BACTERIAL COMMUNITY STRUCTURE AND ITS INFLUENCING FACTORS IN SURFACE SEDIMENTS OF THE NYANG RIVER IN THE DRY SEASON, CHINA

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Abstract. The Nyang River is located in an area sensitive to global climate change, the climate warming changes and human activities have impacted the fragile ecosystem of Plateau Rivers, for which we analyzed the bacterial diversity, community structure characteristics and main influencing factors in the surface sediments of 7 sampling sites in the middle and lower of the Nyang River in Tibet which is in China by high-throughput sequencing technology. The study showed that the abundance of bacterial community in the surface sediments of the middle and lower reaches of the Nyang River that is S1 (upstream of the junction of Bahe Town), S2 (Bahe River), S3 (downstream of the junction of Bahe Town), S4 (upstream of Duobu Hydropower Station), S5 (downstream of Duobu Hydropower Station), S6 (upstream of Bayi Town), S7 (downstream of Bayi Town). The abundance of bacterial community and bacterial diversity at S2 site are relatively high, and the bacterial diversity and abundance of the 7 sampling sites are lower than those of other areas. Five dominant bacteria phyla, including Firmicutes, Proteobacteria, Cyanobacteria, Actinobacteria, Bacteroidetes, and seven dominant bacteria genera, including Exiguobacterium, Hymenobacter, Hyphomicrobium, Leptolyngbya_FYG, Phormidesmis_ANT. L52.6, Tychonema_CCAP_1459-11B were extracted from the surface sediments of the middle and lower reaches of the Nyang River in the dry season. There were significant differences in the dominant bacteria among the samples. Further study found that the influencing factors were flow velocity, environmental factors and nutrients in descending order.

Keywords: *high-throughput sequencing technology, species composition, univariate correlation analysis, redundancy analysis*

Introduction

The Qinghai-Tibet Plateau, known as the third pole in the world, is the birthplace of many great rivers. As the main body of the Qinghai-Tibet Plateau, Tibet, with an average altitude of about 4, 200 meters, is one of the provinces with the largest rivers in China, with a drainage area of 1, 202, 370 km^2. As one of the most environmentally sensitive cold regions in the world, Tibet is characterized by high altitude and cold, low atmospheric pressure, high solar radiation and other unique climatic characteristics and underlying surface conditions.

The rivers in Tibet are characterized by low temperature, low nutrients, strong solar radiation (Zhang et al., 2019) and ecological fragility, and extreme habitats have created

unique bacterial communities (Ling et al., 2022). As the climate warming changes the evaporation and precipitation in Tibet, which causes glacier retreat and permafrost degradation, which on the high altitude environment (Lu et al., 2010) and completely changes the transport routes of river runoff and sediment (Wang et al., 2020). In addition, human activities such as water diversion, dam construction and sewage discharge have disturbed the fragile ecosystem of Plateau Rivers, affecting the river ecosystem and its connectivity t (Wang et al., 2017), resulting in the fragmentation of river habitat (Jiang et al., 2012) and profound influence on river sediments. At the same time, microbial communities have different responses to climate-driven and anthropogenic environmental changes in aquatic ecosystems (Tang et al., 2021), and specific microbial community structures are usually formed in response to various environmental pressures (Gao et al., 2019).

On the other hand, microorganisms regulate global and local biogeochemical processes, fundamentally affect greenhouse gas emissions in the atmosphere and participate in the formation of regional climate, geochemical cycles and biological evolution (Timmis et al., 2020., Fuhrman, et al., 2009). Microbial community plays an important role in the biogeochemical flux and nutrient cycle of river ecosystems (Wang et al., 2022), and the structure and abundance of microbial community affect the ecological environment (Zhang et al., 2021). In the river ecological environment, the sediments are not only the habitats for benthos, but also the places where microorganisms are concentrated during the ecological process of rivers. The number of microorganisms is much higher than that of the overlying water, about 2~1000 times (Luo et al., 2019). In addition, the bacterial community in the sediment of the glacier-fed river contributes more than 75% to the bacterial diversity of aquatic system (Zhang et al., 2021; Papale et al., 2020), Therefore, it is very important to reveal the bacterial community structure of sediments, analyse the influencing factors bacterial community and monitor their changes for the study of plateau river environment and ecology.

The formation process of Yarlung Zangbo River occurs simultaneously with the uplift of Qinghai-Tibet Plateau and the river has unique characteristics in terms of morphology and sediment (Wang et al., 2015). The Nyang River is the largest tributary of Yarlung Zangbo River and an important water source of the Nyang River Basin. It is located in the southeast of the Tibet Autonomous Region, originating from Cuomuguola Glacier Lake in the west of Gongbojiangda County at the southern foot of Nyainqentanglha. It flows into the Yarlung Zangbo River near Geze Village, Bayi Town, Linzhi City, with a total length of 286 km, a total drop of 2, 080 m and a drainage area of 17, 800 km^2. River systems play a key role in the natural water environment and the transport of nutrients. Human activities, including wastewater discharge and river damming, have adverse effects on the ecosystems and river continuums (Wang et al., 2017). Under its special alpine environment, the bacterial diversity of Nyang river is more susceptible to the influence of evapotranspiration, altitude and annual average temperature (Zhang et al., 2021). Meanwhile, with the development of cities as well as the exploitation and utilization of water resources, the dam construction and the increase of human activities in the Nyang River Basin will inevitably affect the microbial community structure in river sediments (Liu et al., 2020).

It has been shown that altitude is the most important factor affecting the change of microbial community (Li et al., 2022, 2021). There are obvious differences between bacterial communities in plain and plateau areas (Xu et al., 2021), for example, the attenuation relation between altitude and distance of antibiotic resistance genes (ARGs)

is observed along the Yarlung Zangbo River (Wang et al., 2020), and the genetic system types of denitrification genes in high altitude rivers have high specificity (Zhang et al., 2021), altitude is the strongest factor affecting comammox bacteria in Qinghai-Tibet Plateau (Shi et al., 2020), geographical isolation and physical and chemical differences lead to differences in the composition of sulfur-oxidizing bacteria communities among hydrothermal sediment samples in Tibet (Zhen et al., 2019). The construction of reservoirs may affect the downstream geochemical environment and ecosystems (Zhao et al., 2020), such as the concentration of microorganisms in sediments (Luo et al., 2019). Relevant scholars have carried out studies on unknown strains in high altitude rivers (Jiao et al., 2022), and the microbial community containing gene plays an important role in nitrogen removal and nitrous oxide mitigation in in high-altitude river sediments (Guo et al., 2022). In addition, high altitude rivers are suffering from microplastic pollution (Liu et al., 2021; Jiang et al, 2019), and bacterial communities play an important role in maintaining glacier-fed aquatic ecosystems (Zhang et al., 2021). Furthermore, the bacterial community structures are great differences for the dry and wet season, meanwhile, within-river spatial differences in river bacterial diversity were more commonly (Hiruy et al., 2022; Chiaramonte et al., 2013; Myrstener et al., 2021; Reza et al., 2018).

Based on this, the bacterial diversity, community structure types and influencing factors in surface sediments of the middle and lower reaches of the Nyang River in the dry season were analyzed by Illumina High throughput sequencing technology in this paper, so as to explore the influence of environmental pressure sources, understand the composition and dominant groups of microbiological communities in sedimentary environment, discuss its response mechanism to plateau habitats, and provide scientific basis for plateau river ecological environment management and the utilization of microbial resources.

Materials and methods

Sample collection

Taking the middle and lower reaches of the Nyang River as the research area, 7 sampling sites in Bahe Town, Duobu Hydropower Station and Bayi Town in China were selected according to the influence degree of human activities on the Nyang River, mainly considering the dam construction and the pollutant discharge factors of settlement site (*Fig. 1*) S1 (29°51′33″N, 93°38′59″E), S2 (29°51′12″N, 93°39′53″E), S3 (29°50′58″N, 93°40′3″E), S4 (29°44′33″N, 94°3′19″E), S5 (29°45′15″N, 94°12′25″E), S6 (29°42′37″N, 94°18′47″E) and S7 (29°35′27″N, 94°24′11″E).

The sampling time was late November 2020. Text with references to tables (*Table 1*). At the sample collection site, 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. Pondus Hydrogenii (pH), temperature (TEMP), oxidation reduction potential (ORP), electrical conductivity (EC) and dissolved oxygen (DO) were measured by portable water quality analyzer (HACH HQ4od, www.hach.com.cn). At the same time, the water samples were collected by the Multi-parameter Water Quality Determinator (6B-3000A, Jiangsu Shengaohua Environmental Protection Technology CO., LTD, www.sahkjcod.com), and the measurements of Chemical Oxygen Demand (COD), Total Nitrogen (TN), Total

Phosphorus (TP), NH3-N and NO2-N. These items were selected according to relevant literature (Guo et al., 2022) and carried out through the Environmental Quality Standards for Surface Water (GB3838-2002) and the Water and Wastewater Analysis Method. The surface sediments which are approximately 3 cm depth, 0.5m from the river bank, shall be collected by grab-type bottom sampler, put into the collection bag and transported back to the laboratory for storage at -80 °C for measuring. See *Table 1* for the water quality index of overlying water of 7 samples.
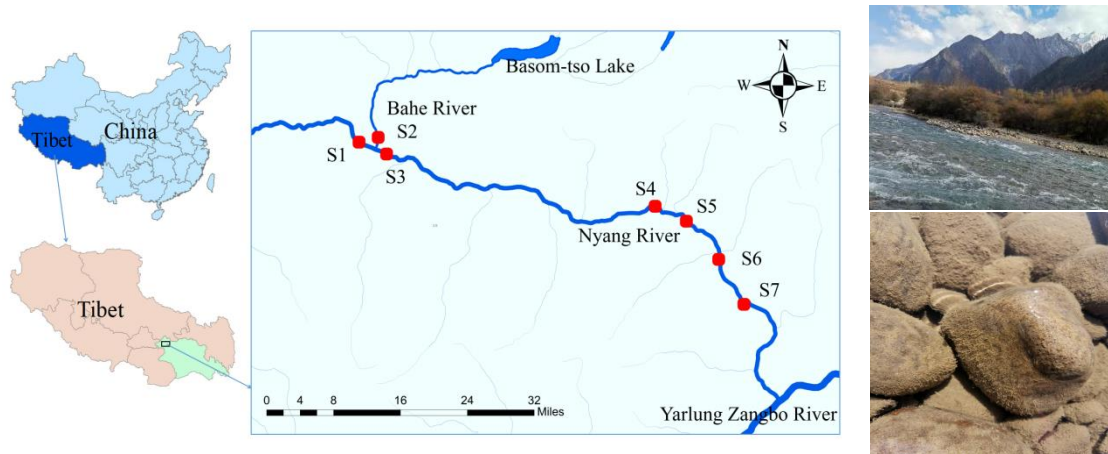
Figure 1. *Distribution diagram of sampling sites station in the middle and lower of the Nyang River*

Table 1. *Water quality index of overlying water*

Samples	V (m/s)	TEMP (°C)	pH	DO (mg/l)	ORP (mv)	EC (µs/cm)	ALT (m)
S1	1.040	5.6	8.20	8.24	146.60	151.60	3214.47
S2	0.392	7.4	7.53	7.96	139.60	111.15	3197.45
S3	0.645	7.8	7.88	7.80	155.30	110.60	3208.22
S4	1.074	7.3	7.75	7.99	139.05	123.90	3064.81
S5	0.919	8.0	8.02	7.78	160.20	119.65	3047.13
S6	0.583	7.0	7.90	8.08	135.20	119.70	2999.04
S7	0.225	8.5	8.25	7.83	146.40	110.20	2955.90

Altitude (ALT), oxidation reduction potential (ORP), electrical, flow velocity (V), conductivity (EC), dissolved oxygen (DO), temperature (TEMP), Pondus Hydrogenii (pH)

Methods

Total DNA extraction was performed according to the instructions of E. Z. N. A. ®soil kit (Omega Bio-tek, Norcross, GA, U.S.), DNA concentration and purity were measured by NanoDrop2000, and DNA extraction quality was detected by 1% agarose gel electrophoresis. Bacterial diversity was measured by PCR amplification of V3-V4 variable regions using primers of 338F (ACTCCTACGGGAGGGAGGAGCAG) and 806R (GGACTACHVG GGTWTCTAAT), and the functional gene nirS was measured by PCR amplification of V3-V4 variable regions using primers of cd3aF (GTSAACGTSAAGGARACSGG) and R3cdR (GASTTCGGRTGSGTCTTGA). The

specific amplification procedure was as follows, predegeneration at 95 °C for 3 min, 27 cycles (denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s) and final extension at 72 °C for 10 min (PCR instrument, ABI GeneAmp®9700). The amplification system was 20 ul, 4 ul 5*FastPfu buffer, 2 ul 2.5mM dNTPs, 0.8 ul primer (5 uM), 0.4 ul FastPfu polymerase, 10 ng DNA template.

The PCR products were recovered using 2% agarose gel, purified by AxyPrep DNA Gel Extraction Kit (AxygenBiosciences, Union City, CA, USA), eluted by Tris-HCl and detected by 2% agarose electrophoresis. Quantitation was performed using QuantiFluorTM-ST (Promega, USA). The purified amplified fragments were constructed in PE 2*300 library according to Illumina MiSeq platform (Illumina, SanDiego, USA) standard operating procedures.

Steps for library construction. (1) linking the "Y"-shaped linker. (2) removing the linker self-connected segments using magnetic bead screening. (3) enrichment of library templates by PCR amplification. (4) sodium hydroxide is denatured to produce single-stranded DNA segments. Sequencing was performed using the Miseq PE300 platform of Illumina (Shanghai Majorbio Bio-pharm Technology Co., Ltd.). The raw data is uploaded to the NCBI database.

The original sequencing sequence uses Trimmomatic software quality control, FLASH software for splicing and Usearch (version 7, http://drive5.com/uparse/))to divide all sequences into OTU. The functional gene databases of Silva database and Gene Bank were compared. Qiime (version 1.9.1) and RDP Classifier (version 2.2, http//source.net/projects/rdp-classifier) were used to analyze the biological information (domain, kingdom, phylum, class and order) of 97% similar OUT representative sequences in samples. The bacterial diversity index was calculated using mothur (version v. 1.30.1). Community column configuration use R (version 3.3.1) language tools for statistics and mapping. Spearman correlation and Heatmap plots used the R packages vegan and pheatmap.

Results and discussion

Water physicochemical indexes analysis

According to the physicochemical indexes of 7 sampling sites (*Table 2*) and surface water environment quality standard analysis, COD in all samples reaches Class I standard of surface water (CCOD ≤ 15 mg/L). The COD at S3 sampling site is smaller than the minimum COD of relevant rivers (Şener et al., 2017), but it is larger than other cross-sectional values, which may be correlated with the discharge of aquaculture wastewater from the adjacent plateau Tibetan pig breeding base. TP all reaches Class IV standard of surface water, TP of the Niyang River main stream had no significant change, the scour slowed down in the downstream water transport process after storage of the reservoir, TP of the overlying water slightly decreased, the phosphorus release in the river sediment was relatively slow or it may be enriched in the bottom mud, resulting in the decrease of TP at S5 and S6 sampling sites.

The concentration rises as it passes the Bayi area. TN of S4 reaches Class II standard of surface water (0.2 mg/L < CTN ≤ 0.5 mg/L), TN of S5, S6 and S7 reaches Class III standard of surface water (0.5 mg/L < CTN ≤ 1.0 mg/L), TN of the other three samples reaches the Class IV standard of surface water (1.0 mg/L < CTN ≤ 1.5 mg/L), which is lower than that of other rivers (Fan et al., 2022), among them, TN at the sampling sites of S1, S2 and S3 is higher than that of other sediments, because it is located in Bahe

Town and the pollution of surrounding villages leads to N, TN of S5 is high due to the outflow of TN from the reservoir, NH3-N of S1, S4 and S6 reached the Class II standard of surface water, and NH3-N of the other 4 samples reached the Class I standard of surface water. S3 was imported into the Bahe River and diluted to 0.074, and NOB of S4 and S7 ammonia oxidizing bacteria (whether changing) consumed oxygen, ammonia oxidation dissolved oxygen decreased, NH3-N decreased TN almost unchanged. DO of S6 has high dissolved oxygen, good nutrition, good carbon source, good denitrification effect, low TP, low TN, insufficient nutrition, low ace, low chao, may take value on the shore, the water surface is wide and relatively gentle, conducive to algae growth, compete for other microorganisms (nutrients), the main C source is COD, higher than S5/S4 upstream, inhibit other bacterial colonies.

Table 2. Water physicochemical data indexes

Samples	COD (mg/l)	TP (mg/l)	TN (mg/l)	NH3-N (mg/l)	NO2-N (mg/l)
S1	8.110	0.230	1.320	0.226	0.025
S2	3.710	0.220	1.010	0.007	0.017
S3	10.220	0.270	1.310	0.074	0.027
S4	3.430	0.260	0.490	0.176	0.011
S5	4.810	0.210	0.901	0.008	0.014
S6	5.520	0.200	0.502	0.189	0.026
S7	6.430	0.250	0.501	0.005	0.028

Chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP)

On the whole, the surface water environmental factors (NH3-N, TN, TP) in the middle and lower reaches of the Nyang River differ in spatial scale, and the water quality index is good, and TP in some sections of surface water is slightly higher.

Sediment bacterial diversity analysis

In community ecology, Alpha diversity refers to the diversity in a specific area or ecosystem, which mainly reflects the number of species in the bacterial community, and can be measured by common measures metrics, such as the observed richness (sobs), the Chao 1 estimator (chao), The ACE estimator (ace), the shannon diversity index (shannon) (Hauptmann et al., 2016), the Good's coverage (coverage), a simpson index-based measure of evenness (shannoneven) and a shannon index-based measure of evenness (simpsoneven) to estimate the abundance and diversity of community species in this environment. The diversity data of surficial sediment samples from 7 sampling sites are shown in *Table 3*.

As shown in *Table 3*, the coverage index of 7 sampling sites was all greater than 0.99, indicating that the test results could fully reflect the real situation of microorganisms in the samples. The chao and ace indexes can reflect the abundance of the community, in which the ace ranges from 662.3918 to 1626.3954, and the chao index ranges from 648.0137 to 1614.3476, which were smaller than those obtained in the existing literature (Sun et al., 2021; Liu et al., 2016; Yang, et al., 2018), the smaller the values, the lower the abundance of the bacterial community, which may be related to the low temperature and low nutrition status of the plateau rivers. The abundance of bacterial community of 7 sampling sites was S2, S3, S1, S7, S4, S5 and S6 in order,

among which the abundance of S2 sampling sites may be related to Basom-tso lake in the upper reaches, and relevant studies need to be carried out. The community abundance of S2 and S3 showed a good correlation with the nutrition at the sampling sites, while the community abundance of S7 was correlated with the sewage discharge from the urban sewage treatment plant upstream of the sampling site.

Table 3. *The index of archaeal diversity*

Samples	Shannon	Ace	Chao	Coverage	Shannoneven	Simpsoneven
S1	4.841	1609.259	1570.711	0.993	0.670	0.020
S2	4.888	1626.395	1614.348	0.995	0.673	0.016
S3	3.608	1620.719	1582.000	0.993	0.502	0.005
S4	2.986	1457.531	1483.324	0.991	0.422	0.003
S5	3.427	1240.184	1189.141	0.994	0.506	0.008
S6	3.107	662.392	648.014	0.997	0.500	0.014
S7	4.450	1487.830	1465.440	0.992	0.623	0.013

The community evenness index (shannoneven) and simpsoneven index are between 0.422~0.672 and 0.003~0.020 respectively. The shannon index can be used for community diversity, which ranges from 2.9864 to 4.8877, indicating that the bacterial diversity of 7 sampling sites decreases due to the influence of altitude, and the ordering results were S2, S1, S7, S3, S5, S6 and S4 in order from largest to smallest, among which the diversity of S2 and S7 was related to the injection of extrinsic microorganism, and the diversity of S1 and S3 as well as the pollutants at the corresponding sampling sites are high. The above analysis showed that the community diversity and abundance of Plateau Rivers were lower than those in other regions.

### *Bacterial community structures and types of sediment samples*

There are 2222 OTUs belonging to 28 phyla, 76 classes, 200 orders, 352 families and 642 genera in the surface sediments of 7 sampling sites in this study. It can be seen that the surface sediments in the middle and lower reaches of the Nyang River are rich in bacterial diversity, and the bacterial species in the special habitats of the plateau are lower than those in the previous literatures (Fan et al., 2022; Yang et a., 2018; Wang et al., 2020).

Analysis of species composition at phylum level

It can be seen from *Figure 2* that the community structure of bacterial communities in surficial sediment samples from different sampling sites in the Nyang River was at phylum level. Among the 7 samples, there were 12 bacterial phyla with relative abundance greater than 1%, which were regarded as dominant bacterial phyla, and other bacteria phyla were classified as others uniformly, as shown in *Figure 2*. The sum of the total relative abundances of these 12 dominant bacterial phyla exceeded 99%, which was sufficiently representative and persuasive.

Firmicutes (0.17%~66.30%), Proteobacteria (10.04%~48.19%), Cyanobacteria (1.55%~74.97%), Actinobacteria (0.97%~24.83%) and Bacteroidetes (1.35%~17.07%) were the top five highest relative abundances in *Figure 2*. The top five relative abundances were 90.02%, 88.30%, 95.13%, 93.69%, 92.41%, 97.86% and 85.88%

respectively. There is a great difference in relative abundance of bacteria phyla in water bodies at different sampling sites (Jani et al., 2018). The average abundance of Firmicutes is the highest, among which S3 and S4 are the second most abundant bacteria correspond to the downstream of the town and the power station reservoir respectively, and their domestic sewage has been effectively degraded, so the abundance of Firmicutes is high, which is also negatively correlated with the Firmicutes and fecal pollution described in the literature (Wu et al., 2015; Chen et al., 2016), showing a good correlation with ammonia and nitrate on the whole. S5 may be from the upstream bacterial transfer, S2 and S7 may be from the influx of exogenous microorganisms during the period.
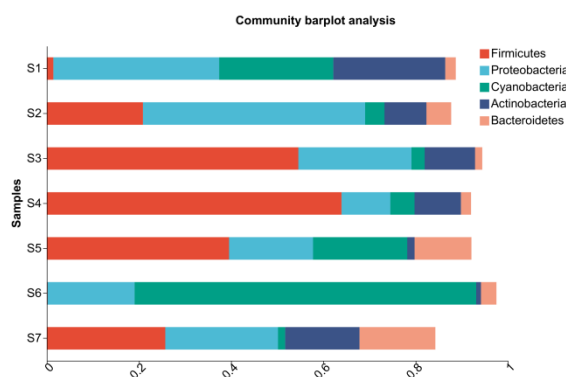
Figure 2. Relative abundance of bacterial species at phylum level in sediment samples

Proteobacteria, the bacteria with the second highest average abundance, is a major contributor to nitrogen removal based on nitrification and denitrification. The content of S2 sample is about 50%, which may be correlated with the inflow of exogenous microorganisms in Basom-tso Lake. The abundance of Cyanobacteria in S6 is as high as 74.97%, which may be related to the dam construction slowing down the downstream hydrological situation, the river has formed a trend of homogenization, and the dry season is easy to cause the flow velocity decreased and tended to be static, and then the occurrence of eutrophication, the specific mechanism needs to be revealed.

The abundance of Actinobacteria in S1 reaches the maximum and can be as high as 24.83%, which exists in cold and extreme habitats in high attitude plateau and is the basis of the microbial food chain (Chrismas et al., 2016). The concentration of nutrients in winter is lower than that in summer, and actinobacteria is active in undernourished environment. while the concentration of Bacteroidetes in S7 reaches 17.07%, which also plays an important role in the decomposition and fermentation of organic matter in the sediment, the high content of S7 and S2 sampling sites may be caused by the untreated domestic sewage received by the river in the nearby towns. The high content of S5 sampling sites may be caused by the relatively concentrated population of nearby villages and the influence of slower water flow and increased organic matter in rivers (Chen et al., 2016). Compared with previous research reports, the water ecosystem of each sampling site has the same composition category (Guo et al., 2019; Şener et al., 2017). In our study, the abundance of each phylum varied greatly, which was correlated with the variable slope, water temperature, hydrological regime, dissolved oxygen, altitude and other factors of the plateau river.

Analysis of species composition at genera level

The average relative abundance of all bacterial bacterial genera in the 7 samples was calculated, the top 7 bacterial genera in the average relative abundance were taken as the dominant bacterial genera, and the relative abundance of the dominant bacterial genera in each sample was shown in *Figure 3*.
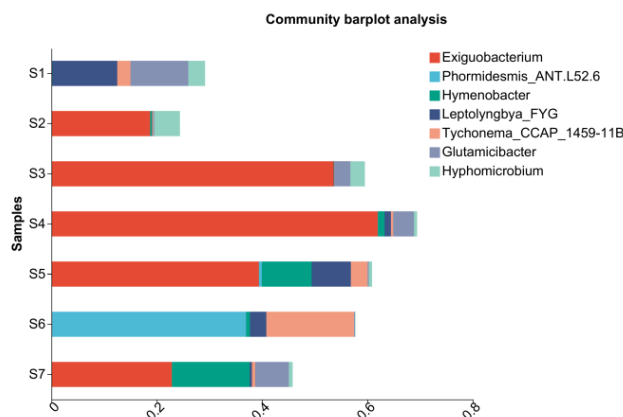
Figure 3. Relative abundance of bacterial species at Genus level in sediment samples

As shown in *Figure 3*, the dominant microorganisms at each sampling site at the genus level differ greatly, and only S3, S4 and S5 show a good correlation. The top 7 bacterial genera with average relative abundance extracted are as follows, Exiguobacterium (0.04~64.36%), Glutamicibacter (0.21-11.52%), Hymenobacter (0.03~15.56%), Hyphomicrobium (0.05~5.12%), Leptolyngbya_FYG (0.05~12.95%), Phormidesmis_ANT. L52.6 (0.01~37.32%), Tychonema_CCAP_1459-11B (0.04~16.86%) respectively. The sum of the relative abundances of the above bacteria in the 7 sampling sites was 30.64%, 25.60%, 61.52%, 72.10%, 61.56% and 58.49% respectively. 48.24%.

The results showed that 7 bacterial genera were underrepresented in S1 and S2, further analysis of the data showed that the top 7 (47.52%) corresponding to S1 were Leptolyngbya_FYG (12.95%), Glutamicibacter (11.52%), norank_f_Leptolyngbyaceae (7.51%), unclassified_f_Rhodobacteraceae (4.33%), Pseudorhodobacter (4.23%), unclassified_f_Microbacteriaceae (3.63%), Hyphomicrobium (3.36%), while the top 7 corresponding relative abundance of S2 (45.89%) were Exiguobacterium (19.68%), Hyphomicrobium (5.12%), Luteolibacter (3.76%), Phreatobacter (7.30%), unclassified_o_Rhizobiales (3.92%), Legionella (3.60%), respectively. norank_f__Saprospiraceae (2.50%).

The maximum average relative abundance of Exiguobacterium is 28.10%, suitable for growth under extreme environment and low nutrient conditions (Kasana et al., 2018) with strong adaptability. The average relative abundance of Exiguobacterium in sediments of the Nyang River is much higher than that of other genera except S1 and S6. The relative abundance of Exiguobacterium in S4 (61.97%), S3 (53.57%) and S5 (39.39%) was higher than that of other dominant bacteria (Fan et al., 2022). Phormidesmis_ANT. L52.6 belongs to Cyanobacteria, which is found that it survives in cold environment by producing special protective coating made of sugar (Kasana et al., 2018), and exists in cold streams and glaciers lacking nutrients (Raabova et al., 2019),

followed by the average relative abundance in the Nyang River sediments is the second highest (5.35%), the maximum relative abundance in S6 was 36.81%, and the relative abundance in the other 5 samples was all less than 1%.

Hyphomicrobium had a good promotion effect on ammonia nitrogen removal (Zhao et al., 2018), which was also corresponding to ammonia nitrogen removal effect at corresponding sampling sites. Hymenobacter strain is a cold-tolerant and heterotrophic aerobic bacterium, growing in low-salt and low nutrition, and can be dormant during glacial period (Klassen et al., 2011). The average relative abundance of Hymenobacter strain in the Nyang River sediments was ranked the third (3.81%), and its relative abundance in S7 (14.79%) and S5 (9.44%) was relatively high, with relative abundance of 1.26% in S4 and less than 1% in the other 4 samples.

Leptolyngbya_FYG is an unclassified genus of Cyanobacteria, widely distributed in Marine, fresh water, swamp and field, with a low biological sample size (Li et al., 2020), ranking the fourth (3.52%) in the average relative abundance among 7 samples of the Nyang River sediments. The relative abundance in S1 (12.33%), S5 (7.46%) and S6 (3.11%) was relatively high, with relative abundance of 1.20% in S4 and less than 1% in other 3 samples. Tychonema_CCAP_1459-11B, is found in colder lakes in northern Europe and slightly eutrophicated water bodies (Wei et al., 2012), ranked the fifth in the average relative abundance of sediments of the Nyang River (3.37%). The relative abundance of Tychonema_CCAP_1459-11B is 16.62% in S6, and 3.17% and 2.53% in S5 and S1 respectively. The relative abundances in the other 4 samples are less than 1%, and they play a weak role.

The above results showed that there are great differences in the composition of microbiological population in rivers under the influence of plateau environment, and the special bacterial population structure and characteristics caused by arctic-alpine and low nutrient conditions are consistent with the analysis results of community bacterial diversity.

Univariate correlation analysis

Based on the correlation between species, network maps are drawn to reflect interactions between species in samples. The interrelationships between different bacterial communities are critical to the structure, function, and stability of bacterial ecosystems. However, the relationship between different bacterial communities has been generally neglected in previous studies on plateau bacterial communities. In this study the interaction between bacterial communities at the genera level was preliminarily investigated to evaluate the role of a genus in the whole community in sediments, as shown in *Figure 4*.

The interrelationships among microorganisms in 7 sampling sites were studied by univariate correlation network diagram, and the species with absolute correlation coefficient no less than 0.5 and significance coefficient $pv < 0.05$ were selected. The results showed that there were 13 bacterial genera were extracted, and all showed positive correlation, among which the 7 genus, such as Pseudorhodobacter, Hyphomicrobium, Phreatobacter, unclassified_o__Rhizobiales, unclassified_f__Sphingomonadaceae, unclassified_f__Rhodobacteraceae, Sphingorhabdus, were Proteobacteria phylum, unclassified_f_Microbacteriaceae was Actinobacteria phylum, Leptolyngbya_FYG, norank_f_Leptolyngbyaceae, Tychonema_CCAP_1459-11B were Cyanobacteria, Luteolibacter was Verrucomicrobia phylum andDeinococcus was Deinococcus-Thermus phylum.
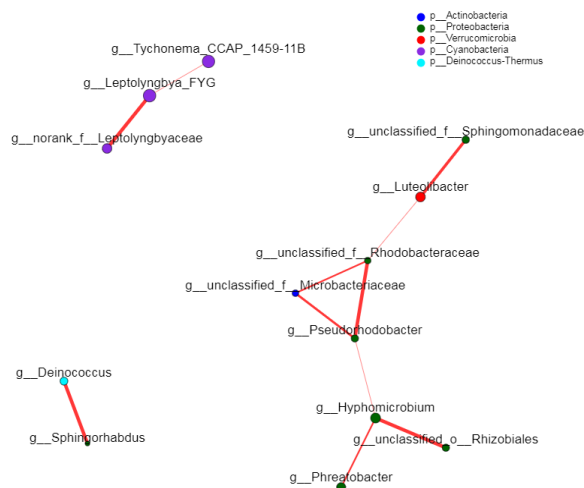
Figure 4. *Univariate correlation network at the genera level in sediment samples*

Generally speaking, Firmicutes and Bacteroidetes with high average abundance can be found to be relatively closed. Existing data show that the correlation between bacteria genera mainly for nutrition, environmental factor, sensitivity, etc., for example, g_unclassified_f__Microbacteriaceae is the most important node, belonging to Actinomyceta phylum has the function of degrading organic matter (Qian et al., 2022), the number of nodes connected to 2 and present positive correlation, with cluster coefficient of 1, the species is basically completely connected with adjacent species g_unclassified_f__Rhodobacteraceae and g__Pseudorhodobacter. The two connected bacteria are denitrifying and phosphorus removal bacteria, and there is no corresponding competition of nutrient sources, thus exhibiting a better symbiotic relationship, while g__Pseudorhodobacter has a positive correlation with g_unclassified_f_Rhodobacteraceae. It may be connected to complex nitrogen phosphorus removal processes.

## Relationship between sediment bacterial diversity and influencing factors

Correlation heatmap analysis

See *Figure 5* for the correlation between water quality and Physicochemical indexes of overlying water of the Nyang River and dominant bacteria at phylum and genera level.

The correlation heatmap shown in *Figure 5a* shows that, at the phylum level (the top 25 dominant bacteria), there is no bacteria phylum associated with COD, WS2 phylum is negatively correlated with TP. Proteobacteria and Chlamydiae are positively correlated with TN. Cyanobacteria is negatively correlated with NH3-N. Armatimonadetes are positively correlated with NO2-N. Firmicutes are positively correlated with the flow velocity, which may be caused by the increase of flow velocity reduces the organic matter and is more conducive to Firmicutes survival, consistent with the analysis result of species composition. Armatimonadetesp_unclassified_k_, norank_d_Bacteria is negatively correlated with the flow velocity. Cyanobacteria is positively correlated with temperature, which is consistent with relevant studies showing that the higher the temperature, especially in summer, is more conducive to the growth of Cyanobacteria, while Rokubacteria and GAL15 are negatively correlated. Chloroflexi and Gemmatimonadetes are negatively correlated with pH. GAL15 is

positively correlated with DO. Rokubacteria is negatively correlated with ORP. Firmicutes and Nitrospinae are positively correlated with EC. Dependentiae, Chlamydiae and Planctomycetes are positively correlated with altitude, possibly due to the influence of the exposure to ultraviolet radiation under high altitude aerobic conditions (Zong et al., 2022).

a. Phylum level   b. Genus level
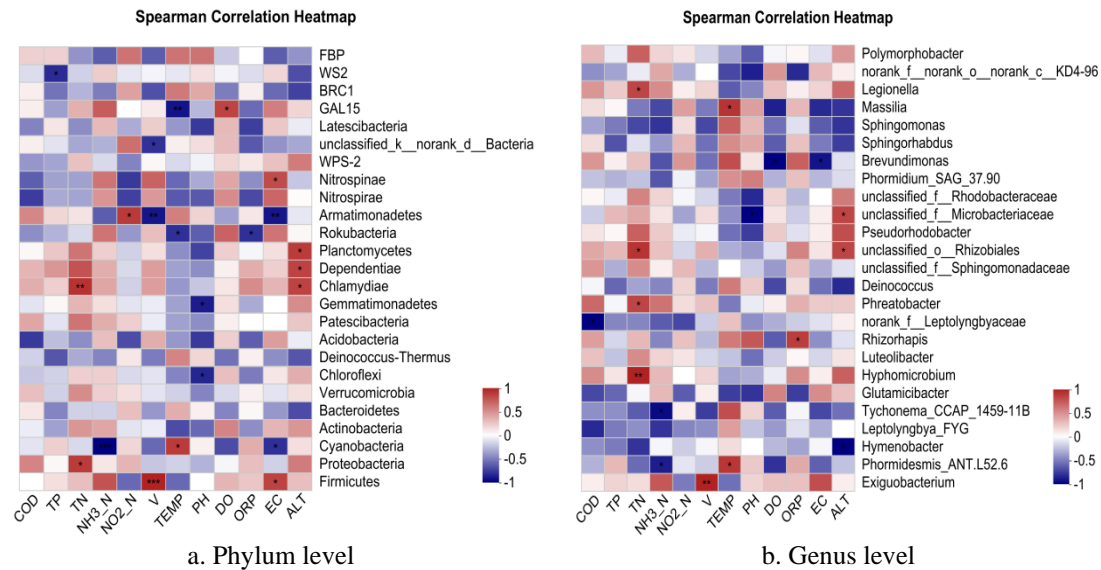
Figure 5. *Heatmap of correlation between environment and species Spearman in sediment samples. X-axis and Y-axis are the influencing factors and species respectively, and the correlation R-values and p-values are obtained through calculation. The R-values are shown in different colors in the figure. If the p-values are less than 0.05, it is marked by*. The legend on the right is the color interval of different R-values. The cluster trees can be selected to present species and influencing factors (e.g. left and top). *0.01 < p ≤ 0.05, **0.001 < p ≤ 0.01, ***p ≤ 0.001. Altitude (ALT), oxidation reduction potential (ORP), electrical conductivity (EC), dissolved oxygen (DO), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP)*

These results indicate that the surface sediments and the influencing factors have a certain impact on the bacterial phyla in the plateau habitats (Staley et al., 2015), and the effects are different to a certain extent, among which EC, flow velocity and water temperature have greater impact. The relative abundance of Firmicutes is positively correlated with the velocity and EC, indicating that the higher the flow velocity and EC, the higher the abundance, which was mainly related to the high slope of the river and the lower water temperature. Proteobacteria is positively correlated with TN, which was related to the fact that this bacterium contains a large number of nitrogen-fixing bacteria. The correlation between Cyanobacteria and TEMP, EC and ammonia nitrogen is related to its nitrogen fixation function. The positive correlation of temperature is that the higher the temperature is, the better the nitrogen fixation effect is, the negative correlation of EC is that the larger the concentration of EC is, the higher the concentration of EC prevents the possibility of obtaining nitrate nitrogen. The reason for the negative correlation of ammonia nitrogen is that the increase of ammonia concentration will promote the reaction to the opposite direction of nitrogen fixation. There was no correlation between Actinobacteria and Bacteroidetes and the influencing factors of plateau environment.

The correlation heatmap shown in *Figure 5b* shows that at the genus level, norank_f__Leptolyngbyaceae is negatively correlated with COD. Due to its biological carbon sequestration, the higher COD is, the more difficult it is to sequestration carbon, thus affecting and reducing the abundance of norank_f__Leptolyngbyaceae. Phormidesmis_ANT.L52.6 and Tychonema_CCAP_1459-11B are negatively correlated with NH3-N. The former can convert carbon dioxide and water into sugars through photosynthesis to achieve organic phosphate mineralization and carbon fixation, while the latter is believed to occur in slightly eutrophical lakes. TAnd positively correlated with Hyphomicrobium TN, Phreatobacter, unclassified_o__Rhizobiales and Legionella, Hyphomicrobium as organic matter degradation bacteria, the the higher abundance of it, the more organic matter degradation, in turn, reduced the ability of TN lower (Zhao et al., 2018), Phreatobacter can inhibit denitrification process, unclassified_o__Rhizobiales consistent with TKN and nitrate are positively correlated (Wu et al., 2019), Legionella is easily affected by nitrogen source to strengthen (Zhang et al., 2022).

Exiguobacterium is positively correlated with V, which can decompose organic pollutants, that is, increasing the flow velocity can degrade organic matter more efficiently. Phormidesmis_ANT.L52.6 and Massilia are positively correlated with temperature. The former has the effect of removing nitrate nitrogen (Wang et al., 2020), and the Massilia has the effect of fixing nitrogen (Guo et al., 2019), unclassified_f__Microbacteriaceae is negatively correlated with PH (Liu et al., 2017), which is inhibited by pH. Brevundimonas is negatively correlated with DO, which may be related to the fact that Brevundimonas belongs to anaerobic degradation of organic matter (Li et al., 2019). Rhizorhapis is positively correlated with ORP the higher the ORP, the higher the REDOX point, the stronger the oxidation capacity and the more likely denitrification occurred. The higher the Rhizorhapis abundance was. TP and NO2-N had no significant correlation with bacterial genera.

Based on the analysis of the influence of twelve influencing factors such as COD and TP on bacteria, it can be found that the flow velocity had a good positive correlation with the highest abundance phylum of Firmicutes and family of Exiguobacterium, i.e. the hydrological situation had a very important influence on bacteria.

Redundancy analysis of bacterial communities and influencing factors

In order to explore the contribution of influencing factors to bacterial abundance and community composition, RDA was used to reveal the correlation between bacterial communities and influencing factors (Guo et al., 2019). The results of redundancy analysis (RDA) between sediment bacterial community and influencing factors of the Nyang River are shown in *Figure 6a*. The analysis results at the phylum classification level show that the factors such as V, TN, TEMP, pH, ALT and EC can account for 85.3% of the variation of bacterial communities in sediments, which is higher than that of Fuhe River (Wang et al., 2023). The samples of S3, S4 and S5 in sediments are similar, while those of S2 and S7 are similar, and the samples of S1 and S6 are not similar. The influence factors such as V, TN and TEMP in sediments has a great influence on the bacterial community in sediments in the middle and lower reaches of the Nyang River.

In *Figure 6a,* the five dominant bacteria species Cyanobacteria are positively correlated with TEMP (Yang et al., 2019; Jing et al., 2019) and pH. In the Nyang River region, there is little rain in winter and strong solar radiation, which is conducive to the

growth of phototrophic microorganisms, and is negatively correlated with EC, V, TN and HRI. excessive flow velocity leads to decrease of cyanobacteria (Yu et al., 2015). Firmicutes is positively correlated with EC, V, pH, negatively correlated with TN, TEMP and ALT. Proteobacteria is positively correlated with TN and ALT (the degradation of organic matter requires more total nitrogen total phosphorus), Proteophyta is the main denitrifying bacteria phyla, which is significantly correlated with TN, and negatively correlated with EC, V, TN and TEMP. Actinobacteria is positively correlated with TN, EC, V and negatively correlated with pH, TEMP and ALT etc.

a. Phylum level   b. Genus level
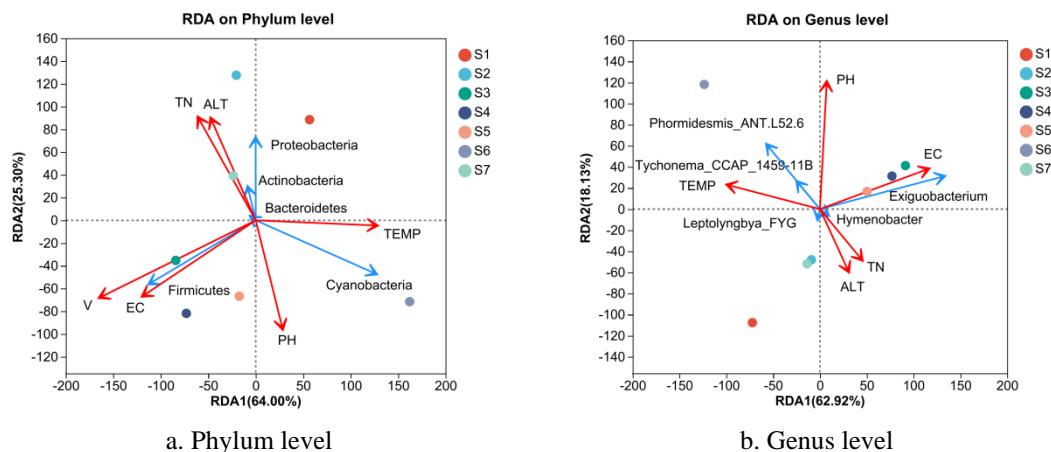
Figure 6. Redundancy analysis of bacterial communities and influencing factors in sediment samples. Altitude (ALT), oxidation reduction potential (ORP), electrical conductivity (EC), dissolved oxygen (DO), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP)

The analysis result under the taxonomy level of genus in *Figure 6b* shows that the influencing factors can account for 90.07% of the variation of bacterial community in sediments. The samples of S3, S4 and S5 in sediments are similar, the samples of S1, S2 and S7 in sediments are similar, while the samples of S6 in sediments has no similar samples. environmental factors such as pH, V, ALT and TEMP have a great influence on bacterial communities of sediments in the middle and lower reaches of the Nyang River. According to the analysis of the four dominant genera and species shown in the figure, Phomidesmis_ANTL52.6 and Tychonema_CCAP_1459-11B are positively correlated with TEMP and PH, and negatively correlated with EC, V, TN and ALT. Hymenobacter is positively correlated with TN, EC, V and ALT and negatively correlated with pH and TEMP. Exiguobacterium survives in extreme environment, such as permafrost soil and glacier, producing proteases and hydrolases or extreme enzymes, which may be helpful in terms of contributing to bioremediation and biodegradation (Kasana et al., 2018), which is positively correlated with EC, V, TN and PH, and negatively correlated with TEMP and ALT. Leptolyngbya_FYG, Leptolyngbya cyanobacteria is involved in photosynthesis, which plays an important role in maintaining ecological balance, with low sample size, such as nitrogen fixation, primary productivity through photosynthesis and algal bloom (Li et al., 2020). There were positive correlations with TN, ALT, TEMP and negative correlation with pH, EC and V.

Redundancy analysis of influencing factors showed that S3, S4 and S5 clustering were effective at phylum and genus level, and the most influential factors were flow velocity, and the influence of each influencing factor on microorganisms was different to some extent.

Analysis of influencing factors of bacterial communities in the Nyang River

In order to further reveal the degradation process of sewage in Plateau River, this study analyzed the bacterial communities of corresponding sampling sites, and found that after analysis.

The bacterial diversity and abundance in the Nyang River are lower than those in other regions, that is, the unique environmental conditions of the plateau restricted the bacterial species and the abundance of corresponding species. Further analysis of the bacterial structure and types also showed that there are only 28 phyla and 642 genera in the Nyang River, among which the five bacteria phyla with the highest average abundance are Firmicutes, Proteobacteria, Cyanobacteria, Actinobacteria, and Bacteroidetes. The abundance of these phyla varied greatly, mainly due to the velocity of Plateau Rivers, water temperature and other factors. The top seven average relative abundance bacteria genera are Exiguobacterium, Glutamicibacter, Hymenobacter, Hyphomicrobium, Leptolyngbya_FYG, Phormidesmis_ANT. L52.6, Tychonema_CCAP_1459-11B, which are underrepresented in S1 and S2, that is, there was a great difference in each sample and plateau and low nutrient conditions caused a variable bacterial community.

According to univariate correlation analysis, 13 species of bacteria extracted from the plateau under the condition of low nutrients showed good positive correlation, which mainly reflected the high dependence of plateau microorganisms on influencing factors. The Heatmap of correlation between the influencing factors and species Spearman further revealed this mechanism. V significantly affects the highest average abundance of the phylum Firmicutes and genus Exiguobacterium, while COD, TP, TN, NH3-N and NO2-N affect only 5 out of 25 bacterial phyla and 7 out of 25 bacteria genera, and the abundance of these phyla and bacterial genera was low, that is, poor nutrition had very weak influence on plateau river bacterial communities. Temperature (3 bacterial phyla and 2 bacterial genera), pH (2 bacterial phyla and 1 bacterial genus), DO (1 bacterial phyla and 1 bacterial genus), EC (4 bacterial phyla and 1 bacterial genus), ORP (1 bacterial phyla and 1 bacterial genus), and altitude (3 bacterial phyla and 4 bacterial genera) as environmental conditions, where EC was also positively correlated with Firmicutes. Obviously, the flow velocity had a more significant effect on bacterial composition.

Further redundant analysis of bacterial communities and environmental factors also proved that flow velocity was the most important factor affecting bacterial composition, and the flow velocity was the most influential factors for both phylum and genus bacteria. EC was also the second most influential factor for phylum and genus bacteria. The influence of pH, temperature and altitude on microorganisms was still stronger than those of COD, TP, TN, NH3-N and NO2-N. In other words, as a plateau river, the influence of bacterial community in the Nyang River is flow velocity, environmental factors and nutrients in descending order.

Based on the above analysis, it can be found that in addition to the good quality of TP water, its bacterial diversity and abundance are also lower than that of other rivers, and its bacterial community is also extremely variable. As a hydrological situation, the flow velocity is the most important reason, followed by environmental factors such as EC, pH, temperature and altitude, and the least influence is nutrients.

Conclusion

Seven sampling sites were set up in the middle and lower reach of the Nyang River in the dry season to detect surficial sediment microorganisms. The results showed that the water quality indexes of the Nyang River except TP were class II surface water and above. Further analysis of surface sediments bacterial composition founded that Firmicutes, Proteobacteria, Cyanobacteria, Actinobacteria and Bacteroidetes were dominant microorganisms in the Nyang River and their diversity and abundance were significantly lower than those in other regions, and the dominant bacterial abundance and species varied greatly. The analysis of influencing factors showed that the influencing factors bacterial community in the Nyang River in the dry season was flow velocity, environmental factors and nutrients in order. Based on the analysis of bacterial community structure and influencing factors in surface sediments, it provides useful thinking for studying the self-purification mechanism of high-altitude rivers.

Data availability. All data generated or analyzed during this study are included in this published article.

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.

Competing 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.

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