

PREDICTION OF MICROBIAL COMMUNITY STRUCTURE AND FUNCTION IN SURFACE SEDIMENTS OF BAHE CASCADE HYDROPOWER STATION IN THE DRY SEASON IN CHINA

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Abstract. Bahe river is the secondary tributary of the Yarlung Zangbo river, and the largest tributary of Nyang River in China, for which we analyzed the microbial diversity, community structure and metabolic function diversity in the surface sediments of 3 cascade hydropower stations upstream and downstream and their adjacent tributaries were analyzed by high-throughput sequencing. The study has shown that the abundance of microbial community in the surface sediments of Bahe cascade hydropower station were as follows S7(downstream of Laozui Hydropower Station), S3(downstream of 606 Hydropower Station), S5(Zhulaqu of Bahe tributary), S4(upstream of Xueka Hydropower Station), S1(upstream of 606 Hydropower Station), S2 (Zimuzhongnongbaqu of Bahe tributary) and S6(downstream of Xueka Hydropower Station/upstream of Laozui Hydropower Station), and the microbial diversity and abundance of 7 sampling points were lower than those of other areas. The predominant bacteria in the surface sediments of Bahe cascade hydropower stations are Proteobacteria, Cyanobacteria, Frimicutes, Actinobacteria, Bacteroides, etc. The dominant bacteria are Exiguobacterium, Tychonema_CCAP_1459-11B, norank_f_unclassified_o_Oxypotobacteria_Incertae_Sedis, Hyphobacterium, Sphingorhabdus, Leptolyngbya_FYG, Phytobacter, Pseudoarthrobacter, Pseudoarthrobacter, Unclassified_f_Microbacteriaceae. Predominant bacteria varied significantly between samples. Further study shown that total phosphorus, temperature and flow velocity were the main factors affecting microbial community structure in plateau rivers. And the cascade effect also affects the microbial community structure, the functional genes, functional proteins, dominant enzymes and FAPROTAX functions of the river in S3 and S4. However, cascade effect was failure in S6, S7.

Keywords: *microbial diversity, cascade effect, influencing factors, high-throughput sequencing*

Introduction

With the development of economy, the demand for energy is growing day by day. The development of hydropower can not only provide clean energy for human beings, improve water supply and flood management, but it can also bring about the promotion of social and economic benefits (Chen et al., 2021). According to the statistics of International Journal of Dams in 2011, the theoretical reserves of global hydropower resources are more than 4.02×10^7 GWh. So far, hydropower has provided 16% of the world's total electricity, accounting for 45% of renewable power. The hydropower complex will not generate atmospheric greenhouse gas emission (Rosa et al., 2004). In order to achieve the carbon neutralization target by 2050, it is still necessary to increase the installed capacity of 2.6 billion kw of hydropower (Yang et al., 2022). China is the country with the largest installed hydropower capacity in the world. The Qinghai-Tibet

Plateau is known as an Asian water tower. Nearly 70% of China's hydropower potential is located on the Qinghai-Tibet Plateau. Tibet is also known as The Water Tower of China with a total water resource of 448.2 billion m³. According to the China's 13th Five-Year Plan for the hydropower development, issued by the National Energy Administration, the installed capacity of hydropower resources development in China is about 660 GW and the annual power generation is about 3×10⁶ GWh. China's hydropower resources are concentrated in the southwest area, accounting for two-thirds of the country's total hydropower resources. Among them, Tibet ranks first with a technological development capacity of 1.76 million kW making it a key region in China's hydropower development (Duan, 2021). Salween River and Brahmaputra River originate here. Developing hydropower resources has huge potential to provide abundant, cheap and clean electricity, develop local economy and reduce greenhouse gas emissions.

The construction of hydropower station has led to significant changes in the original river hydrology, water environment, aquatic biological habits and the structure and function of ecosystem, and significantly changed the metabolic characteristics of aquatic microbiota (Chen et al., 2020). During the operation of hydraulic engineering, such as river dam construction and other hydrological controls, the river continuity is destroyed, the hydrological and hydrodynamic mechanisms, sediment particle size distribution, downstream groundwater exchange, river nutrient conditions and ecosystems are changed, which cascade the geochemical and microbial processes in river corridors (Li et al., 2020), affecting the sediment microbial communities in downstream connected lakes (Huang et al., 2016), thereby changing the bacterial community of the Earth's river.

As the main form of exploitation and utilization of water energy resources, the cascade development of river has a profound influence on river ecosystem while promoting regional economic development. Cascade dams in rivers usually form a series of reservoirs with different characteristics, such as small reservoirs with short hydraulic retention time (HRT) and large reservoirs with long HRT and vertically stratified water. In reservoir cascades, the hydrological environmental conditions of the downstream reservoirs are affected by the discharges of the nearest upstream reservoir, which may result in bacterial community characteristics different from those of a single reservoir or lake. The cascade reservoirs led to the fragmentation of the river ecosystem to a greater extent, affecting the migration of fish and the like, obstructing the diffusion of terrestrial species and destroying their continuity, thus leading to a significant reduction of species diversity in the river buffer zone (Naiman et al., 2000). The cascade reservoirs have a significant impact on the structure and function of the riparian ecosystem.

Sediments can be classified into river, lake and marine sediments according to the aquatic environment. Generally, the organic matter content in sediments of rivers and lakes in freshwater systems is relatively high (Zhang et al., 2021), and many rivers originating from the Himalaya Mountains and the Qinghai-Tibet Plateau, with sediments and nutrients that are easy to deposit, can often create large-area, high-yield alluvial plains (Winemiller et al., 2008). Streams carry biological and abiotic substances, while sediments are the source or sink of the nutrient cycle (Zhang et al., 2019), and sediment bacterial communities are more complex and stable than planktonic bacterial communities. The impact of river damming on trophic biogeochemistry has become the main focus of research (Chen et al., 2022), which

indicates that sediment bacterial communities are affected by dam regulation, and the effects of oxygen on microorganisms generally depends on the tolerance of enzymes in microbial cells (Qin et al., 2021).

The Bahe river Basin presents typical climate characteristics of chillness and oxygen shortage on the plateau. The glacier landform is developed. In winter, groundwater is main recharge. The anoxic area contains microorganisms that can live in anaerobic bacteria, which is of microbiological significance, especially in the plateau freshwater anoxic area that received little attention (Gracas et al., 2011). The influence of cascade hydropower stations on the microbial composition of river microbiota needs to be explored. At present, there is no research on the result of microbial population in surface sediment of cascade hydropower station of Bahe river. The cascade hydropower reservoirs have formed their own hydrological characteristics, elemental biogeochemical cycle and phytoplankton ecology (Yang et al., 2020), and there is relatively little research on the response of fluvial microorganisms to the cascade hydropower station operation in plateau rivers. Based on this, in this paper, the microbial diversity, community structure and metabolic function in the upstream and downstream surface sediments of Bahe cascade hydropower stations were analyzed by Illumina Miseq high-throughput sequencing, so as to reveal the structure morphology of microbial community in the surface sediments of the basin under the influence of cascade hydropower stations, and explore its response mechanism of the microbial community to plateau habitats. It provides a scientific basis for the ecological protection and ecological restoration in hydropower development of plateau river cascade hydropower stations.

Materials and methods

Sample collection

According to the dam construction and operation of Bahe cascade hydropower stations, 7 sampling points were selected for three cascade hydropower stations, namely, 606, Xueka and Laohuzui (*Fig. 1*). S1 (upstream of 606 hydropower station), S2 (Zimuzhongnongbaqu of Bahe tributary), S3 (downstream of 606 hydropower station), S4 (upstream of Xueka Hydropower Station), S5 (Zhulaqu of Bahe tributary), S6(downstream of Xueka Hydropower Station/upstream of Laozui Hydropower Station) and S7(downstream of Laohuzui Hydropower Station). According to the construction of hydropower station, the distribution of sampling points are main stream (S1, S3, S4, S6, S7) and tributary (S2, S5), and the elevation gradient of 7 sampling points is 3204~3370 m.

Bahe river originates from the eastern end of the Nyingchi-tanggula Range Mountains. The upper source is composed of two branches, Pasang river and Milaqu river. The total length is 89 km. The drainage area is 4229 km², and the annual average runoff is 49.5×10^8 m³. The water source is supplemented by glacial meltwater, rainwater and groundwater. The cascade hydropower station of Bahe river has 606 power station, Xueka power station and Laohuzui Hydropower Station from the upstream in sequence. The controlled drainage are 5760, 5230 and 4140 km² respectively, and the annual average power generation is 0.27×10^8 , 1.82×10^8 and 4.955×10^8 kW·h respectively. The total storage capacity of Basom Lake, the water resource storage upstream of 606 Hydropower Station, is about 3.5×10^8 m³ (Li et al., 2012), increasing the power supply of Linzhi Power Grid and Tibet Central Power Grid.

The 606 Hydropower Station and Xueka Hydropower Station are pilot power stations. The distance between Chongjiu Reservoir Dam and Xueka Hydropower Station Dam is 20 km, and the flow of 2.4 km downstream of Xueka Hydropower Station decreases in winter. Laohuzui Hydropower Station is located on the main stream of Bahe River, which is hydropower station after dam, about 5.5 km away from the outlet of Bahe River, 95 km away from Bayi Town of Linzhi City in the east, about 300 km away from Lhasa City in the west, and the normal storage level is 3297.00 m. The regulated storage capacity of the reservoir is 7.1 million m³.

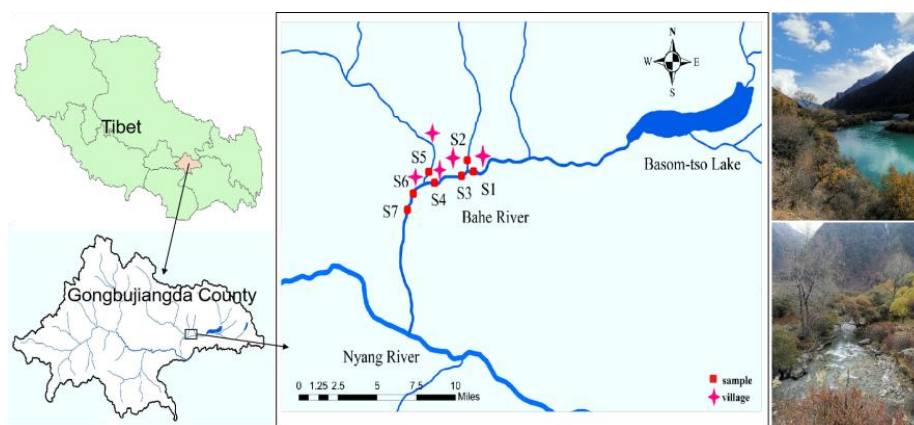


Figure 1. Location distribution of sampling points of Bahe Cascade Hydropower Station in Gongbujiangda County, Linzhi City, Tibet, China

Methods

The sampling time was late November 2022. Dissolved oxygen (DO), oxidation reduction potential (ORP), Pondus Hydrogenii (pH) and temperature (TEMP) adopted HACH multifunctional detector (HACH HQ4od, www.hach.com.cn) for detection. The altitude (ALT) as well as longitude and latitude were measured by Mobile Mapper10(Thales Navigation, www.magellangps.com). Flow velocity (V) was measured by the Doppler FLOW-ADC-10T. The concentration of total phosphorus (TP), total nitrogen (TN) and ammonia nitrogen (NH₃-N) was determined by colorimetry with Sheng Aohua multi-parameter water quality detector (6B-3000A, Jiangsu Shengao Environmental Protection Technology CO., LTD, www.sahkjcod.com) (Guo et al., 2022), and the grab-bucket bottom sampler was used to collect the surface sediment 0.5 m offshore and 3 cm underground, which were put into the collection bag, and transported back to the laboratory and stored in a refrigerator at -80°C for testing.

Surface sediment samples from 7 sampling sites were analyzed by 16S rRNA gene assay and SPSS24 software was used for statistical analysis. In order to understand the functional characteristics of microorganisms and genes, 7 samples obtained in this experimental study (in the environment of -80°C) were entrusted to Shanghai Majorbio Bio-pharm Technology Co., Ltd. for testing (<http://www.majorbio.com>).

In the sequencing experiment, the DNA of the sample was extracted by Fast DNA Spin Kit (Fast DNA Spin Kit for Soil, MP, USA) [12], followed by 1% agarose gel electrophoresis. PCR instrument (ABI GeneAmp® Model 9700) 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'). Three repeated PCR amplification products from the same sample were mixed and detected by using 2% agarose gel electrophoresis. According to the preliminary quantitative results of electrophoresis, the PCR products were detected and quantified with QuantiFluor™-ST blue fluorescence quantitative system (Promega Inc.), and then mixed proportioned to the sequencing volume required for each sample. The Illumina official linker sequence was added to the outer end of the target region by PCR, and the PCR product was recovered by gelatinized with AxyPrep DNA gel recovery kit (AXYGEN), then eluted with Tris-HCl buffer and detection by 2% agarose electrophoresis. After the NaOH denaturation, a single-stranded DNA fragment can be obtained, one end of the single-stranded DNA fragment and a complementary primer base are fixed on a chip, a target DNA fragment to be detected is synthesized, "bridge" is formed after denaturation and annealing, DNA polymerase and dNTP are added, the surface of the reaction plate is scanned by laser, and the nucleotide types polymerized by the template sequence are read. and finally, chemically cutting the 'fluorescent group' and the 'stop group' to recover the viscosity of the 3' end, continuing to polymerize a second nucleotide, cycling and reciprocating, and counting the sequence of the template DNA fragment obtained in each round, so that the sequencing of the Illumina MiSeq is finished.

The microbial diversity index was calculated using *mohur* (version v. 1.30.2 <https://mothur.org/wiki/calculators/>), the community histogram was statistically plotted using the R language tool, the community Heatmap R language (version 3.3.1) *vegan* package was plotted, the Spearman correlation Heatmap was plotted using R language (version 3.3.1) (*sheetmap* package), and the KEGG-based database was used to predict the bacterial function and use the function prediction software (*PICRUSt2*) to predict the bacterial function and use the Lc-bio Technologies Platform (<https://www.omicstudio.cn/tool/81>) to map the metabolism.

Results and discussion

Determination of physical and chemical indexes

Physical and chemical indexes of overlying water quality of 7 samples are shown in *Table 1*.

Table 1. *Physical and chemical indexes of overlying water quality*

Samples	S1	S2	S3	S4	S5	S6	S7
N	29°58'36"	29°58'41"	29°58'35"	29°58'01"	29°58'18"	29°57'55"	29°53'33"
E	93°43'52"	93°43'30"	93°43'20"	93°41'25"	93°40'52"	93°40'30"	93°39'28"
V (m/s)	0.30	1.36	0.02	1.25	0.51	0.50	0.33
TEMP (°C)	7.6	5.5	8.1	9.6	7.2	8.6	8.8
pH	8.96	8.61	8.79	8.75	8.61	8.62	8.43
DO (mg/l)	11.36	13.93	13.43	13.24	11.03	11.27	10.39
ORP (mv)	90.0	146.1	124.9	180.9	119.2	128.2	136.2
ALT (m)	3370.25	3369.00	3352.00	3327.00	3318.00	3315.00	3204.42
TP (mg/l)	0.008	0.004	0.014	0.031	0.046	0.039	0.018
TN (mg/l)	0.074	0.366	0.450	0.112	0.385	0.186	0.279
NH ₃ -N (mg/l)	0.043	0.251	0.394	0.043	0.359	0.033	0.059

According to the analysis of physical and chemical indexes of water quality and environmental quality standard of surface water at 7 sampling points, TP concentration at Bahe river sampling point was between 0.004-0.046 mg/l, and TP concentration at Bahe river sampling point has no significant change. After reservoir impoundment, scouring in the downstream water conveyance process slowed down. TP concentration of overlying water slightly decreased. Phosphorus release in surface sediments were relatively slow or it might be concentrated in sediment, so that TP concentration at sampling point S7 decreased (TP of S1, which is also located downstream of the reservoir, is also small.).

The potential sources of nitrogen is that the pollution of surrounding villages, and biogenic material is rich in lake or reservoir. TN concentration of S1, S6 and S4 is less than 0.2 mg/l, TN concentration of S2, S3, S5 and S7 is less than 0.5 mg/l, which is lower than that of other rivers (Fan et al., 2022). TN of S3 is higher than that of other points, because it is adjacent to village and downstream river is dammed, small lake section is formed in canyon river, flow velocity is small, biogenic material is rich in villages, pollution enrichment results in N accumulation, S2 and S5 are tributaries of Bahe river, TN is relatively high, TN of S7 is relatively high, TN of S4 is higher than that of S1, TN of S6 is higher than that of S4, and TN of S6 is higher than S4 and maybe caused by influence of tributaries S3 and S5, and villages upstream of S2 and S5 are relatively dense. The N source of S7 is from Laohuzui Reservoir. TP of S1, S3, S4 and S6 show an increasing trend, which may affect the increase of total nitrogen in sediments from the upstream to the downstream of the reservoir (Wang et al., 2022). TP is higher at S6 before the reservoir than at the point S7 at the downstream of the reservoir, and P maybe enriched or degraded in the reservoir. The potential nutrients in the reservoir are high and the microbial abundance is high. the NH₃-N concentration of S1, S4, S6 and S7 is less than 0.06, and the NH₃-N concentration of S2, S3 and S5 samples is less than 0.4(mg/l).

According to Spearman correlation coefficient analysis, when the confidence (double-test) of N (latitude) and TEMP was 0.05, which shown a significant negative correlation ($p_v = -0.786$). when the confidence level (double-test) is 0.01, TN and NH₃-N show significant positive correlation, the proportion of NH₃-N in S1(57.26%), S4(87.68%), and S5(38.04%) in the study basin is higher. This is mainly related to the insufficient oxidation of NH₃-N into nitrate after the cascade hydropower station of 606 and Xueka Hydropower Station are formed, while the proportion of S6(17.98%) and S7(21.25%) is low. This is mainly related to the fact that there is no cascade connection between Xueka Hydropower Station and Laohuzui Hydropower Station, which has no effect on the oxidation of NH₃-N. Generally speaking, the environmental factors (NH₃-N, TN, TP) of river surface water of Bahe cascade hydropower station are different in spatial scale and have a certain correlation.

Alpha diversity analysis

Respectively calculating chao (community richness), ace (community richness), Shannon (community diversity), Simpson (community diversity), coverage (community coverage), Shannoneven (community evenness) index by using Alpha diversity, estimating the abundance and diversity of community species in the environment. The diversity data of surface sediment samples from the seven sampling points is shown in *Table 2*.

As shown in *Table 2*, the coverage index of 7 sampling points was greater than or equal to 0.98, which indicates that the test results can fully reflect the real situation of

microorganisms in the sample. The chao and ace indexes can reflect the richness of the community (the number of organisms is much higher than ours), among which the ace ranges from 1856.471 to 3016.348, and the chao index ranges from 1610.729 to 2944.627. Compared with the ace and chao indexes obtained in the existing literature (Yang et al., 2018; Luo et al., 2020; Chen et al., 2018) (ace = 6132~10089, chao = 6224~9871), the ace index shows a decreasing trend at sections S1, S4 and S5, which is verified again to have cascade effect. The increase of abundance after S6 and S7 indicates that the cascade effect disappears. The smaller their value means the lower the abundance of microbial community is, which may be related to the low temperature and low nutrition state of plateau rivers, and the maximum richness of the sampling site S7, which may be affected by the dam construction and the enrichment of the reservoir source material during the operation of the hydropower station in winter. In tributary S2 and S5, community abundance shown a good correlation with nutrition at sampling points, community evenness index shannoneven range 0.370~0.758 respectively, community diversity can be used with Shannon index between 2.521~5.870, which were smaller than those obtained in the existing literature (Chen et al., 2020, 2018; Zhang et al., 2019; Luo et al., 2020). Relevant research shows that the construction and operation of hydropower station will reduce the microbial diversity of river water body, indicating that the microbial diversity of 7 sampling points decreases under the influence of altitude. And the ranking results are in turn S7, S3, S5, S4, S1, S2 and S6, in which the diversity of S3 and S5 is related to exogenous microbial injection, and the microbial diversity of S7 sampling point downstream of dam is the highest, consistent with the research (Chen et al., 2018). The abundance, activity and diversity of bacteria in the sediment of the dam reservoir gradually increased downstream. The above analysis shows that the diversity and abundance of communities in the plateau rivers are lower than those in other areas, and the hydroelectric cascade dams have an effect on microorganisms.

Table 2. Schedule of microbial diversity

Samples	Shannon	Simpson	Ace	Chao	Coverage	Shannoneven
S1	4.831	0.040	2472.545	2421.224	0.983	0.648
S2	4.120	0.105	2936.185	2429.415	0.983	0.567
S3	5.352	0.033	1966.750	1960.678	0.990	0.723
S4	5.080	0.017	1856.471	1800.629	0.988	0.702
S5	5.344	0.022	2756.924	2714.979	0.982	0.698
S6	2.521	0.289	2073.269	1610.729	0.988	0.370
S7	5.870	0.009	3016.348	2944.627	0.980	0.758

Microbiological structure composition

Species composition analysis at genus level

The samples are drawing in average, and the species with abundance less than 0.01 were included as others. It can be seen from *Figure 2* that the microbial communities of surface sediment samples from different sampling points of Bahe Cascade Hydropower Station was at the phylum level. Among the 7 samples, there are 13 microbial and bacterial phyla with relative abundance of more than 1%, and they are regarded as dominant phyla, and other bacterial phyla are classified as others. Bacterial community composition varied at phylum taxonomic levels as shown in *Figure 2*.

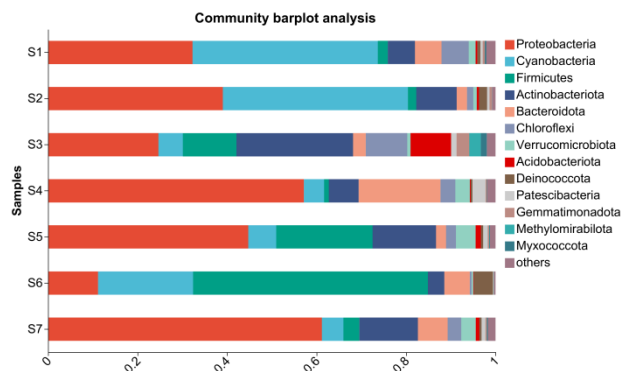


Figure 2. Relative abundance of microbial species in surface sediment samples at phylum level

In Figure 2, the five bacterial phyla with the highest average relative abundance were Proteobacteria (11.17%~61.19%), Cyanobacteria (4.52~41.43%), Firmicutes (1.05%~52.48%), Actinobacteria (3.67%~26.05%) and Bacteroides (2.26%~18.29%). The relative abundance of the first 5 species at each sampling point was 87.94%, 93.63%, 71.01%, 87.70%, 88.97%, 94.26% and 89.29%. The results of community composition and related literature are similar (Wang et al., 2018).

Further analysis showed that the relative abundance of bacteria in different sampling points was different, wherein, Proteobacteria has the highest average abundance, which is close to that of Lancang River, Yangtze River and an equatorial river (Dumestre et al., 2002), S4 and S7 have the highest abundance in 7 sections. Proteobacteria is the main contributor of denitrification in nitrification and denitrification. The abundance change of S6 and S7 shows that the dominant species group is changed from Firmicutes to Proteobacteria after the flow passes through the Laohuzui Hydropower Station, which is also consistent with the concentration of ammonia nitrogen.

Cyanobacteria was the second rank of average abundant bacteria. There were grazing pollution in S1 and S2, which may stimulate the growth of cyanobacteria. The abundance reached 41.43% and 41.36% respectively. S7 was in dry season, when the reservoir discharges water, the water flow is large, the water retention time is short, and the relative abundance of cyanobacteria is low. Firmicutes have the highest relative abundance at S6, possibly due to spore-forming ability and gram-positive cell walls of most thick-walled phyla, which further support their tolerance to adverse environmental conditions such as oligotrophic environment (Schimel et al., 2007). Actinobacteria is a kind of gram-positive bacteria that can absorb nutrients and is ubiquitous in freshwater habitats (Li et al., 2017). Bacteroidetes is mainly contributed by tributaries, but grows better in unsaturated rivers and has good cascade accumulation effect.

At the Phylum level, the bacterial communities in the sediments of Bahe cascade hydropower stations are mainly Proteobacteria, Cyanobacteria, Firmicutes, Actinobacteria and Bacteroides. The relative abundance of dominant phylum has obvious fluctuation among different sampling points. The tributaries carry microbial communities from different habitats, which mainly cause the “cumulative effect” along the river (Moitra et al., 2015; Nino-Garcia et al., 2016). The construction of cascade hydropower station has obviously changed the composition of sediment microbiota. Local water environmental conditions make specific species more competitive and dominate local bacterial communities, leading to ‘environmental selection’ leading to ‘species classification’ (Staley et al., 2015; Szekeley et al., 2013).

Analysis of species composition at genera level

The average relative abundance was obtained for all microbial bacteria genera in seven samples, and the relative abundance in each sample is shown in *Figure 3*.

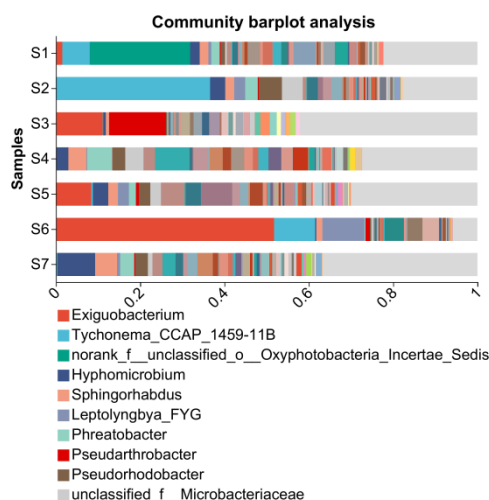


Figure 3. Relative abundance of microbial species in surface sediment samples at genus level

As shown in *Figure 3*, the difference of dominant microorganisms at sampling points at genus level is relatively large. The bacterial genera with the first 10 extraction average relative abundances are *Exiguobacterium* (0.00~51.76%), *Tychonema_CCAP_1459-11B* (0.06~36.60%), *norank_f_unclassified_o__Oxyphotobacteria_Incertae_Sedis* (0.00~23.80%), *Hyphomonas* (0.32~8.94%), *Sphingorhabdus* (0.77~5.19%), *Leptolyngbya_FYG* (0.00~10.02%), *Phytobacter* (0.02~6.00%), *Pseudoarthrobacter* (0.05~13.47%), *Pseudorhodobacter* (0.31~5.51%), *unclassified_f__Microbacteriaceae* (0.07~4.92%) The total relative abundance of the above bacteria at 7 sampling points is 40.56%, 58.56%, 26.46%, 20.76%, 24.91%, 75.26% and 22.93% respectively.

Further analysis showed that the relative abundance of bacteria in different sampling sites was different. Among them, the average relative abundance of *Exiguobacterium* is the largest (10.40%), which is suitable for growth under extreme environment and low nutrient conditions (Kasana et al., 2018). In sediments of Bahe river, except S3 and S6, the average relative abundance of *Exiguobacterium* is much higher than that of other fungi genera. S3 and S6 are reservoirs in impoundment area of hydropower station, among which S6 is dam reservoir of Laohuzui Hydropower Station. It shows that the genus has strong adaptability to impoundment reservoirs. *Tychonema_CCAP_1459-11B* was found in relatively cold lakes and slightly eutrophic water bodies in Northern Europe (Komarek et al., 2005). The average relative abundance of sediments in Bahe river ranks the second (7.68%), and its relative abundance in tributary S2 is 36.60%, followed by that in main stream S6 and S1 (9.73% and 6.55%), respectively, may be affected by grazing and untreated domestic water in nearby villages. The relative abundance of the other four sampling points was less than 0.5%, which played a weak role.

The average relative abundance of *norank_f_unclassified_o__Oxyphotobacteria_Incertae_Sedis* in the sediment of Bahe cascade power station ranks third (3.43%), the

maximum abundance is 23.80% at S1, and the relative abundance of other 6 sampling points is less than 0.2%, which may be related to the low water flow velocity in this section. Hyphemicrobium has a good promotion effect on ammonia nitrogen removal (Zhao et al., 2018), which is also corresponding to the ammonia nitrogen removal effect of corresponding sampling points. The average relative abundance of 7 sediment samples from Bahe cascade hydropower station ranks the fourth (3.15%). The highest abundance is 8.94% at S7, followed by S5 (3.65%), S2 (3.56), S1 (2.29%) and S4 (2.77), and the lake abundance of the other two sampling points, S3 and S6, are all less than 0.5%. Leptolyngbya_FYG is an unclassified genus of cyanobacteria, widely distributed in oceans, freshwater, marshes and fields, with low biological sample size (Li et al., 2020). The average relative abundance of 7 samples from Bahe cascade power station ranks the sixth (2.41%), and the relative abundance in S6 (10.02%), S2 (2.65%) and S5 (2.60%) is higher. The relative abundances of the other four samples were all less than 1%. Phreatobacter (0.02~6.00%), Pseudarthrobacter (0.05~13.47%), Pseudorhodobacter (0.31~5.51%) and unclassified_f_Microbacteriaceae (0.07~4.92%).

The above results show that the composition of microbial population in the surface sediments of cascade hydropower stations is quite different under the effect of plateau environment. The unique microbial population structure and characteristics are caused by alpine and low-nutrient conditions, which are consistent with the analysis results of community microbial diversity.

Relationship between surface sediment microbial diversity and influencing factors

Correlation heatmap analysis

The correlation between the overlying water quality and physical and chemical indexes of Bahe cascade hydropower station as influencing factors and dominant bacteria at phylum and genus levels is shown in Figure 4.

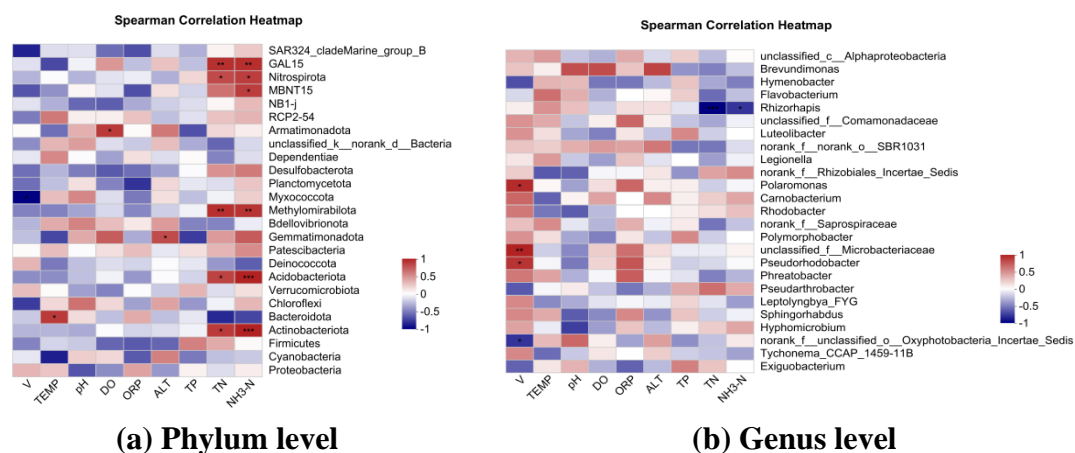


Figure 4. Heatmap of Spearman correlation between Bahe River environment and species. The X-axis and Y-axis are the influencing factors and species respectively, and the correlation R and p -values are obtained by calculation. R values are displayed in different colors in the figure. If p is less than 0.05, it is marked with*. The legend on the right is the color interval of different R values. The cluster tree of species and influencing factors can be selected (such as the left side and the upper side), * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$

According to the correlation heat map, as shown in *Figure 4a*, it is found that at the phylum level (the top 25 dominant bacteria), there is Gemmatimonadota with significant positive correlation with altitude, possibly due to the absence of phylum significantly correlated with PH, ORP and TP under high-altitude aerobic conditions (Zong et al., 2022). Bacteroides with significant positive correlation with temperature, Armatimonadota with significant positive correlation with DO, Myxococota with significant negative correlation with V, Actinobacteriota, Acidobacteriota, Methylomirabilota, Nitrospirota and GAL15 with NH₃-N and TN have significant positive correlation. In addition, MBNT15 was positively correlated with NH₃-N.

As shown in *Figure 4b* by correlation heat map, it is found that at genus level, norank_f__unclassified_o__Oxypotobacteria_Incertae_Sedis shows significant negative correlation with V, and unclassified_f__Microbacteriaceae, Pseudorhodobacter, Polaromonas. Rhizorhapis shows significant negative correlation with NH₃-N and TN.

These results indicate that the river surface sediments and the influencing factors have a certain effect on microbial flora in the plateau habitat, and the effects of NH₃-N and TN are significant, which is closely related to the poor nutrition in the high-altitude and low-pressure habitats. The high relative abundance of Actinobacteriota shows a significant positive correlation with NH₃-N and TN, indicating that the higher the NH₃-N and TN is, the higher the abundance is, which was related to that the bacteria contained a large number of nitrogen-fixing bacteria. Bacteroidota has a significant correlation with TEMP. Based on the analysis of the influence of nine influencing factors such as COD and TP on bacteria, it can be found that V, NH₃-N and TN had significant positive correlation on 5 of the first 25 dominant species, which had a very important effects on bacteria. In general, cascade power plants have been shown to have a cumulative effect on nutrients NH₃-N and TN, which in turn further influence microbial community composition.

Redundancy analysis of microbial community and influencing factors

The results of redundancy analysis (RDA) between microbial community and influencing factors in surface sediments of Bahe cascade hydropower station are shown in *Figure 5*. The results of analysis at the level of phylum classification show that factors such as V, ALT, TP, TEMP, TP and DO can explain 86.11% of the variation of sediment bacterial community, and the interpretation degree is higher than that of Fuhe River (Wang et al., 2023) and Jialing River (Wang et al., 2018). The samples of S4, S5 and S7, S3 and S6, S1 and S2 are similar. TP, V, TEMP, ALT and other influencing factors have great impact on the sediment microbial community in the cascade hydropower stations of Bahe river.

For the four dominant strains in *Figure 5a*, Firmicutes, as an autotrophic bacteria degrading organic matter, is positively correlated to TN, TEMP and TP, which is related to the fact that the growth of bacteria requires a large amount of nitrogen and phosphorus to form cells and the whole process is endothermic reaction, which is negatively related to ALT, V, DO, etc. This maybe mainly related to the lower flow velocity, the easier it gets to obtain energy and the inverse ratio of DO to the concentration of nutrients. Due to the cascade effect, the lower TP and TN in the water, the higher the corresponding value in the sediment, resulting in the higher abundance of Cyanobacteria in the sediment, and the higher the Cyanobacteria entering the water under the action of water flow.

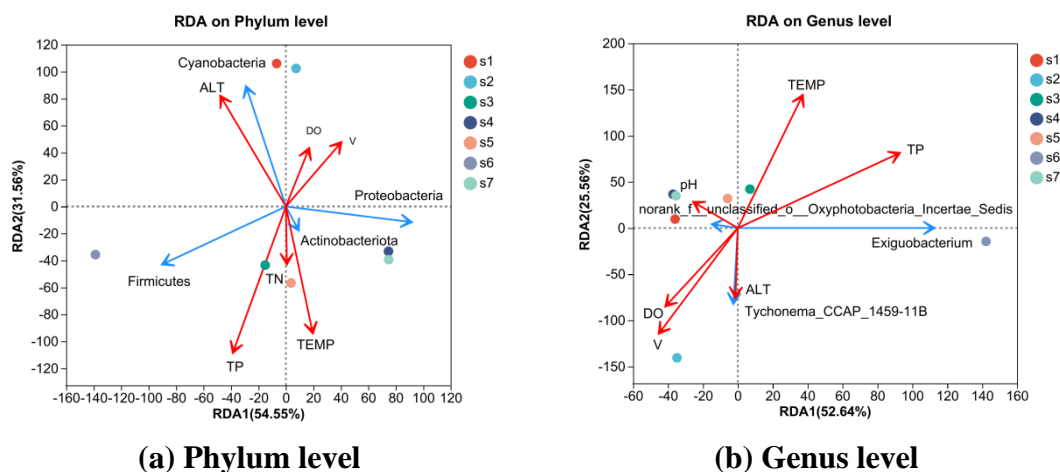


Figure 5. Redundancy analysis of microbial community and influencing factors of surface sediment sample

Finally, Cyanobacteria is negatively correlated with TP and TN. In addition, the higher the flow velocity, the more likely the microorganism will emerge from the water surface and the faster the reoxygenation of the water body will be. Meanwhile, the water temperature surface of plateau rivers is higher than that of the lower layer, and the flow velocity will aggravate the water heat exchange. Proteobacteria is positively correlated with DO, TEMP, V, TN, and TP and ALT. As the main denitrification bacteria, Proteobacteria is positively correlated with TN. However, in view of the carbon nutrition competition between TP and TN, Proteobacteria has a negative correlation with TP. In the low nutrient state, the increase of V increased the probability of Proteobacteria obtaining nutrients. Meanwhile, the denitrification of Proteobacteria under the low nutrient state was less affected by DO, but the increase of V resulted in the increase of DO. The result showed that DO was proportional to Proteobacteria. The decrease of ALT was conducive to the increase of water temperature and the occurrence of denitrification, indicating that the high abundance of Proteobacteria was correlated with high nutrient availability (Fierer et al., 2007). Actinobacteria, which degrades organic matter, is positively correlated with TP, TEMP and TN, and negatively correlated with DO, V and ALT, etc. The reason is that organic matter degradation requires heat absorption and consumption of TP and TN (which is consistent with the results of heat map analysis). The degradation of organic matter by Actinobacteria is easily disturbed by the flow rate, so it is negatively correlated with V and DO. On the whole, ALT was positively correlated with Cyanobacteria and negatively correlated with Proteobacteria, Actinobacteria and Firmicutes, indicating that ALT affected the composition of the microbial community.

According to *Figure 5b* of the analysis results under genus classification level, the influencing factors can explain 78.2% of the change of bacterial community in sediments. The samples of S1, S3, S4 and S5 in sediments are similar, and there are no similar samples of S2, S6 and S7, environmental factors such as TEMP, TP, V and DO in sediments have a great influence on microbial community of surface sediments of cascade hydropower stations of Bahe river. According to the analysis of three dominant genera in the figure, *Exiguobacterium* is positively correlated with TP, TEMP, and negatively correlated with ALT, pH, HE, DO, V. *Tychonema_CCAP_1459-11B* is

positively correlated with V, DO, ALT, and negatively correlated with pH, TEMP and TP, *norank_f_unclassified_o_Oxypotobacteria_Incertae_Sedis* is positively correlated with pH, DO, V, and negatively correlated with HEI, TEMP and TP. The redundant analysis of influencing factors shows that the clustering effect of S4 and S5 at phylum and genus level is obvious. The influencing factors that have the greatest influence on the sample microorganisms were velocity and temperature, and the influence of each influencing factor on microorganisms was different to some extent.

The results of redundancy analysis showed that TP, TEMP and V were the main factors affecting the microbial community structure, which was different from the results of correlation heat map analysis, such as V, NH₃-N and TN. It was probably related to that NH₃-N and TN did not affect the higher abundance microorganisms. In addition, the analysis shows that the microbial community structure is influenced by cascade effect.

Prediction of microbial function of surface sediment in Bahe cascade hydropower station

Microbial community functional proteins and their changes

To analyze the metabolic characteristics of microbial communities, we predicted the metabolic characteristics of microbial communities by PICRUSt (Langille et al., 2013). About 80% of the genes are involved in metabolism, genetic information processing and environmental information processing (*Fig. 6*).

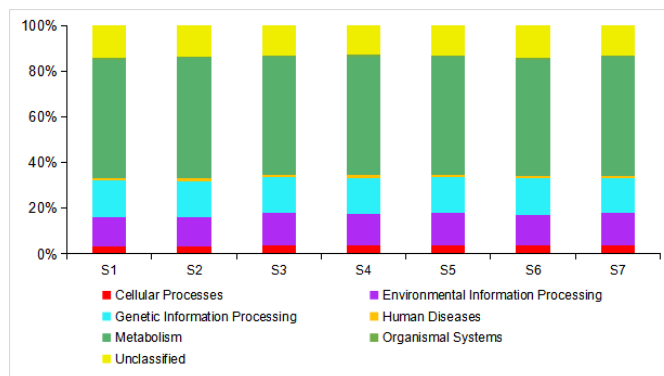


Figure 6. Metabolic characteristics of Bahe cascade hydropower station

PICRUSt program is widely used to predict the metabolic characteristics of microbial communities (Li et al., 2018), and the results show that the construction of hydropower station significantly changes the functional genes of microbial community of hydropower station. Taking into account the predicted changes in metabolic characteristics at the DNA level, these results suggest that communities with different functions in the water microbial community have been replaced after the construction of the hydropower station (Chen et al., 2020).

In *Figure 7*, according to PICRUSt function prediction based on COG database, there are significant differences ($p_v < 0.05$) in functional gene composition of S1~S7 at 7 sampling points as shown in the figure, wherein, there is no significant correlation between Extracellular structures and Cytoskeleton and other functional genes, and little significant correlation between Lipid transport and metabolism and Transcription and

other functional genes (the former has seven significantly related functional genes. the latter having only four significantly related functional genes). The highest relative abundance of S with unknown function was 10.23% and highest in 7 samples, S6 and S2 respectively. S1, S4, S5, S7, S3, 10.98%, 10.94%, 10.86%, 9.84%, 9.79%, 9.79%, 9.39%, respectively. The lowest abundance factor is that the highest abundance of extracellular structure W is 0.00009%, which is almost negligible. E and F, which represent the function of nitrogen metabolism, can show that cascade effect is formed between S1, S3 and S4, and the cascade effect in S6 and S7 shows the disappearance of cascade effect. C, which represents energy, and G, which represents carbon, also show the same sign.

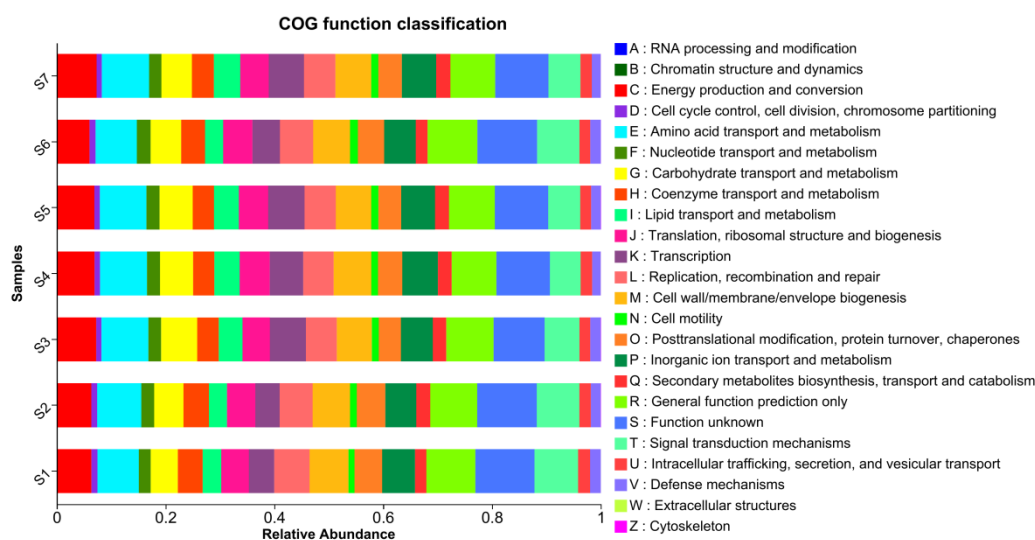


Figure 7. PICRUSt function prediction

In Bahe river, the average relative abundance of COG is ranked in the first seven places (S, R, E, T, M, C, P). Except that the function of S is unknown, the rest are R (general function prediction), E (amino acid transport and metabolism), T (signal transduction mechanism), M (biosynthesis of cell wall, cell membrane and membrane structure), C (energy generation and conversion), P (inorganic ion transport and metabolism), synthesis of these functional proteins and small cell proteins. Energy consumption and signal transmission of functional protein. Secondly, functional proteins such as L type (copy, reconstruction and repair), K type (transcription), O type (post-translational modification), H type (coenzyme), I type (mass transport and metabolism), Q type (secondary metabolite biosynthesis, transportation and catabolism), U type (intracellular synthesis) function proteins are closely related to life activities such as microbial growth and reproduction, i.e. large molecular functional proteins are mainly synthesized by gene transcription at the cell level. which provides favorable preconditions for the efficient execution of the vital activity of the cells. The higher the average relative abundance of the above-mentioned COG functional classes indicates that they play a greater role in the growth and reproduction of microbial cells in high protoplasm. The dominant COG functional classifications are similar to those in the sub-highlands, but the W, Z, A and B COG functional classifications related to microbial growth and signal transduction are inhibited in the plateau.

There are 4309, 4307, 4248, 4297, 4360, 4246, 4356 functional proteins in S1~S7, and the sequence is S5, S7, S1, S2, S4, S3, S6. In *Figure 8*, according to COG family information, the top 10 COGs of 7 samples were collected to obtain 18 kinds of COG functional proteins, in which 5 functional proteins of G0642, G1028, G0438, G0745, G0596 were dominant functional proteins in 7 sampling points, which indicated that these COG functional proteins are important for microbial cell activity. It is an important function of all cellular activities and responses. At the same time, the above functional proteins also showed the cascade effect in S1, S3 and S3, but the cascade effect disappeared after S6 and S7. COG2814 is a membrane transporter and a dominant functional protein at and below the downstream of 606 Hydropower Station. COG0583 and COG1309 functional proteins are transcription regulatory factors and are dominant functional proteins at S3, S4, S5 and S7. COG2226 functional protein is methyltransferase required for conversion of dimethyl menadione (DMKH2) to menadione (MKH2), and S1, S2, S6 and S7 are dominant functional proteins. COG0515, COG1357, COG4636 are located at S1 and S2, S6 is the dominant functional protein. G2197 was the dominant functional protein at S3 and S5. G1595 is a dominant functional protein at S3, G0463 is a dominant functional protein at S1, G1960 is a dominant functional protein at S4, S5, S7, G1629 is a dominant functional protein at S4, and G4995 is a dominant functional protein at S2.

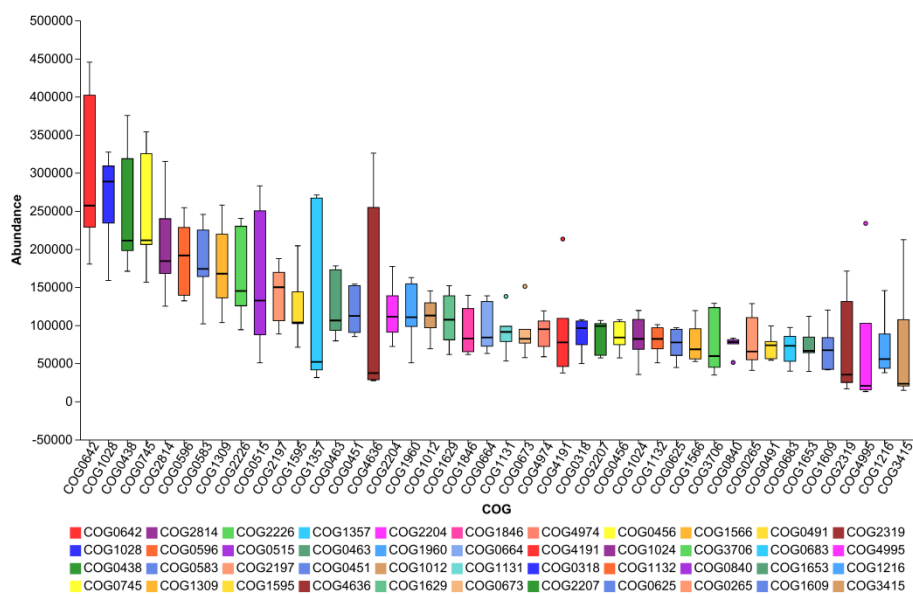


Figure 8. Box diagram of COG sample abundance statistics

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

In *Figure 9*, according to the enzyme information corresponding to the OTU, the abundance of the 7 sampling points were ranked as S2, S7, S4, S3, S5. S1. S6 plots the functional Heatmap for the enzymes with the top 30 abundances under the 7 sampling points. Seven enzymes, including DNA-directed DNA polymerase (2.7.7.7) were dominant at all sampling sites. The proportion of DNA-directed DNA polymerase was 1.214%, 1.200%, 1.174%, 1.140%, 1.087%, 1.031%, 1.024%, respectively. The

percentages of the seven sampling points were 1.214%, 1.200%, 1.174%, 1.140%, 1.087%, 1.031% and 1.024% for S7, S4, S5, S3, S1, S6 and S2, respectively, in descending order. Only 2.7.11.1 showed an increasing trend at S6, and the other 9 enzymes showed a decreasing trend at S6, which mainly showed the failure of cascade effect.

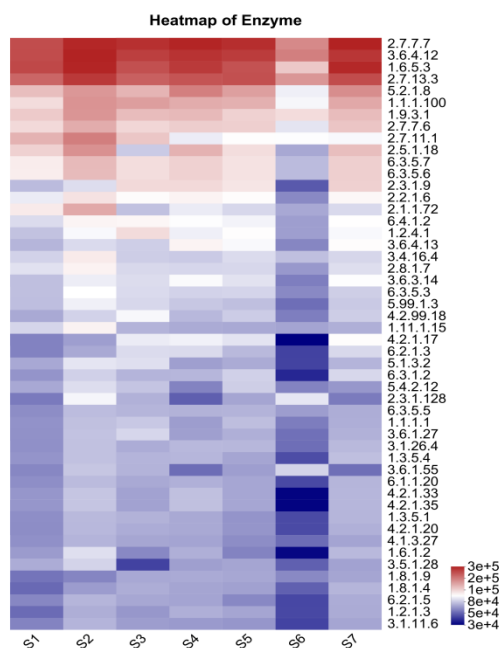


Figure 9. Function heatmap diagram. The abscissa is the name of the sample and the ordinate is the enzyme number. The change of different functional abundance in the sample is shown by the color gradient of the color block. The legend is the value represented by the color gradient

DNA helicase (3.6.4.12) deoxyribonucleic acid helicase, which is involved in ATP hydrolysis, releases ADP, phosphate and energy. DNA helicase uses the energy of ATP hydrolysis to release double-stranded DNA. S4, S1, S6, S7, S5, S3, S2 were 1.110%, 1.094%, 1.089%, 1.087%, 1.086%, 1.052%, 1.046%, respectively. ATP phosphohydrolase (DNA helix expansion) acts on anhydrides to promote cellular and subcellular motility (not 3.6.4.12 in KEGG, but 3.6.4.13 RNA helicase). Genes for carbohydrate and lipid metabolism and atp-related enzymes (enzymes 2.7.13.3 and 3.6.4.12), with sufficient oxygen and aerobic respiration, result in more active carbohydrate and lipid metabolism, with higher 3.6.4.12 leading to faster microbial reproduction. Enzyme 2.7.7.7 is involved in DNA replication, enzyme 2.7.13.3 catalyzes the conversion of ATP to ADP and protein l-histidine to protein n-phosphate-l-histidine, thereby releasing the reaction energy (Liu et al., 2022).

1.6.5.3 (NADH: ubiquinone reductase (H (+)-translocating), a flavonoid protein containing iron-sulfur clusters, present in mitochondria and aerobic bacteria, catalyses translocation of hydrates (transfer of electrons from NADH to ubiquinone) and is fully oxygen active). The percentage of S7, S1, S4, S2, S5, S3, S6 were 1.174%, 1.115%, 1.069%, 1.034%, 0.961%, 0.954% and 0.727%, respectively. Histidine kinase (2.7.13.3) belongs to transferase and participates in the metabolism of bacterial bi-component system. S5, S7, S6, S1, S2, S4, S3 were 0.975%, 0.961%, 0.957%, 0.943%, 0.928%,

0.922% and 0.873%. 5.2.1.8 (Peptidylprolyl isomerase) in S4, S7, S5, S1, S2, S3 and S6 were 0.728%, 0.674%, 0.630%, 0.582%, 0.551%, 0.551%, 0.496%, respectively, 5.2.1.8 is an enzyme that catalyzes the cis/trans isomerization of a peptidyl group and a partially folded peptide bond (Dimou et al., 2017; Thomloui et al., 2017).

1.1.1.100 (3-oxoacyl-[acyl-carrier-protein] reductase) 3-ketoacyl-[acyl-carrier-protein] reductase with 0.622%, 0.580%, 0.574%, 0.570%, 0.562%, 0.562%, 0.493% at the seven sampling sites in descending order of S3, S7, S5, S4, S2, S6, and S1, respectively. It is a component of fatty acid synthase type II (Klein et al., 1992), which is a key enzyme in fatty acid biosynthesis and performs the first reduction step in the elongation cycle. The gene, known as *fabG*, was cloned for functional expression in *E. coli* and subsequently purified to homology. The enzyme shows an optimum temperature at 44°C and reaches a wide pH optimum between pH 7 and pH 9 (Holsch et al., 2008).

1.9.3.1 (Cytochrome-c oxidase), the percentage of S6, S2, S1, S4, S3, S7, S5 were 0.652%, 0.556%, 0.543%, 0.530%, 0.527%, 0.519%, 0.477%, respectively. Cytochrome c oxidase (EC 1.9.3.1) is one of the most important enzymes in nature. It participates in the terminal oxidation step of energy metabolism enzymes, catalyzing the four-electron reduction of oxygen to water in the last step of cell respiration (Stevens et al., 1979). This protein is ubiquitous in all aerobic cells. In prokaryotes, cytochrome c oxidase is a plasma membrane protein. In eukaryotes, the enzyme is localized to the inner mitochondrial membrane. In both cases, it is inserted into the lipid bilayer and released only by detergent treatment (Capaldi et al., 1983).

FAPROTAX function prediction

FAPROTAX is an artificially constructed database that maps prokaryotic taxa (if any) to metabolic or other ecologically relevant functions (e. g., nitrification, denitrification). The functions of FAPROTAX are concentrated in marine and lacustrine biogeochemistry, especially the sulfur, nitrogen, hydrogen and carbon cycles. In *Figure 10*, Faprotax provides a functional prediction of bacterial data. 67 functional groups of Bahe surface sediments were determined by FAPROTAX method. The dominant groups in Bahe river in winter were related to chemoheterotrophy heterotrophy, phototrophy, aerobic_chemoheterotrophy, photoautotrophy, oxygen autotrophy and cyanobacteria.

Chemoheterotrophic chemical heterotrophic aerobic_chemoheterotrophy These functions are usually associated with bacteria as decomposers and contribute to in situ repair and organic matter recovery in all ecosystems (Peng et al., 2018; Kampfer et al., 1993), and bacteria in winter surface sediments tend to be chemically heterotrophic. Phototrophy phototrophy, photoautotrophy autotrophy oxygenetic_photoautotrophy and cyanobacteria maybe related to long sunshine duration. The top ten functional groups changed in the sections. Among them, chemotrophy, oxygenic_photoautotrophy, aerobic_chemoheterotrophy, meeting, animal_parasites_or_symbionts and human_pathogens_all showed an increasing trend in S3 and S4, which may be related to the fact that the DO in this section is too large to favor the chemical reaction. Phototrophy, photoautotrophy and cyanobacteria showed a decreasing trend in S3 and S4, and continued to decrease after the increase in S6 and S7. This may be related to the trend of decrease in S3. S4 resulted in enrichment and after S6 the water quality was correlated with S7 after rapid degradation of lean nutrients. Functional prediction shows that cascade effect exists between S3 and S4, while cascade effect in S6 and S7 fails.

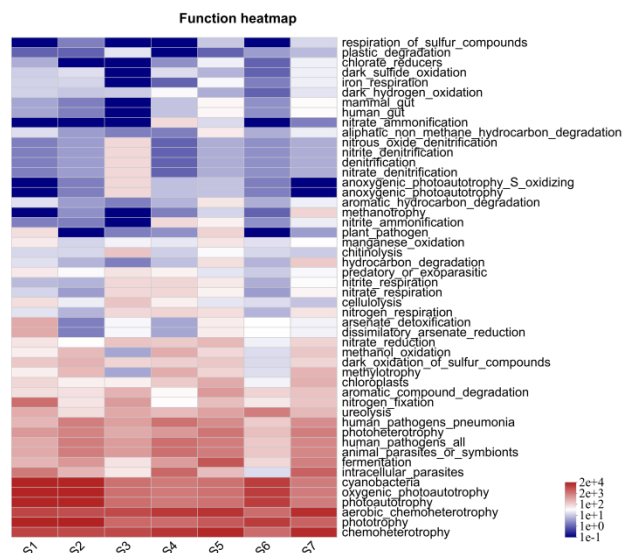


Figure 10. FAPROTAX Function heatmap. The abscissa refers to the name of the sample or group, and the ordinate refers to the name of the function. The variation of abundance of different functions in the sample is displayed through the color gradient of the color patch. The value represented by the color gradient is on the right side of the figure

Conclusion

The research carried out water quality physical and chemical index monitoring and 16S rRNA gene sequencing of 7 sections in dry season on Bahe river of cascade 3 hydropower station in dry season, and carried out analysis on water quality physical and chemical indexes, diversity of microbial community, dominant microbial community, redundant analysis of influencing factors, functional protein, functional gene, dominant enzyme, FAPROTAX function prediction, etc. The results show that:

(1) Monitoring results of physical and chemical indexes of water quality show that Bahe river is a class II standard of surface water, which is carried out through the Environmental Quality Standards for Surface Water (GB3838-2002), and there is a cascade effect (accumulation effect) of TN and TP between sampling sections.

(2) The results of microbial community diversity analysis show that the diversity and richness of the communities in the plateau river are lower than those in other areas, and the species number and richness in the plateau are lower than those in other areas. The diversity of the river is influenced by the construction of the power station, which is mainly represented by the cascade effect of S3 and S4.

(3) According to the analysis of dominant microorganisms in sediments, the dominant bacteria at the gate level were Proteobacteria, Cyanobacteria, Frimicutes, Actinobacteria, Bacteroides, Exiguobacterium, Tychonema_CCAP_1459-11B, norank_f_unclassified_o_Oxypotobacteria_Incertae_Sedis, Hyphomicrobium, Sphingorhabdus, Leptolyngbya_FYG, Phytobacter, Pseudarthrobacter, Pseudoarthrobacter, Unclassified_f_Microbacteriaceae, and the changes of 7 sections were significant. The effects of high cold and low nutrition were significant.

(4) Analysis of microbial diversity and influencing factors showed that TP, TEMP and V were the main factors influencing microbial community structure in plateau river. Further study found that cascade effect also affected microbial community structure.

(5) Functional gene, functional protein, dominant enzyme and FAPROTAX function prediction again prove that the construction of the power station has influence on the above indexes, and cascade effect will occur in S3 and S4, while cascade effect in S6 and S7 will fail.

By means of water quality physical and chemical index monitoring and 16S rRNA gene sequencing, the influence of hydropower station construction on Bahe river is analyzed. It is also the first time that diversity, dominant microorganism and biodiversity are applied to cascade effect analysis of river in plateau area, which provides a brand-new evaluation technical means for river hydropower development.

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Conflict of interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval. All activities were performed according to Chinese laws and regulations. No ethical conflicts exist in the realization of this work.

Consent for publication. All authors have reviewed and approved the present version to be published.

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