumina was added to the outer end of the target region by PCR. AxyPrep DNA gel recovery kit (AXYGEN Company) was used to cut the gel to recover the PCR product, and then the PCR product was eluted by Tris-HCl buffer solution and detected by 2% agarose electrophoresis. After being denatured by NaOH, a single-stranded DNA fragment was obtained. One end of the single-stranded DNA fragment and the base of complementary primer were fixed on the chip to synthesize the target DNA fragment to be detected. After denaturation and annealing, a “bridge” was formed and added with DNA polymerase and dNTP. The surface of the reaction plate was scanned by laser to read the nucleotide type polymerized on the template sequence. Eventually, the “fluorescent group” and the “terminating group” are chemically cut, restoring the 3' end viscosity, the polymerization of the second nucleotide is continued, cycle back and forth, counting the template DNA fragment sequences obtained in each round, The sequencing of Illumina MiSeq is complete.

Alpha diversity metrics were used to estimate the abundance and diversity of community species, such as chao, ace, shannon, simpson, shannoneven (Cassol et al., 2025). Use mothur microbial diversity index (version v. 1.30.2 <https://mothur.org/wiki/calculators/>) calculation, community bar chart using R language tools statistics and drawing, community Heatmap R language (version 3.3.1) is used for vegan mapping, while Spearman correlation Heatmap uses R language pheatmap package(version 3.3.1) mapping based on KEGG database (Lu et al., 2016., Ni et al., 2019, Office, 2024), using function prediction software (PICRUSt2) to predict bacteria function and using the league sichuan platform (<https://www.omicstudio.cn/tool/81>) map metabolism. Netwoa (version1.11) software was used for collinearity analysis to show the distribution between samples and species. Redundancy analysis (RDA) is a PCA analysis constrained by environmental factors. RDA analysis and mapping in R language vegan package (vsesion 2.4.3) are used to obtain the correlation between sediment microbial communities and environmental factors (Huang et al., 2024).

Results and discussion

Alpha diversity analysis

The index of Chao, Shannon, coverage, Shannoneven and Simpstoneven were calculated by using Alpha diversity to analyze the abundance and diversity of community species in this environment. The diversity of surface sediment samples from the 14 sampling sites is shown in *Figure 2*.

As shown in *Figure 2*, the coverage index of the 14 sampling sites is greater than or equal to 0.975, indicating that the sample sequencing coverage is good and the test result can fully reflect the true situation of the microorganism in the sample. Shannon index can be used for community diversity, which is within the range of 1.781~6.155. The Shannon index from the upper reaches of Niyang River to the lower reaches of Niyang River shows a gradually decreasing trend, which is similar to the sampling site of Parlung Zangbo River, i. e. U6 is higher than D6, which indicates that the diversity of surface sediment microbial community at the sampling sites of Niyang River and

Parlung Zangbo River gradually decreases after the wastewater is discharged from the sewage treatment plant; the main stream of Yajiang River main stream shows a gradual increase trend from U3 to D4, and decreases to 1.781 at U5 point and then rises to 5.546 at D5, and on the main stream of Yajiang River main stream, the Shannon index at the downstream after the sewage treatment plant is increased compared with that at the upstream. the variation trend is similar to that of the observation sampling sites, i.e. d7 is higher than U7.

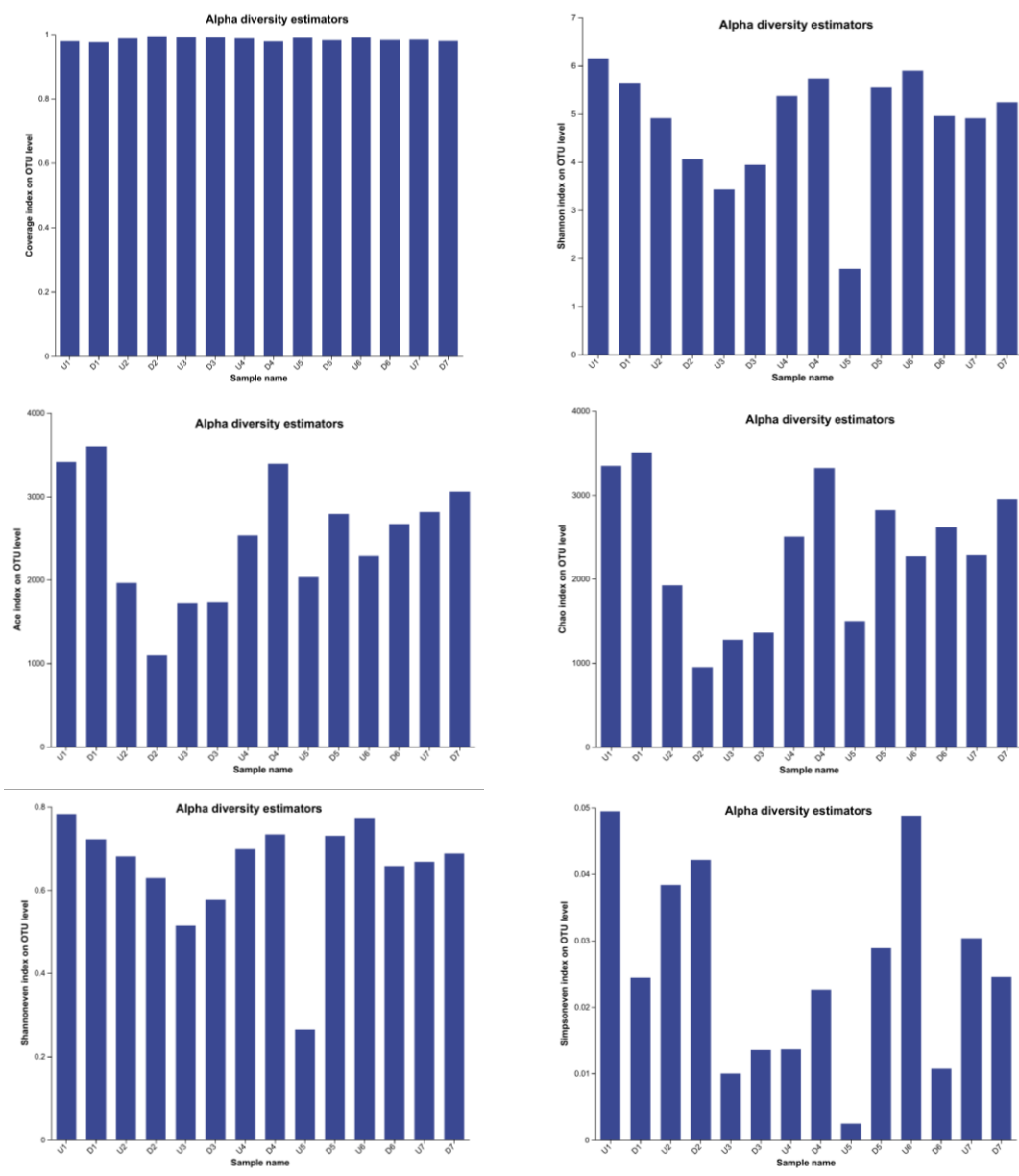


Figure 2. Microbial diversity Index

The index of chao and ace can reflect the richness of the community. Among them, the ace ranges from 1094.286 to 3000.745 and the chao index ranges from 951.250 to 3507.311. Compared with the ace and chao indices obtained in the existing literatures (François et al., 2001; Wu et al., 2015) they are smaller. Except that U2 in the upper

reaches of Bayi district is higher than D2, the index of ace and chao in the lower reaches of other towns in plateau is higher than that in the upper reaches. The smaller the values are, the lower the richness of microbial community is, which may be related to the low temperature and low nutrient state of plateau rivers, and the richness of D1 sampling site is the largest. Simpson even indices ranged from 0.265 to 0.782/0.002 to 0.049, respectively. Using ANOVA and post-hoc multiple comparison tests, we assessed the difference between index groups. We also checked the data for meeting the assumptions of normality and homogeneity of variance before conducting. There was no significant difference in index value between upstream and downstream sampling sites ($p \geq 0.254$), and there was significant difference in indexes between different sampling sites ($P < 0.05$); there was significant difference between Niyang River and main stream of Yajiang River main stream ($p < 0.05$); there was significant difference between main stream of Yajiang River main stream and three tributary groups ($p < 0.05$); there was significant difference between upstream of main stream of Yajiang River main stream and upstream of three tributaries ($p < 0.05$), indicating that the evenness of tributaries is higher than that of main stream. and the evenness of the upstream of the branch stream is larger than that of the upstream of the main stream.

Microbial structure and composition

In this study, 6193 OTUSs of surface sediments from 14 sampling sites belong to 49 phylums, 151 classes, 365 orders, 597 families and 1187 genera. It can be seen that the surface sediments of the lower reaches of the Yarlung Zangbo River have abundant microbial diversity, and compared with the existing documents (Fan et al., 2022; Fierer et al., 2007), the microbial species in the special habitats of the plateau are low.

Analysis of species composition at phylum level

The samples were drawn out, and the species with abundance less than 0.01 were combined into others. As shown in *Figure 2*, the microbial community structure of surface sediment samples at different sampling sites of urban rivers in Nyingchi City was at the gate level. In 14 samples, there were 11 species of microbial bacteria with relative abundance of more than 1%, which were regarded as dominant bacteria, and other bacteria were classified as others, as shown in *Figure 3*.

The five bacterial phyla with the highest average relative abundance in *Figure 3* are Proteobacteria (10.07%~70.10%), Cyanobacteria (2.48%~85.63%), Actinobacteria (1.88%~24.02%), Bacteroidetes (0.62%~29.15%), Firmicutes (0.31%~13.00%), and the first five relative abundance of each sampling site are 83.11%, 81.83%, 87.35%, 83.63%, 82.00%, 83.27%, 85.87%, 77.87%, 98.51%, 85.89%, 85.89%, 84.40%, 92.19% and 93.72% and 81.83%. Their community composition and findings from relevant literature are similar (Li et al., 2018).

Further analysis showed that there were great differences in the relative abundance of bacteria in different sampling sites. Proteobacteria at U1, D1, U2, D2, U4, D4, D5, U6, D6, U7, D7 was the most abundant dominant phylum, D2 was bacteroidota, and U3, D3, U5 was cyanobacteria. Among them, the average abundance of Proteobacteria is the highest at 39.35%, which is relatively close to that of Lancang River, Yangtze River and Chidao River (Wu et al., 2015, Wu et al., 2022, Liu et al., 2022). The dominant bacteria group in urban domestic sewage (Luo et al., 2020) is 62.26%, 50.43%, 39.08% and 28.21% respectively from the high to the bottom of Zayü River, Parlung Zangbo River,

Niyang River and the main stream of Yarlung Zangbo River. Only the abundance of Niyang River U1, U2, Parlung Zangbo River U6 and upper U4 of main stream flowing through Mainling County are respectively higher than those of D1, D2, D6 and D4 of downstream sampling sites, and the abundance of other downstream sampling sites is higher than that of upstream.

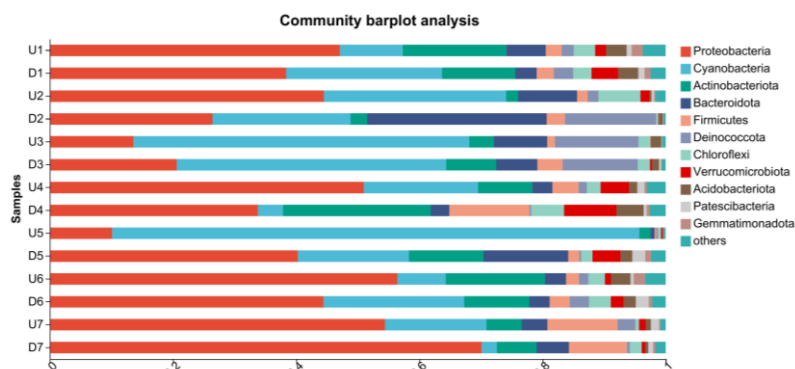


Figure 3. Relative abundance of microbial species in sediment samples at phylum level

The average abundance of Cyanobacteria was the second bacteria, and the average abundance was 37.43%, 21.90%, 15.35% and 9.49%, respectively in the main stream of Yarlung Zangbo River, Niyang River, Parlung Zangbo and Zayü River. U2, U3, U4, U5, U7 were higher than those of D2, D3, D4, D5, D7 in the lower reaches of the town, and the abundance of the other sampling sites was lower than that of the upper sampling sites. Among them, U3, D3 and U5 sampling sites on the main stream of the Yarlung Zangbo River are Cyanobacteria with the highest abundance. There are villages along the river above the U3 point. Although D3 point is located at the downstream of the sewage treatment plant, the sewage treatment plant is not located at the most downstream of the town due to the construction of urban new area, and untreated sewage may be discharged into the river course. U5 is located in the pasture. Therefore, the pollution of the three sampling sites stimulates the growth of cyanobacteria. The abundance of Cyanobacteria at the sampling sites is 54.51%, 43.79% and 85.63% respectively. The high abundance of Cyanobacteria in U2 may be related to the fact that the construction of the Duobu dam in the upstream reached has slowed down and downstream hydrological regime and formed a trend of homogenization in the river course. Moreover, in the dry season, the flow rate tends to become smaller and tends to be static, thus resulting in nutrient accumulation. The specific mechanism of Cyanobacteria remains to be revealed later.

Actinobacteria is a gram-positive bacterium that absorbs nutrients and is ubiquitous in freshwater habitats (Dwulit-Smith et al., 2018, Hungund et al., 2022). The average abundance of Actinobacteria was the third. The average abundance was 13.35%, 9.82%, 8.34% and 6.10% in Parlung Zangbo, Yarlung Zangbo River, Niyang River and Zayü River, respectively. Only U1 and U6 were higher than those at D1 and D6 in the downstream of the town, and the abundance in the other sampling sites was higher than that in the upstream sampling sites. Bacteroidota (actually Bacteroidetes) was the fourth bacteria in average abundance. The average abundance was 12.14%, 6.00%, 4.70% and 3.36% in the main stream of Niyang River, Yajiang River main stream, Zayü River and Parlung Zangbo River, respectively. In addition, U2, U5, U7 are lower than downstream

D2, D5, D7, and the upstream abundance of other sampling sites is greater than that of downstream sampling sites. The abundance of D2 sampling site is 29.15%, which is the dominant strain with the highest abundance. It also plays an important role in the decomposition and fermentation of organic matters in sediment, possibly caused by the domestic sewage from nearby towns received by the river.

The average abundance of Firmicutes is the fifth. The average abundance of Firmicutes is 10.42%, 4.13%, 2.67% and 2.52% in Zayü River, Yajiang River main stream, Parlung Zangbo River and Niyang River respectively. Except that U7 is higher than D7, the abundance in the downstream of other towns is higher than that in the upstream, indicating that the domestic sewage in other points has been effectively degraded or diluted, so the abundance of Firmicutes is high, which is also negatively correlated with Firmicutes and fecal pollution as described in the literature (Peng et al., 2018; Kämpfer et al., 1993), and generally shows a good correlation with ammonia and nitrate. The abundance of Firmicutes at Zayü River sampling site is obviously higher than that at other sampling sites. U7 may come from the microbial transmission in the upper reaches, while D7 may come from the inflow of other microorganisms from the flood gully during the period, thus reducing the Firmicutes of D7.

The case of relative abundance difference between sites was found by T-test, at the phylum level, Proteobacteria dominates the bacterial community in the plateau river sediments in Nyingchi, Yarlung Zangbo River, and the relative abundance of the dominant bacteria of Proteobacteria, Cyanobacteria, Actinobacteria, Bacteroidetes and Firmicutes, Proteobacteria, Cyanobacteria, Actinobacteria and Firmicutes ($p \leq 0.05$) are significantly different among sampling sites, that is, the distribution difference of the above dominant bacteria at different sampling sites under plateau environment is significant; meanwhile, the discharge of sewage treatment tail water also causes significant changes in the composition and abundance of dominant bacteria at upstream and downstream points.

Species composition analysis at genus level

The average relative abundance of all microbial genera in the 14 samples was obtained, and the relative abundance in each sample is shown in *Figure 4*.

As shown in *Figure 4*, the relative abundance of dominant microorganisms varies greatly at each sampling site at the genus level is quite different. The top 10 bacteria genera in the ranking of average relative abundance are: *Tychonema_CCAP_1459-11B* (0.03~40.31%), *norank_f_Leptolyngbyaceae* (0.07~82.00%), *Pseudorhodobacter* (0.23~16.70%), *Sphingorhabdus* (0.53~7.75%), *Hymenobacter* (0.02~25.07%), *Deinococcus* (0.01~14.51%), *norank_f_Rhizobiales_Incertae_Sedis* (0.07~8.73%), *Hyphomicrobium* (0.03~6.91%), *Rhodobacter* (0.15~9.68%), *Polaromonas* (0.02~5.59%). The sum of the relative abundances of the above bacteria in the 14 sampling sites was 20.72%, 31.50%, 22.62%, 57.71%, 63.26%, 54.06%, 38.89%, 18.68%, 88.71%, 28.84%, 29.61%, 42.12%, 30.43%, 39.46%.

Further analysis showed that there were significant differences in the relative abundance of bacteria genera in water bodies at different sampling sites. Among them, *Tychonema_CCAP_1459-11B* has the highest average relative abundance (10.37%), which is found in relatively cold lakes and slightly eutrophic water in Northern Europe (Wei et al., 2012). Its relative abundance in U3 sampling site is 40.31%. It may be affected by grazing and untreated domestic water in nearby villages. The relative abundance in D3 and D6 is next, 28.78% and 21.20% respectively. Although D3 point is

located at the downstream of the sewage treatment plant, the construction of urban new area makes the sewage treatment plant not in the most downstream of cities and towns, and untreated sewage may be discharged into the river course. The abundance of sampling sites U4, D5, D1 and D2 is 12.74%, 12.2%, 11.32% respectively. 9.50%, the relative abundance of other sampling sites is less than 2 percent. They play a weaker role. The average relative abundance of norank_f_Leptolyngbyaceae and Pseudorhodobacter ranked the second (7.11%), and the maximum relative abundance was 82% at U5, 3.66% at U2, 3.20% at U7, 2.96% at D1, and less than 2% at other sampling sites.

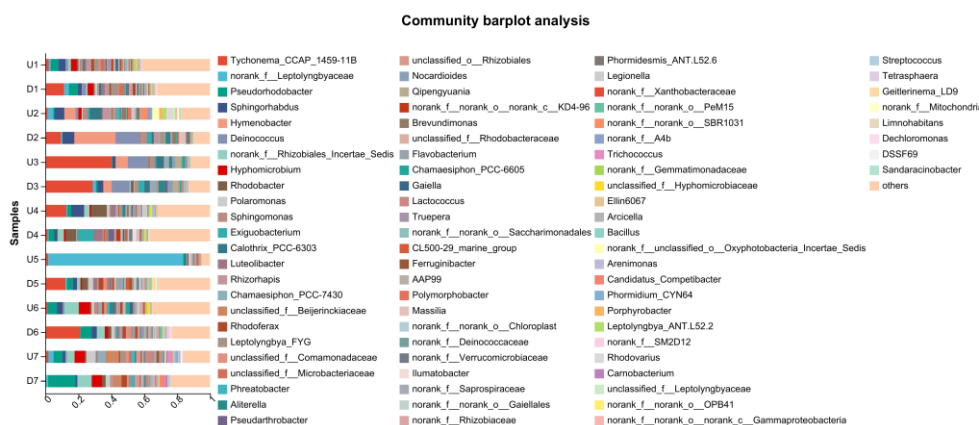


Figure 4. Relative abundance of microbial species in sediment samples at genera level

The average relative abundance of Pseudorhodobacter ranked the third (4.13%), the maximum relative abundance was 16.70% in the D7 sampling site, D6, U6, U7, D1, U1, D4, D5 and U4 was 6.83%, 5.75%, 5.40%, 5.39%, 4.73%, 3.54%, 3.50% and 2.47%, and the relative abundance of other sampling sites was less than 2%.

The average relative abundance of Sphingorhabdus ranked the fourth (3.41%), in U4 and D2 was 7.75% and 7.12%, and 5.87%, 4.43%, 3.57%, 3.54%, 3.17%, 2.64%, 2.43% in U2, U1, D3, U6, D6, D5 and D1 respectively. The relative abundance of other sampling sites was less than 2%.

The above results show that the microbial population composition in the surface sediments of urban rivers on the plateau varies greatly under the action of plateau environment, except for norank_f_Leptolyngbyaceae, Hymenobacter, Deinococcus and Rhodobacter, there are significant differences ($p \leq 0.05$), which had a good response to the unique climate environment of the plateau, especially the alpine and low-nutrient conditions have great influence on the microbial population structure and characteristics, which is also consistent with the results of microbial diversity analysis. At the same time, the discharge of tail water from sewage treatment also caused significant changes in the composition and abundance of dominant bacteria genera at upstream and downstream sampling sites, especially, the upstream and downstream common genera of the 7 discharge points of the top ten dominant genera were 6, 4, 7, 5, 5, 5, and 5 respectively, and there are no common dominant genera in all sampling sites.

Co-occurrence network analysis

In order to elucidate the co-existence relationship of microorganisms at the phylum level in 14 samples of urban rivers in plateau habitats, the collinearity network diagram

of microbial community at the phylum level is drawn by analyzing Networks software. As shown in *Figure 5*, the phylum with the highest weighting degree at each sampling site is located at the center of the network diagram, and their degree is basically 14, and the collinearity and robustness are good; there are 25-47 species of phylum at the 14 sampling sites. In order to facilitate the analysis and screening of the dominant phylum at the top 5 of the weighting degree, accounting for 89.97% of the weighting degree of all bacteria genera, it is highly representative. The dominant bacteria in 14 sampling sites were p_Proteobacteria, p_Cyanobacteria, p_Acidobacteriota, p_Bacteroidota, p_Deinococcota, p_Firmicutes, p_Verrucomicrobiota, p_Chloroflexi. The highest weight of the genera in each sampling site was p_Cyanobacteria at D3, D4 and U5, p_Bacteroidota at D2, and p_Proteobacteria at the other sampling sites. The analysis results were consistent with the relative abundance at phylum level.

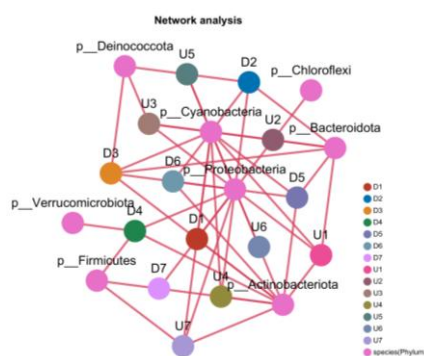


Figure 5. Co-occurrence network of microbial species in sediment samples at phylum level

Relationship between sediment microbial diversity and influencing factors

Correlation heat map analysis

The correlation of overlying water quality and physical and chemical indexes as influencing factors with dominant bacteria at phylum and genus level is shown in *Figure 6*.

The correlation heat map (*Fig. 6a*) showed that at the phylum level (the first 25 dominant bacteria), there is a negative correlation with flow velocity: Dependientiae, SAR324_cladeMarine_group_B; Cyanobacteria and Firmicutes are negatively correlated with pH; Patescibacteria showed a positive association with DO; Gemmatimonadota, Bdellovibrionota, SAR324_cladeMarine_group_B, WPS-2 showed positive correlation with N; Proteobacteria showing a positive correlation with E and unclassified_k_norank_d_Bacteria showing a negative correlation with E; Proteobacteria and Firmicutes are negatively correlated with ALT; positively correlated with UV presentation is cyanobacteria, The negative correlation is shown by Firmicutes, Proteobacteria; associated with the TP being positively related is proteobacteria, Bdellovibrionota, Dependientiae, Nitrospirota, Methylomirabilota, NB1-j; The positive correlation with TN is unclassified_k_norank_d_Bacteria; The positive correlation with NH₃-N was found with Actinobacteriota, acidobacteriota, unclassified_k_norank_d_Bacteria, Nitrospirota.

The correlation heat map (*Fig. 6b*) shows that at the genus level, the positive correlation with V is Chamaesiphon_PCC-7430, the positive correlation with TEMP is

Sphingomonas, Exiguobacterium, and negative correlation with Polaromonas, unclassified_f_Microbacteriaceae, unclassified_o_Rhizobiales; norank_f_Rhizobiales_Incertae_Sedis with negative correlation with pH; and Pseudorhodobacter, norank_f_Rhizobiales_Incertae_Sedis, Hyphomicrobium, Polaromonas, Rhodoferrax, unclassified_f_Beijerinckiaceae, unclassified_f_Comamonadaceae, Hymenobacter exhibiting a negative correlation, Deinococcus, Sphingomonas, Exiguobacterium, Calothrix_PCC-6303; Sphingomonas was positively correlated with ORP. positively related to the e presentation is pseudorhodobacter, norank_f_Rhizobiales_Incertae_Sedis, hyphomicrobium, Polaromonas, unclassified_f_Beijerinckiaceae, unclassified_f_Comamonadaceae, the negative correlation is presented by unclassified_f_Comamonadaceae, Deinococcus, Sphingomonas, Calothrix_PCC-6303.

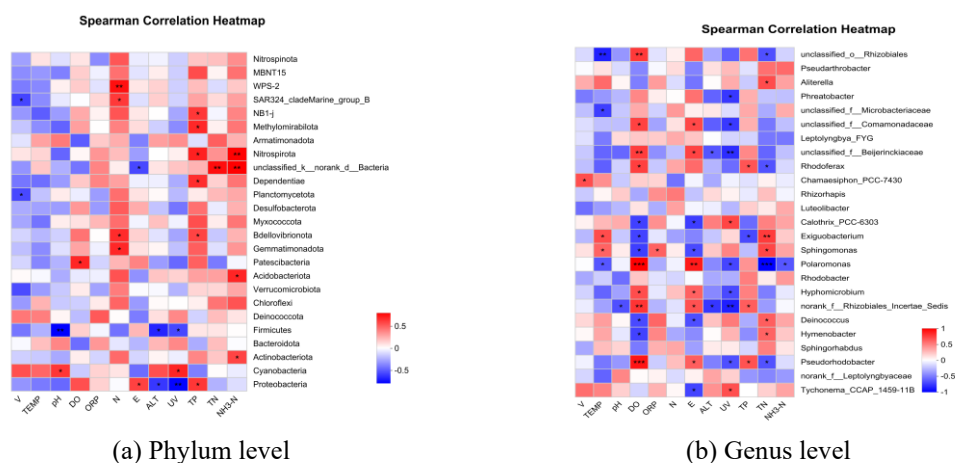


Figure 6. Heatmap of correlation between environment and species Spearman. The X-axis and Y-axis are factors and species, respectively, and the correlation R-value and p-value are obtained by calculation. The R values are shown in different colors in the graph, and if the p value is less than 0.05, it is marked by *, and the legend on the right is the color interval of different R values; the species and influencing factor cluster tree (such as left side and upper side) can be selected to be presented; * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$

The negative correlation with ALT was norank_f_Rhizobiales_Incertae_Sedis, unclassified_f_Beijerinckiaceae; Tychonema_CCAP_1459-11B, Calothrix_PCC-6303 showed positive correlation with UV while Pseudorhodobacter, norank_f_Rhizobiales_Incertae_Sedis, Hyphomicrobium, Polaromonas showed negative correlation; Pseudorhodobacter, norank_f_Rhizobiales_Incertae_Sedis and Rhodoferrax showed a positive correlation with TP, while Exiguobacterium showed a negative correlation; positive correlation with TN is the presence of Hymenobacter, Deinococcus, Sphingomonas, Exiguobacterium, Aliterella, while the negative correlation with TN is the presence of Pseudorhodobacter, Polaromonas, Rhodoferrax; Polaromonas showed negative correlation with NH₃-N.

These results indicated that UV, V, TEMP, ALT, DO, TP, E and other factors had certain effects on the microbial community in the plateau habitat. The analysis showed that DO, E, TP, NH₃-N and TN had great influence on the microbial community, which was closely related to the trophic deficiency in the high-altitude and low-pressure habitats.

Redundancy analysis of microbial community and influencing factors

The results of redundancy analysis (RDA) between microbial communities and influencing factors in surface sediments of plateau urban rivers are shown in *Figure 7*. The analysis results at phylum level show that TN, UV, DO, E, TP, ALT, TEMP, TP, pH and other influencing factors can explain 90.08% of the changes of sediment bacterial communities, which is higher than that of Fuhe River (Wang et al., 2023) and Jialing River (Wang et al., 2018). The samples of U1, D4, D5, D2, U3, D3, U2, U5, U6, D6, U4, U7, D7 in sediments are similar. UV, V, TEMP, ALT, DO, TP, E and other factors in sediments have great influence on the microbial community in sediments of Bahe cascade hydropower station. The three dominant bacteria in *Figure 7a*, Cyanobacteria positively correlated with ORP, pH, V, ALT, UV, N, TEMP, and negatively correlated with TP, DO, E, TN, NH₃-N.

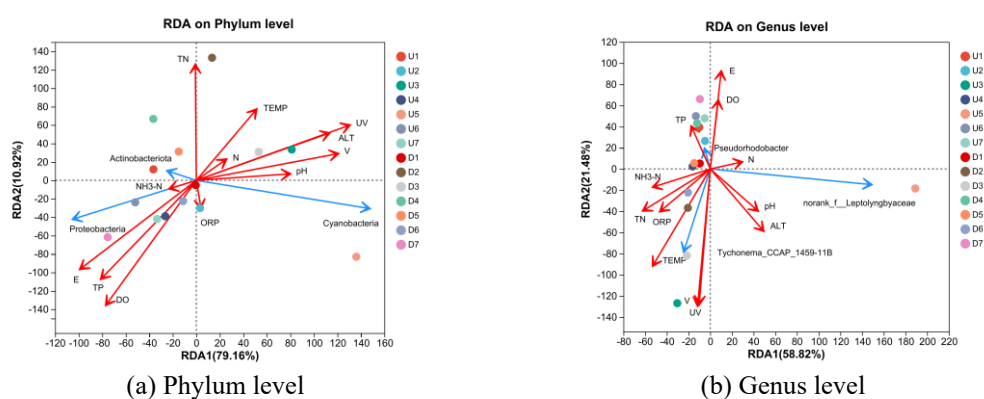


Figure 7. Redundancy analysis of microbial communities and influencing factors in surficial sediment samples

Proteobacteria is positively correlated with NH₃-N, E, TP, DO and ORP, and negatively correlated with pH, V, ALT, UV, TEMP and TN. As the major denitrifying bacteria phylum, Proteobacteria is positively correlated with TP. However, in view of the carbon and nutrient competition between TP and TN, Proteobacteria has a negative correlation with TN. The increase of V reduces the probability of obtaining nutrients by Proteobacteria under the poor nutrition state, which is contrary to the conclusion that the increase of flow rate strengthens the probability of obtaining nutrients by Proteobacteria, which is mainly related to the fact that this flow rate is more than three times that described in the literature; meanwhile, the denitrification of Proteobacteria in the hypotrophic state is less affected by DO. However, an increase of V leads to the increase of DO, the result show that DO is proportional to Proteobacteria, the reduction of ALT is beneficial to the rise of water temperature and the occurrence of denitrification reaction, indicating that the high abundance of Proteobacteria is associated with high nutrient availability (Fierer et al., 2007); Actinobacteria and NH₃-N, E, TP, DO, TN shows a positive correlation, with TEMP, ALT, UV, V, pH, the ORP shows a negative correlation, as a bacterial phylum for degrading organic matter, This is due to the fact that organic degradation requires absorption of heat and consumption of TP, TN. The degradation of organic compounds by Actinobacteria was easily disturbed by the flow velocity and was negatively correlated with V. On the whole, ALT and N were positively correlated with Cyanobacteria and negatively correlated with

Proteobacteria and Actinobacteria, i.e. ALT affected the composition of microbial community.

Figure 7b of the analysis results at the level of genus classification shows that the influencing factors can explain 80.3% of the bacterial community change of the surface sediments. In the sediments, U1, U2, U3, U4, U5, D1, D5, D6 and D7 samples are similar, U6, D2, D3 and D4 samples are similar, and U5 samples are not similar; UV, V, TEMP, E, DO, ALT, TN and other environmental factors in the sediments have great impact on the microbial community of urban river sediments in the plateau. According to the analysis of three dominant genera and species in the figure, norank_f_Leptolyngbyaceae has positive correlation with N, pH, ALT, DO and E, and negative correlation with TP, NH₃-N, TN, ORP, TEMP, V and UV; Tychonema_CCAP_1459-11B has positive correlation with V, UV, ALT, pH, TEMP, ORP, TN, NH₃-N, and negative correlation with TP, DO, E and N; Pseudorhodobacter has positive correlation with TP, E, DO and N, and negative correlation with NH₃-N, TN, ORP, TEMP, V, UV, ALT and pH.

The redundancy analysis of influencing factors showed that the clustering effect of U5 at the phylum and genus level was not obvious. The influencing factors that had the greatest impact on the sample microorganisms were UV, V, E, TEMP, ALT, TN. There were certain differences among the influencing factors on microorganisms. Moreover, there is some difference between the analysis results of correlation heat map with UV, TN, TP, DO, E, N as the main influencing factors, which may be mainly related to the fact that NH₃-N and TN do not affect microorganisms with high abundance. In addition, the analysis shows that the microbial community structure is affected by different river headwaters.

Prediction of microbial function of river sediments in cities and towns on plateau

Functional proteins of microbial community and their changes

The PICRUSt program is widely used to predict the metabolic characteristics of microbiota (Li et al., 2018). As shown in *Figure 8*, the average relative abundance of COG classification ranks the top seven (S, R, E, T, M, C, P) in plateau urban rivers, except for unknown S function, the rest are class R (general function prediction), E (amino acid transport and metabolism), T (signal transmission mechanism), M (biosynthesis of cell wall, cell membrane and membrane structure), C (energy generation and conversion), and P (inorganic ion transport and metabolism). These functional proteins are closely related to the microbial growth and reproduction and other life activities, providing the prerequisite for efficient conduct of cell life activities. The functional proteins L (replication, reconstruction and repair), G (carbohydrate transport and metabolism), J (protein translation, ribosome structure and formation), O (post-translational modification, protein folding and molecular chaperone), H (coenzyme) and I (plasma transport and metabolism) are mainly involved in small molecule protein synthesis, energy consumption and signal transmission. The higher the average relative abundances of the above COG functional classes, it indicates that they play a more important role in the growth and reproduction of microbial cells in the plateau habitat. The dominant COG functional classifications were similar to the relevant studies in sub-plateau region, but the high-altitude areas inhibited the COG functional classes W, Y, Z, A and B, which are related to microbial growth and signal transduction.

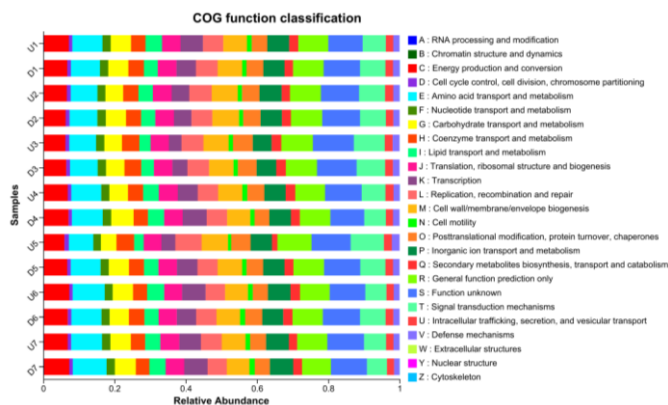


Figure 8. PICRUSt function prediction

As shown in Figure 9, from U1, D1, U2, D2, U3, D3, U4, D4, U5, D5, U6, D6, U7, and D7, there are 4319, 4344, 4286, 4202, 4216, 4230, 4360, 4339, 4287, 4323, 4364, 4319, 4323, 4342, respectively, which are U6, U4, D1, D7, D4, D5, U7, U1, D6, U5, U2, D3, U3, and D2 from the most to the least. According to the information of COG family, the top 10 COGs of abundance in 14 samples were summarized, 19 functional proteins were obtained, among which 4 functional proteins of COG0642, COG0438, COG0745, COG0596 were dominant in 14 sampling sites, indicating that these functional proteins of COG are important to microbial cell activities and are important functions of all cell activities and reactions.

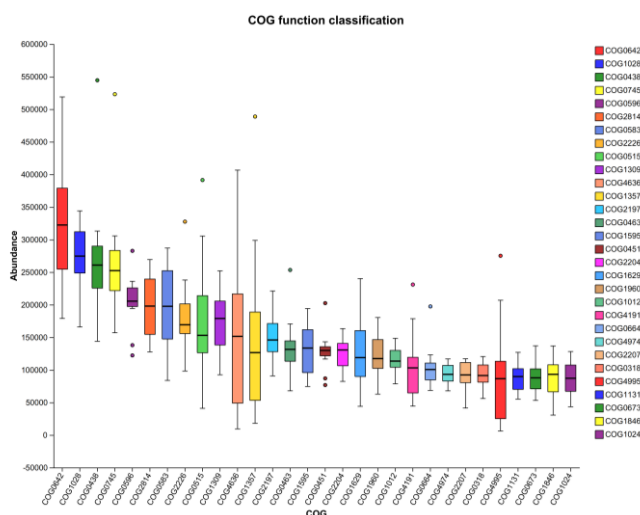


Figure 9. Box diagram of COG sample abundance statistics

COG1028 is a dehydrogenase/reductase, except U5, the other 13 sampling sites ranked in the top 4, among which U1, D1, U2, D2, U3, D3, U4, D4, U6 ranked first, D5, D6 ranked second, and U7 and D7 ranked third and fourth, respectively; COG2814 is the membrane transporter, and U1, D1, U2, U3, D3, U4 are the dominant functional proteins; COG0583 is the dominant functional protein in U1 and D1; COG2226 functional protein is the methyltransferase required for the conversion of Demethylated menadione (DMKH2) to menadione (MKH2), and D1, U2, D2 are the dominant

functional proteins; COG0515 is the dominant functional protein on D1, D2, U3, D3, U4, D4, U5, U7, D7; COG1309 is the dominant functional protein in U1, D1, U3, U4, D4, D5, U6, D6, U7, D7; the COG4636 is located at U2, D2, U3, D3, U5 is the dominant functional protein. COG1357 is in U2, U3, D3, U5 is the dominant functional protein. COG2197 in U1, D4, U5, U6, U7 is the dominant functional protein. COG0463 is the dominant functional protein in U5; COG1595 is the dominant functional protein in D4; COG2204 is the dominant functional protein in D7; COG1629 is in U2, D2, U4 is the dominant functional protein. COG1960 in U1, U6, D7 is the dominant functional protein. G4995 is a dominant functional protein in D3.

Among the 14 sampling sites, only 4 COGs were common, i.e. COG0642, COG0438, COG0745, COG0596, and another 15 dominant but non-shared COGs were obtained; The single-sample T-test shows that the dominant COG of the 14 sampling sites has significant difference ($p = 0.008 < 0.01$). That is, the difference of the dominant COG of different sampling sites is very obvious; meanwhile, the tail water discharge of sewage treatment also causes the significant change of the composition and abundance of the dominant COG of the upstream and downstream sampling sites.

Enzyme system and abundance in microbial community of Bahe Cascade hydropower station

In order to analyze the metabolic characteristics of the microflora, we performed functional annotation of KEGG by Tax4Fun to obtain the annotation information of OTU at each functional level of KEGG and the abundance information of each function in different samples. About 99.8% of the genes were involved in metabolism, genetic information processing and environmental information processing.

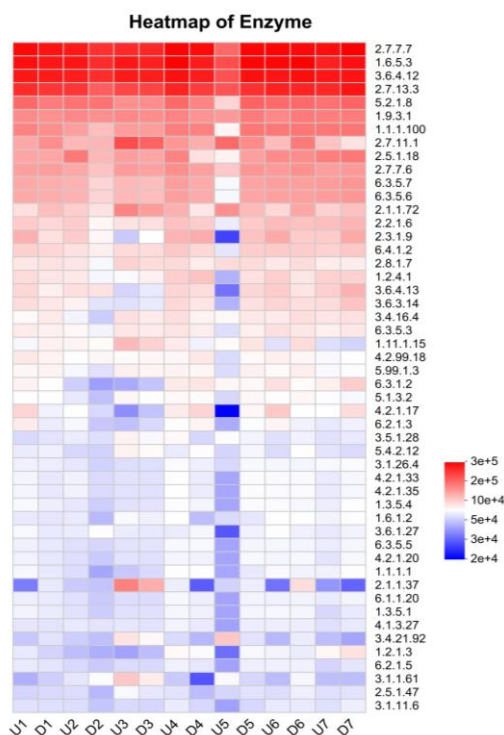


Figure 10. Function heatmap diagram. The abscissa is the sample name, and the ordinate is the enzyme number. The color gradient of the color block is used to show the abundance change of different functions in the sample. The legend is the variation represented by the color gradient

As shown in *Fig. 10*, The effect of oxygen on microorganisms usually depends on the tolerance of enzymes in microbial cells. There are obvious differences in river microbial communities in plain and plateau areas. The type of denitrification gene system in rivers at high altitude have higher specificity, and the bacterial communities in high altitude rivers play an important role in maintaining the glacier-fed aquatic ecosystems. The plateau basin presents typical plateau cold anoxic climate characteristics, glacier landform development, and groundwater recharge in winter. The anoxic zone contains microorganisms capable of living in anaerobic bacteria, which has microbiological significance, especially the plateau fresh water anoxic zone that few people pay attention to this area (Li et al., 2018, Zhu et al., 2024).

According to the corresponding enzymes OTU information, the abundances of the 14 sampling sites were sorted from large to small as D7, U4, D6, D5, U6, U7, U1, D1, D4, U3, D3, U2, D2 and U5. Functional Heatmap maps were drawn for the top 50 abundances of the 14 sampling sites. According to the summary of the top 10 enzyme abundances, there are 6 dominant enzymes at all sampling sites, namely NADH. DNA-directed DNA polymerase (2.7.7.7) is mainly used for DNA replication; DNA helicase (3.6.4.12) is involved in ATP hydrolysis to release ADP, phosphate and energy, and DNA helicase uses the energy of ATP hydrolysis to release double-stranded DNA; NADH: ubiquinone reductase (H⁺-translocating, 7.1.1.2) reduced coenzyme I, which mainly participates in substance and energy metabolism in cells, produced in the citric acid cycle in glycolysis and cell respiration, and acts as a carrier and electron donor of biohydrogen on mitochondrial inner membrane through oxidative phosphorylation process. It transfers energy to ATP synthesis through oxidative phosphorylation processes in the inner mitochondrial membrane; histidine kinase (2.7.13.3) belongs to the group of transferases, participate in the metabolism of the bacterial two-component system; DNA helicase (3.6.4.12) is a DNA helicase, the energy generated by ATP hydrolysis is loaded onto the DNA single strand by a helicase loader, act on the hydrogen bond of DNA double strand; glutaminyl-tRNA synthase (glutamine-hydrolysing, 6.3.5.7) is a glutamine hydrolase, that has the special property of hydrolyzing glutamine to glutamic acid during the aminoacylation of tRNA. It plays an important role in the regulation of protein synthesis and gene expression.

There were only 6 dominant enzymes in the top ten of 14 sampling sites were shared, and 10 dominant but non-common enzymes were obtained. The single-sample T-test showed that there were significant differences in the dominant enzymes of the 14 sampling sites ($p = 0.001 < 0.01$), i.e. the difference of the dominant enzyme in different sampling sites was very obvious; meanwhile, the discharge of wastewater treatment tail water also caused a significant change in the composition and abundance of the dominant enzyme in the upstream and downstream sampling sites.

FAPROTAX function forecast

FAPROTAX is an artificially constructed database that maps prokaryote taxa (e.g., genera or species) to metabolism or other ecologically relevant functions (e.g., nitrification, denitrification). The functions of FAPROTAX focus on marine and lake biogeochemistry, particularly the sulfur, nitrogen, hydrogen and carbon cycles. Faprotax enables functional prediction of bacterial data. As shown in *Fig. 11*, the FAPROTAX method was used to determine 69 functional groups of surface sediments of urban rivers in the plateau. The dominant groups in winter were related to

phototrophy, photoautotrophy, cyanobacteria, oxygenic_photoautotrophy, chemoheterotrophy and aerobic_chemoheterotrophy.

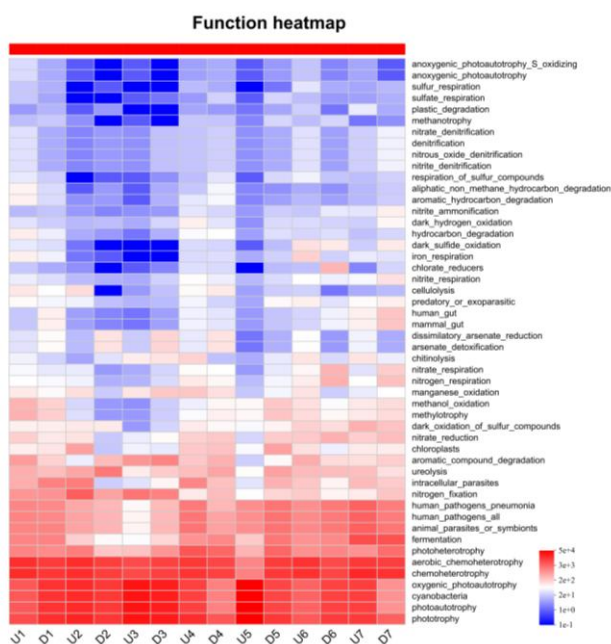


Figure 11. FAPROTAX Function heatmap. The abscissa is the sample name or group name, and the ordinate is the function name. The variation of the abundance of different functions in the sample is shown through the color gradient of the color block. The right side of the figure is the numerical value represented by the color gradient

Phototrophy, photoautotrophy, cyanobacteria and oxygenic_photoautotrophy may be related to longer sunshine duration in the relevant area. Chemoheterotrophy and aerobic_chemoheterotrophy, these functions are generally associated with acting as bacteria involved in decomposition and contribute to in-situ remediation and organic matter recovery in all ecosystems (Peng et al., 2018; Kämpfer et al., 1993), bacteria in sediments in winter water tend to autotrophic in light energy, which is mainly related to higher nutrients in rivers in winter, and is also consistent with Cyanobacteria as a dominant microorganism in rivers.

Only 6 of the top ten dominant Functional groups of 14 sampling sites are shared, namely, aerobic_chemoheterotrophy, chemoheterotrophy, cyanobacteria, oxygenic_photoautotrophy, photoautotrophy, phototrophy, and the above-mentioned Functional groups are closely related to the unique illumination and nutrients in Tibet respectively. In addition, 11 dominant but non-common functional groups are obtained but not shared. The single-sample T-test showed that there were significant differences in the dominant functional groups of 14 sampling sites ($p = 0.013 < 0.05$), i.e. the difference of the dominant functional groups of different sampling sites were significantly different; meanwhile, the discharge of tail water for sewage treatment also causes significant changes in the composition and abundance of upstream and downstream functional groups, among which, the 7 sewage treatment tail water have the greatest influence on cyanobacteria (12.10%), phototrophy (5.90%), photoautotrophy (4.55%), phototrophy (11.15%), cyanobacteria (19.77%), chemoheterotrophy (10.83%), photoautotrophy (9.22%).

Conclusion

In the research, physical and chemical index monitoring and 16S rRNA gene sequencing of 14 sections of water quality in the upstream and downstream reaches of 7 plateau towns in dry season were conducted, and the physical and chemical indexes of water quality, microbial community diversity, dominant microbial community, influencing factors, functional protein, functional gene, dominant enzyme and FAPROTAX functional prediction were analyzed. The results showed that:

(1) The monitoring results of physical and chemical indexes of water quality showed that except TN, all of them reached Class II standard.

(2) The results of microbial community diversity analysis showed that the diversity and richness of microbial communities in 14 sampling sites of Zayü River, Parlung Zangbo, Niyang River and the main stream of Yarlung Zangbo River are lower than those in other regions, and there are significant differences among different sampling sites. The tail water discharged from the sewage treatment plant has an impact on the diversity of microbial community in the surface sediments of the sampling sites.

(3) A total of 49 phyla, 151 classes and 1189 genera were detected in the surface sediments from 14 sampling sites. At the phylum level, the relative abundances of Proteobacteria, Cyanobacteria, Actinobacteria, Bacteroidetes and Frimicutes ranked in the top 5 species were as high as 77.87%-98.51%, and the sum of the relative abundance of the top 10 bacteria genera in the genus level was between 18.68%-88.71%, but there were differences among different sampling sites, and their unique microbial communities and characteristics were formed in the plateau habitat, and their representativeness was very strong by collinear analysis.

(4) The analysis of sediment microbial diversity and influencing factors shows that DO, E, TP, NH₃-N and TN have great effects, and their effects are different to some extent; the redundancy analysis of influencing factors shows that at the level of phylum and genus, UV, V, E, TEMP, ALT and TN are the most influential factors, and the effects of all influencing factors on microorganisms are different to some extent, which may be mainly related to the effects of NH₃-N and TN on microorganisms with high abundance, but have close correlation with the poor nutrition of high altitude and low pressure habitats. In addition, that microbial community structure is affected by the domestic wastewater discharged from different river headwaters and sewage treatment plant.

(5) The prediction of functional protein, dominant enzyme and FAPROTAX function also proved again that different rivers in plateau habitat have influence on the above indexes, and the domestic wastewater discharged from sewage treatment plant has influence on the diversity of microbial community in surface sediment of sampling site.

Therefore, through analyzing the correlation between microbial community and abundance changes and environmental factors to reflect the change of water environmental factors, reflect the quality of water ecological environment, and provide guidance for water quality improvement, water ecological protection and management. However, based on the influence of different spatial dimensions, the specific mechanism of interaction between microbial community structure and environmental factors in the surface sediments of the 14 sampling sites is relatively complicated and further in-depth and comprehensive exploration is needed in the future.

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