

Taxonomic and Functional Responses of Sediment Bacterial Community to Anthropogenic Disturbances in the Yarlung Tsangpo River on the Tibetan Plateau

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ABSTRACT. River sediments, functioning as sink and source for nutrients, are one of the most diverse bacteria habitats in freshwater ecosystems. Recently, more attention has been paid to the sediment bacteria because of their important roles in biogeochemical cycling. Our study addresses the question of how sediment bacterial community varies taxonomically and functionally along one of the largest plateau rivers in the world (the Yarlung Tsangpo River) subject to anthropogenic pressures. The results indicated that wastewater discharge resulted in asynchronous change of biomass and diversity in sediment bacteria, and they were both inhibited by the construction of dam. Moreover, significant alterations in the community composition were observed between upstream and downstream of the dam, while functional variation was not detected due to the bacterial functional redundancy. In addition, revealed by General Additive Model (GAM) and Canonical Correspondence Analysis (CCA), elevation was the endemic driver on the Tibetan Plateau shaping the sediment bacterial community diversity and composition along with pH, while nutrient condition was responsible for functional variation. Furthermore, little synchronized dynamics of co-occurrence was exhibited in sediment bacteria in the Yarlung Tsangpo River. In conclusion, anthropogenic disturbances influenced the sediment bacterial community at the compositional level rather than the functional level in the typical high-elevation river. Therefore, our study provides potential evidence that diversity, composition and function of sediment bacterial community should all be taken into consideration on the Tibetan Plateau.

Keywords: 16S rRNA gene sequencing, dam, environmental variable, microbial community taxonomy and function, Plateau river

1. Introduction

Rivers are unique freshwater systems owing to their constant turnover (Mitsova-Boneva, 2007; Jackson et al., 2014; Zhou et al., 2016), and river sediments, functioning as sink and source for nutrients (Jozsa et al., 2015), are one of the most diverse bacteria habitats in freshwaters (Bermejo et al., 2003). According to previous studies, sediment bacteria are essential participants of freshwater ecosystems, catalyzing important biogeochemical cycling (Rubin and Leff 2007; Tatariw et al. 2013) and acting as vital components of aquatic food chain (Battin et al., 2009). More specifically, bacteria have unique capability to transform nutrients from one form to another, in particular for carbon and nitrogen through carbon fixation and denitrification (Findlay, 2010). Given that different bacterial groups exhibit various functional roles, the occurring variations in bacterial community may have implications for biogeo-chemical processes (Ruiz-González et al., 2013). Moreover, sediment bacterial communities can be highly di-

verse under environmental disturbances due to their small size and rapid growth rates (Zeglin, 2015), making them one of the most sensitive indicators of environmental change in aquatic ecosystems. It is reported that sediment bacterial community compositions were shaped by nutrients and pH in the Santa Ana River in the United States and in the Jiulong River Watershed in China (Hu et al., 2014; Ibekwe et al., 2016) while in the Nanfei River, metals and nutrients had higher impact on sediment bacterial community composition (Xie et al., 2016).

However, the existing studies identified the important drivers shaping sediment bacterial structure while the pattern of diversity and functional variations is still lacking. In addition, according to previous studies, hydrochemistry in rivers is a function of climate, geology, topography and anthropogenic activities (Jordan et al., 2014; Jiang et al., 2015; Tong, 2015) which are closely linked to variation of sediment bacterial community (Eid et al., 2017). Since significant differences of hydrodynamic and hydrochemistry were observed between plain and high-elevation rivers (Huang et al., 2011; Liu et al., 2015), it is deduced that sediment bacterial community may exhibit distinct characteristics in high-elevation rivers. Since the majority of studies have focused on plain rivers, such as the Danube River (Savio et al., 2015) and the Mississippi River (Staley et al., 2015), few results were reported on

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high-elevation rivers.

The Tibetan Plateau in China contributes to distinct energy and hydrological cycling and the Yarlung Tsangpo River vitally takes part in social and ecological development in southwest of China (Immerzeel et al., 2010; Aufdenkampe et al., 2011). The elevation gradient of the river ranges from 300 to 5590 m with the average of 4200 m and the total length of the river is more than 2840 km (Sang et al., 2016). In recent decades, the river is experiencing growing anthropogenic activities, such as wastewater discharge and river damming, which may profoundly affect ecosystem functions and reduce ecosystem services (C. Liu et al., 2008; Zhou et al., 2016; Wang et al., 2017). The extensive hydropower potentials in this river have raised great attention and the only hydropower station in the main stream, the Zangmu Dam, began to operate in late 2015 with a reservoir capacity of $8.66 \times 10^7 \text{ m}^3$. Dam construction may result in alterations in hydromorphology and disturbance of normal hydrological regime of natural ecosystems, as the impoundment by dams can change the aquatic environment physically, chemically and biologically (Chai et al., 2009; Zhou et al., 2015). Consequently, more attention has been paid to the effect of discharge by anthropogenic activities on water quality of great rivers in southwest China, and the alteration of biogeochemical nutrient distribution by construction of hydropower stations. Moreover, since sediment bacteria are adaptive and dynamic, they can easily be affected by a range of natural and anthropogenic stressors, especially under rapid urbanization and hydropower development (Saxena et al., 2015). However, recent studies only concentrate on water and sediment physical distribution along the Yarlung Tsangpo River (Hren et al., 2007; Huang et al., 2011; Jiang et al., 2015) and few research of sediment bacterial community distribution was available so far. Furthermore, as cascade dams have been planned in the mainstream of the river, it is important to investigate anthropogenic impacts on biogeochemical nutrients transportation and sediment bacterial community variation, minimizing the adverse effect caused by anthropogenic disturbances in the future. In addition, the Yarlung Tsangpo River is currently a poorly dam-

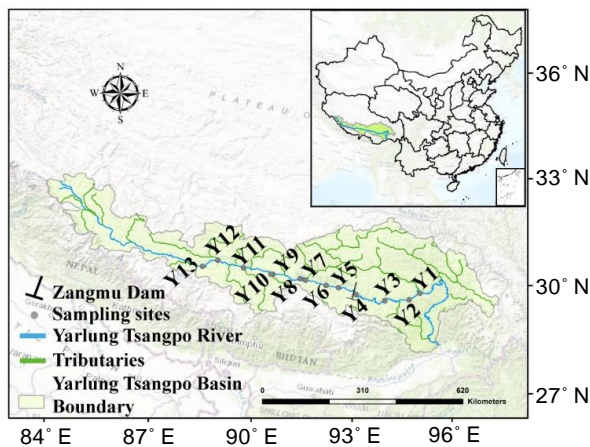


Figure 1. Overview and detailed map of the Yarlung Tsangpo Basin with sampling sites.

regulated river, and its comparison with the highly dam-regulated rivers, such as the Lancang-Mekong River and the Jinsha-Yangtze River, can contribute to the characterization of cascade dam effect on sediment bacterial community, providing scientific support for dam planning and management in the future.

In our study, next-generation 16S rRNA gene sequencing combined with various bioinformatics tools and multivariate statistical methods was applied to investigate the variation of sediment bacterial community along the Yarlung Tsangpo River. The objectives of our study were to reveal the spatial pattern of sediment bacterial diversity, composition and function along the river, to determine the anthropogenic impacts on distribution of sediment bacterial community, and to uncover the important environmental drivers influencing sediment bacterial community in the Yarlung Tsangpo River. Therefore, our present study provides insights for mapping the spatial distribution of bacterial communities in high-elevation and human-impacted large rivers, and broadens our knowledge of the environmental variables governing the sediment bacterial distribution in river ecosystems on a global scale.

2. Materials and Methods

2.1. Study Area and Field Sampling

Our sampling was conducted in November 2016, extending over a gradient of about 950 km (Figure 1) and the elevation gradient ranged from 2900 to 4200 m. The temporal changes were not considered so that an intensive longitudinal sampling effort could be achieved. Much of the upstream catchment is rural with a relatively low population and the big cities (such as Lhasa) lie in the middle reaches. According to previous studies, the middle reaches of the mainstream of the Yarlung Tsangpo River are the integrated development areas for agriculture, and industries and tourism are also being rapidly developed, resulting in the increase of wastewater discharge in this region (Huang et al., 2011; Jiang et al., 2015). Sampling site location parameters were measured by a GPS device (GPSMAP 62s, Garmin, KS, USA) and details are presented in Table S1. Sediments were collected using an Ekman-Brige grab sampler (Hydro-Bios, Altenholz, Germany) in a sediment depth of 6 cm. In all sampling sites, the sediment samples were collected in the left bank, the right bank and the middle of the river, and then the three samples were homogenized as one. A 50 mL aliquot homogenized sediment was stored at $-20 \text{ }^\circ\text{C}$ in the field. In the laboratory, they were kept at $-80 \text{ }^\circ\text{C}$ until the deoxyribonucleic acid (DNA) extraction. A 500 mL aliquot homogenized sediment was freeze-dried (Labconco, MO, USA) in the laboratory for the subsequent physicochemical analysis.

2.2. Physicochemical Analysis

The velocity was measured in the field with a Flow-Tracker ADV (SonTek, CA, USA). In the laboratory, prior to the determination of grain size distribution by the Mastersizer 2000 laser diffractometer (Malvern, UK), the freeze dried

sediments were screened with a 2-mm sieve to remove large particles. The pH and electrical conductivity (EC) were determined at a 1 : 5 sediment to water ratio (w/w) by a multi-parameter water quality analyzer (HQ40d, Hach, CO, USA). Total organic carbon (TOC) of sediment was determined by an Elementar Liqui TOCII analyzer (Frankfurt, Germany). The two-step acid-alkaline method was applied to extract reactive silica (RSi) in the sediment (Faust and Raes, 2012) and the extracted silicate was measured with the molybdate blue spectrophotometric method. A modified alkaline-extracted regression method with a Si/Al ratio correction was used to estimate biogenic silica (BSi) in the sediment (Nilsson et al., 2005). Total nitrogen (TN) and total phosphorus (TP) were measured by the Kjeldahl method and the sulfuric acid-perchloric acid oxidation method, respectively (Ebina et al., 1983). Inorganic nitrogen ($\text{NO}_x\text{-N}$ and $\text{NH}_4^+\text{-N}$) were extracted with 2 M KCl and measured by the flow injection autoanalyzer (Lachat QC8500, CO, USA), according to the manufacturer's protocols. The sensitivity of the detector is 0.33 and 0.23 for $\text{NO}_x\text{-N}$ and $\text{NH}_4^+\text{-N}$, respectively. The wavelengths of the colorimeter were set as 550 and 660 nm for $\text{NO}_x\text{-N}$ and $\text{NH}_4^+\text{-N}$ detections, and the corresponding detection limits are 0.001 mg/L and 0.003 mg/L (Li et al., 2011). The available phosphorus (AP) was extracted by

NaHCO_3 and then determined by the molybdenum blue method using a spectrophotometer (Persee, Beijing, China). All above-mentioned measurements were conducted in triplicate to ensure the precision of the analysis and blank samples were also included in all measurements to improve the accuracy of the analysis. In addition, the standard curves of TOC, RSi, BSi, TN, $\text{NO}_x\text{-N}$, $\text{NH}_4^+\text{-N}$, TP and AP were all established ($R^2 > 0.999$) prior to field sample measurement.

2.3. DNA Extraction and Sediment Bacterial Biomass Determination

The DNA was extracted from 0.5 g sediment in each sample using the MoBio PowerSoil Extraction kit (CA, USA) following the manufacturer's protocols. DNA quality and quantity were verified using agarose gel electrophoresis (1%) with a spectrophotometry (NanoDrop ND 2000, Thermo Scientific, DE, USA).

The extracted DNA was applied to measure total bacteria abundance in sediment samples. The polymerase chain reaction (PCR) of the total bacteria genes was operated in a 7500 RT-PCR system (Applied Biosystems, Darmstadt, Germany). In addition, PCRs of each sample were conducted in triplicate. The PCR amplification and primer sets were validated in

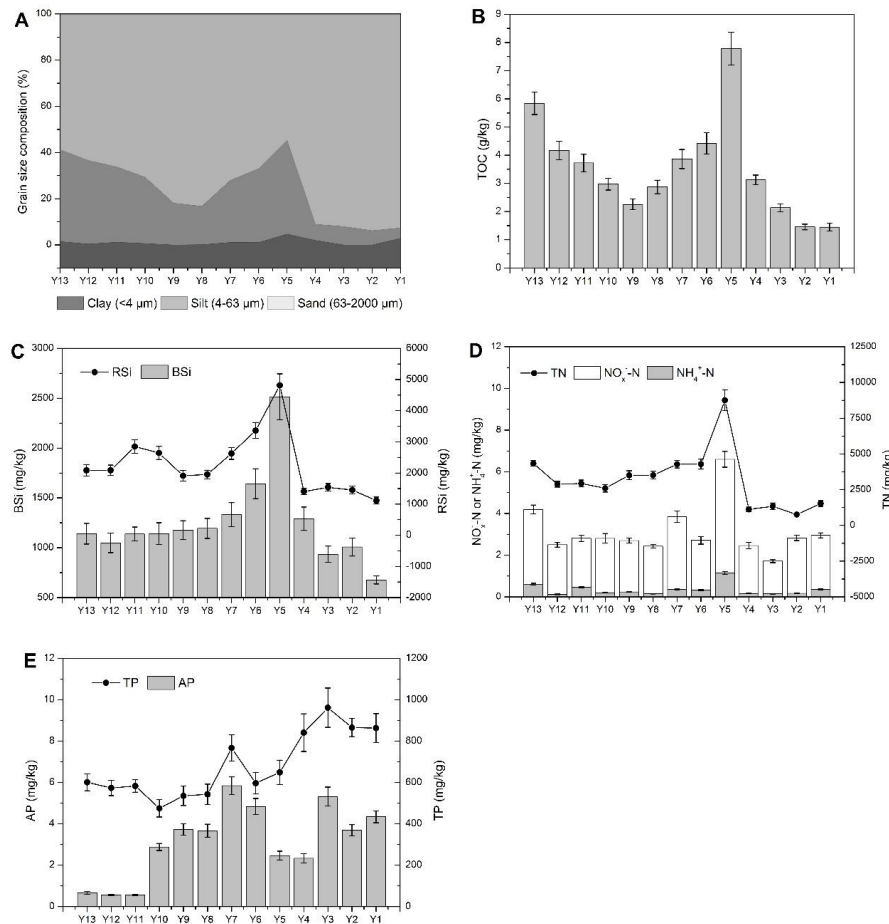


Figure 2. Main physicochemical characteristics of sediment samples.

previous studies (Chen et al., 2017).

2.4. 16S rRNA Gene PCR Amplification and High-throughput Sequencing

The extracted DNA was also used for high-throughput sequencing. The V4 hypervariable region of the 16S rRNA genes was amplified using the 515f/806r primer set to identify each sample (Yan et al., 2015). The details of the PCR amplification condition for 16S rRNA genes can be found in previous study (Li et al., 2015). In addition, triplicate PCRs were applied for each sample and the amplicons were quantified by a PicoGreen dsDNA Assay kit (Invitrogen, CA, USA). Then, the obtained PCR product was sequenced by the Illumina Hiseq 2000 platform following the manufacturer's protocols.

2.5. Data Preprocessing and Statistical Analysis

The details of the data preprocessing can be found in previous study (Li et al., 2015). Briefly, low quality reads, chimeric and singleton operational taxonomic units (OTUs) were all removed. QIIME (version 1.8.0) was applied to match the filtered reads with corresponding samples, and reads with 97% similarity were clustered into OTUs (Xie et al., 2016).

The Spearman correlation analysis was applied to identify environmental parameter correlation. The complexity of environmental parameters in each site was presented by Euclidean distance matrices. A Generalized Additive Model (GAM) was set up to analyze the dynamic of observed species in response to environmental variations. A principal component analysis (PCA) was conducted based on the Bray-Curtis dissimilarity matrices to group the sampling sites. A canonical correspondence analysis (CCA) was conducted to reveal the environmental drivers of variation of sediment bacterial community composition. In addition, BIOENV analysis and Man-

tel test were also applied to examine the strength of the association between each environmental variable and bacterial community. The undirected bacterial species network was constructed by calculating the pairwise Spearman correlations between species across all sediment samples with the threshold of 0.95 (Li et al., 2016). Cytoscape (version 3.5.1) was applied to visualize the network results. Moreover, sediment bacterial functional profiles were predicted using high-throughput 16S rRNA data by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt, <http://picrust.git-hub.com>) which can transform OTU profile into taxonomic profile of significant related genomes in Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The average value and the standard deviation (SD) of the replicates were calculated. A one-way ANOVA followed by Tukey's test was applied to determine the significant differences ($p < 0.05$). All statistical analyses were conducted with SPSS (version 17.0, Chicago, IL, USA) and packages vegan, WGCNA, pheatmap and mgcv of R (version 3.2.1, Vienna, Austria).

3. Results

3.1. Spatial Physicochemical Characteristics of River Sediment

The sediment characteristics of the Yarlung Tsangpo River are shown in Table S1 and Figure 2. And the Euclidean distance of environmental parameters between each sampling site and the Y13 was presented in Figure S1. It is observed that the complexity of environmental condition increased along the river and stayed invariant in downstream sites. The highest value was observed in upstream of the dam, indicating that the environmental condition in the Zangmu Reservoir was remarkably different from that in other sites. More specifically, the velocity in the Zangmu Reservoir was at low level and increased immediately downstream of the dam. The pH decreased along the river and was closely positively correlated with the elevation according to the Spearman correlation test (Table S2). The EC stayed stable in upstream sites and varied greatly in midstream sites. The sediment in the Yarlung Tsangpo River was composed of clay ($< 4 \mu\text{m}$), silt ($4 \sim 63 \mu\text{m}$) and sand ($63 \sim 2000 \mu\text{m}$) and sand was the main component in the river sediment (Figure 2A). The percentage of silt decreased in upstream sites, increased in midstream and dropped dramatically downstream of the dam.

TOC concentration ranged from 1.45 to 7.78 g kg^{-1} with the highest value in the Zangmu Reservoir and a significant decrease ($p < 0.05$) downstream of the dam. Generally, RSi and BSi were both retained in the Zangmu Reservoir and dropped significantly ($p < 0.05$) downstream of the dam. However, RSi stayed unchanged in the downstream sites while BSi decreased continually. In our study, $\text{NO}_x\text{-N}$ and TN both increased significantly ($p < 0.05$) in the Zangmu Reservoir and decreased sharply downstream of the dam. $\text{NH}_4^+\text{-N}$ was detected in all sampling sites at low concentration level. Phosphorus showed a different trend from nitrogen. In upstream sites, high TP and low AP coexisted and they increased synchronously in mid-stream sites. However, TP kept increasing

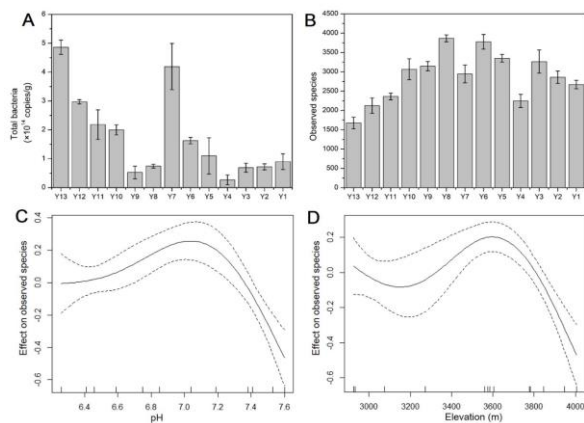


Figure 3. The 16S rRNA gene copies of total bacteria (A) and observed species (B) of samples. pH (C) and Elevation (D) effect curves from a GAM model fitted to observed species data (solid lines), with two 95% confidence bands (dashed lines).

while AP remained at the low level in dam-associated sites.

3.2. Sediment Bacterial Diversity Variation and Environmental Drivers

The total bacterial gene copies ranged from 2.66×10^{13} to 4.86×10^{14} copies g^{-1} (Figure 3A). The upstream sites exhibited the highest total bacterial gene copies while the biomass remained much lower in downstream sites. In the midstream sites, the total bacterial abundance increased significantly ($p < 0.05$) after the inflow of the Lhasa River (Y7) and decreased downstream of the dam (Y4).

The filtered sequences in all sampling sites varied from 30,155 to 55,793 reads, which can be assigned to 10534 OTUs. According to the α -diversity of sediment bacterial community, the observed species, ranging from 1674 to 3866, increased in upstream sites (Figure 3B). The Chao1, Shannon and Simpson index presented similar pattern with that of the observed species (Table S3). It is also noted that α -diversity index all decreased downstream of the Zangmu Dam, indicating that dam construction inhibited the diversity of sediment bacterial community.

A GAM model was applied to investigate the relationships between observed species and environmental variables. There was a linear relationship (degree of freedom = 1) between observed species and EC, sediment grain size, TOC, RSi, BSi, TN, NO_x -N, NH_4^+ -N and TP. The statistical results demonstrated that pH and elevation contributed significantly to observed species variation and explained the greatest variation of sediment bacterial diversity (Table S4). The model also revealed that observed species and pH changed as a single-peak curve with the peak value of about 7.1, and the curve between observed species and elevation peaked at 3600 m (Figures 3C and 3D).

3.3. Spatial Variation of Composition and Predicted Function of Sediment Bacterial Community and Their Environmental Drivers

Based on the taxonomy results, the complexity of sediment bacterial community was presented by the Bray-Curtis distance between each site and the Y13 (Figure S2A). In general, the Bray-Curtis distance increased along the river and reached the highest value in downstream of the Zangmu Dam (Y4). Moreover, a total of 12 dominant bacterial phyla were identified in sediment samples in the Yarlung Tsangpo River (Figure 4A and Figure S2B). Each of the 12 phyla constituted for more than 1% of the bacterial abundance and Proteobacteria was the most dominant phylum, ranging from 31.99% to 54.90%. In addition, the relative abundance of Verrucomicrobia all exceeded 25% in upstream sites and Firmicutes was generally more than 20% in downstream sites, also making them the dominant bacterial phyla in the sediment. A total of 10 typical phylum and class bacteria were chosen to reveal the spatial variation of bacterial distribution (Figure S2). High relative abundances of α -Proteobacteria, δ -Proteobacteria and Verrucomicrobia were observed in upstream and midstream sites while γ -Proteobacteria, Firmicutes and Nitrospirae were higher in downstream sites. Moreover, Betaproteobacteria, Planctomycetes and Spirochaetes were at high level only in midstream sites, and Bacteroidetes varied little along the river except the sharp decrease after the dam.

The spatial change of sediment bacterial community composition was determined by PCA (Figure 5A). The two principal components explained 15.25% (PC1) and 13.08% (PC2) of the sediment bacterial community variance, respectively. The 13 sampling sites can be divided into 3 groups, including the upstream (Y13, Y12 and Y11), midstream (Y10, Y9, Y8, Y7, Y6 and Y5) and downstream (Y4, Y3, Y2 and Y1) sites. Since Y5 and Y4 were far from each other in Figure 5A, it is suggested that the sediment bacterial community was remark-

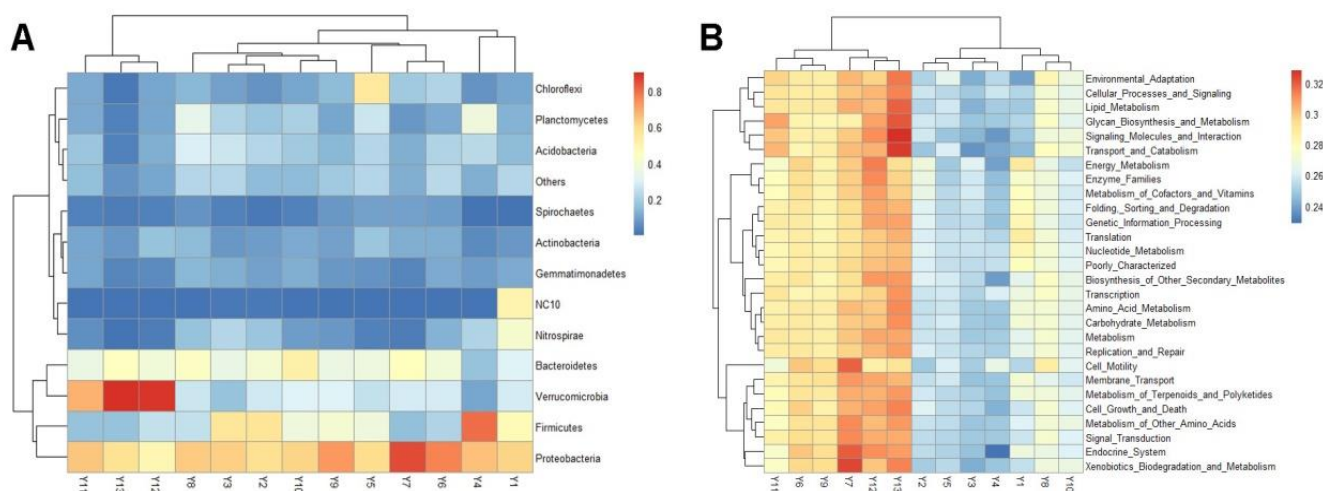


Figure 4. Heat map of the 13 most abundant bacteria at the phylum level (A) and the top 28 predicted functions analyzed by PICRUST (B). Dendrograms are based on the Bray-Curtis similarity measure.

ably different before and after the Zangmu Dam. The 13 environmental variables were measured to identify the significant variables shaping the sediment bacterial community along the river. It is demonstrated by Mantel test that TOC, elevation, pH and grain size were the variables most strongly correlated to taxonomic composition (Figure 5B and Table S5). Then, the four important drivers were chosen for the subsequent CCA analysis and the results revealed that 27.48% (CCA1) and 16.08% (CCA2) of the variation of sediment bacterial community composition were explained (Figure 5B). Grain size was positively linked with downstream sites and TOC was positively correlated with upstream and midstream sites. Elevation separated upstream and midstream sites from downstream sites while pH clustered upstream sites from midstream sites.

In order to overcome high-cost metagenomic sequencing, bioinformatics tools to predict functional diversity by using phylogenetic distributions of functional traits have been applied in our study. According to the PICRUSt results, the top 28 level-2 functional categories are presented in Figure 4B, which belonged to Metabolism, Cellular processes, Environmental information processing and Genetic information processing level-1 categories. Membrane transport, Amino acid metabolism and Carbohydrate metabolism were the most dominant functions of sediment bacterial community in the Yarlung Tsangpo River, constituting about 10% of the total gene abundance, respectively.

The spatial variation of sediment bacterial community functions was presented in Figure 5C, in which the two prin-

cipal components explained 39.94% (PC1) and 18.47% (PC2) of functional variance, respectively. Similar to community composition, the 13 sampling sites can also be clustered into 3 groups, including upstream, midstream and downstream sites. Interestingly, no significant difference was observed in upstream (Y5) and downstream (Y4) sites of the Zangmu Dam. Prior to CCA, the BIOENV analysis was conducted to select the important environmental drivers shaping functional variation of sediment bacterial community. The combination of EC, BSi and NO_x-N was the most significant drivers influencing the variation ($r = 0.3424$). According to CCA results, 25.99% (CCA1) and 4.54% (CCA2) of functional variation can be explained by the 3 environmental parameters (Figure 5D).

3.4. Sediment Bacterial Co-occurrence Network in the Whole River

In order to investigate the relationship of sediment bacteria co-occurrence in the Yarlung Tsangpo River, a co-occurrence network was constructed based on the correlations of relative abundance of OTUs (Figure 6). The network consisted of 216 nodes (OTUs) and 166 edges (correlations). The average network distance between all pairs of nodes (average path distance) was 1.93 edges and the modularity index was 0.96, suggesting that the network has a modular structure. The OUT-1544 (Acidobacteria) had the highest degree of association (degree = 10) in the network. It is also noted that the average significant correlation per node was 1.54 edges, indicating that the sediment bacterial network was not comprised of highly

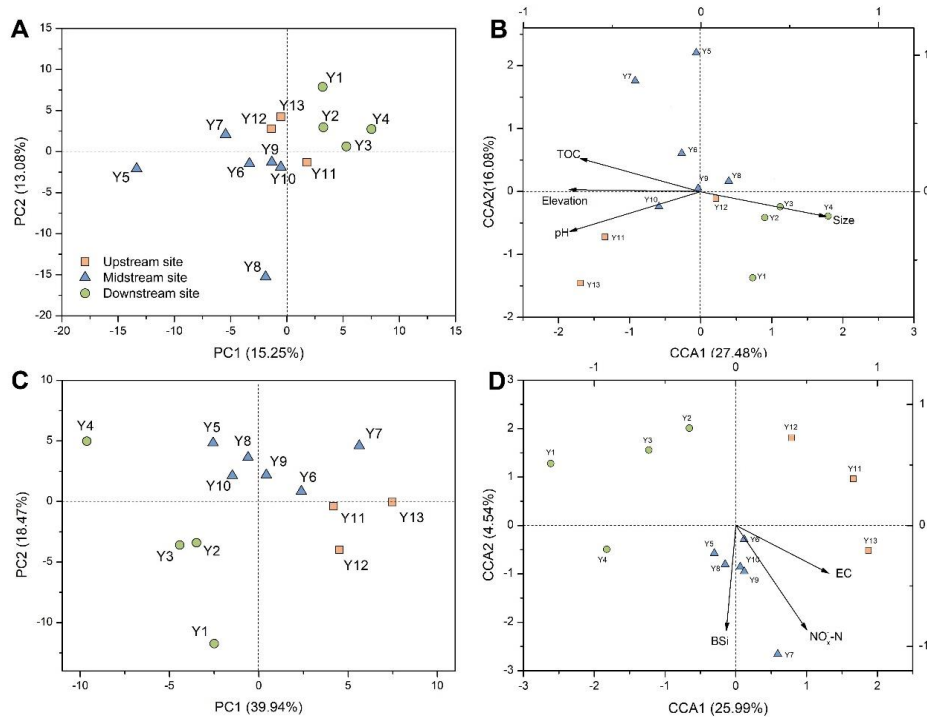


Figure 5. PCA analysis of bacterial community (A) and predicted function (C) of the sediment samples from the Yarlung Tsangpo River. Relationship between bacterial community (B)/ predicted function (D) and environmental variables according to CCA. In panel B and D, symbols were spotted by bottom and left axis and arrows were spotted by top and right axis.

connected OTUs.

4. Discussion

4.1. Spatial Change of Physicochemical Parameters of Sediment in the Yarlung Tsangpo River

Upstream sites of the Yarlung Tsangpo River were characterized by higher velocity, increasing sand portion and stable EC. The higher velocity was related to the steep landscape of the upstream area which was the endemic geographic feature in high-elevation rivers. And the rapid flow may wash away the resuspended silt portion, making sand the main component of the sediment, which was different from the dominance of silt/clay speciation in the Lancang River (a typical plain river) (Liu et al., 2015). The increase of EC in midstream sites can be attributed to the inflow of the Lhasa River, a tributary passing through the city of Lhasa, which contained urban wastewater discharge and increased ion concentration in sediment. In the Zangmu Reservoir, velocity stayed at a low level due to the change from lotic system to lentic system by river damming, which was also observed in many large river systems (Nilsson et al., 2005). The decrease of velocity enhanced the sedimentation of suspended particles (Yan et al., 2015), especially particles measured between 4 to 63 μm (silt). According to the study in the Yangtze Estuary, higher silt may lead to high level of EC as more ions can be released from silt (Wang et al., 2015), which is also confirmed in the present study.

Moreover, the nutrients (C, Si, N and P) showed various pattern in the midstream sites. Carbon, silicon and nitrogen all increased in midstream sites and decreased sharply downstream of the dam, indicating the significant sedimentation and retention by the construction of dam (Teodoru and Wehrli, 2005; Yang et al., 2013). Agriculture has long been developed in the midstream area of the river, and recently, industries and tourism have also expanded rapidly (Huang et al., 2011), increasing non-point pollution and nutrient emissions into the river. Since silicon was not widely used in anthropogenic activities, its increase in the Zangmu Reservoir was due to dam retention, which was supported by previous studies (Humborg et al., 2006; Ran et al., 2013). Different from other nutrients, TP kept increasing in midstream and dam-associated sites, which was in good agreement with the upward trend reported in the plain rivers (Liu et al., 2015), suggesting that phosphorus in sediment was more sensitive to wastewater discharge than to river damming in the Yarlung Tsangpo River.

4.2. Anthropogenic Disturbances on Sediment Bacterial Community

The sediment bacterial biomass and diversity showed complex variation in midstream sites where there existed dense anthropogenic activities. The biomass and diversity varied differently downstream of the Lhasa River, which loaded growing amount of nutrients (N and P) into the mainstream. Increased nutrient concentration has been shown to stimulate sediment bacterial numbers (Wakelin et al., 2008). Moreover, the effluent

discharge has been proved to contribute to biotic homogenization (Ruiz-González et al., 2013) as the increase of nutrients accelerated the growth of bacteria which can utilize nutrients more efficiently and contributed to their dominance in the community, resulting in the co-occurrence of high biomass and low diversity in our study. However, the biomass and diversity both decreased downstream of the dam, indicating the inhibition effect on sediment bacterial community by dam construction. The impoundment of dams would disturb river continuum (Vannote et al., 1980), resulting in the alteration of hydrological parameters. In consequence, the synchronous decrease of biomass and diversity downstream of the dam was due to the quick response of bacteria to environmental alterations (Liu et al., 2012).

According to taxonomy results, Proteobacteria, an important contributor to biogeochemical processes (Xia et al., 2013; Ligi et al., 2014), dominated the sediment bacterial community in the Yarlung Tsangpo River. In our study, α -, β -, and δ -Proteobacteria all increased in middle reaches where wastewater discharge and river damming occurred. Similar results were also reported that damming has led to greater relative abundance of α -Proteobacteria (Zhou et al., 2016). Since α - and β -Proteobacteria were involved in nitrogen fixation (Liu et al., 2012) and δ -Proteobacteria take part in denitrification (Li et al., 2016), it is deduced that the nitrogen cycling process might be affected by anthropogenic disturbances in the Yarlung Tsangpo River. Besides Proteobacteria, Bacteroidetes also increased in midstream sites. As they are believed to degrade high-molecular-weight organic compounds (Drury et

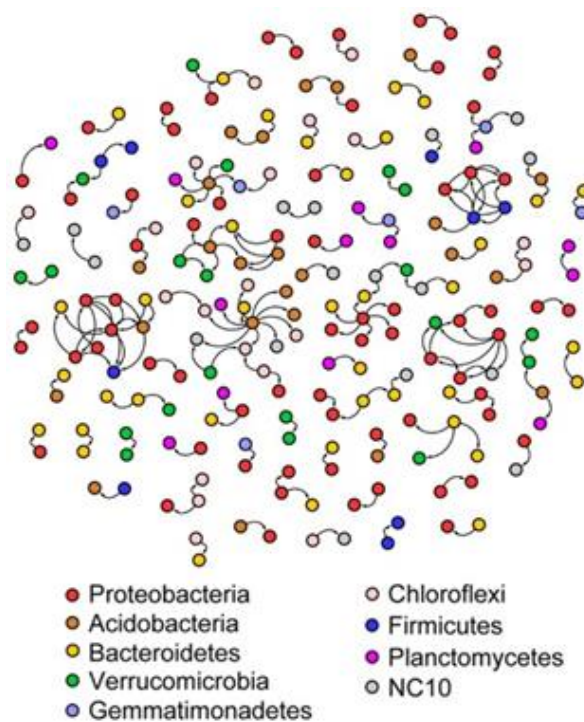


Figure 6. Network of co-occurring OTUs based on correlation analysis. A connection stands for a strong (Spearman's $r > 0.95$) and significant ($p < 0.01$) correlation.

al., 2013), the elevated wastewater discharge in midstream areas was responsible for the stimulation of Bacteroidetes.

Different from midstream sites, upstream sites were also dominated by Verrucomicrobia, however, the ecological roles of which remained largely unknown (Jordaan and Bezuidenhout, 2016) and more investigations are needed in the future. In downstream sites, Firmicutes increased remarkably downstream of the Zangmu Dam and made up a great portion of sediment bacterial community. They have been commonly found in freshwater sediment (Bueche and Junier, 2016) and can survive in extreme environments (Li et al., 2011), such as in the low carbon and nitrogen environment downstream of the Yarlung Tsangpo River.

In the term of bacterial community composition, it is reported that similarity in bacterial community composition may be attributed to biotic homogenization (Drury et al., 2013). Since the midstream sites were distinct from upstream and downstream sites in the Yarlung Tsangpo River, it is deduced that anthropological modifications inhibited biotic homogenization along the river. Shifts in the sediment bacterial community composition include selections for more resistant or contaminant-specific species with an associated change in overall relative abundances (Jordaan and Bezuidenhout, 2016). Since river damming is an important anthropogenic disturbance on natural water systems and the hydrological and nutrient distributional conditions have changed (Zhou et al., 2016), the adaptive bacteria would be sorted and dominate the community in the altered environment (Jordaan and Bezuidenhout, 2016). Nevertheless, different from sediment bacterial community composition, the predicted functions in upstream and downstream sites of the dam did not exhibit significant variation, indicating that the Zangmu Dam only influenced the sediment bacterial community at the compositional level rather than the functional level. According to previous studies, the variable community composition often co-occurred with consistent functional genes (Rousk et al., 2009; Khatri et al., 2017) as functional redundancy can safeguard bacterial communities by maintaining important ecosystem processes (Eid et al., 2017). As a result, the taxonomically-defined community may not present comprehensive response of microbial ecology when interpreting ecological processes. And further validation of functional inference tools are necessary since discrepancy existed between compositional and functional variations.

4.3. Environmental Drivers and Co-occurrence Relationship of Sediment Bacterial Community

Sediment bacterial communities can be highly changeable in natural rivers due to their rapid responses to environmental disturbances and stresses (Crump et al., 2012; Zeglin, 2015). In the Yarlung Tsangpo River, pH was a vital factor governing the sediment bacterial diversity and composition, which was also observed in the Dongting Lake in China (Johannes Rousk et al., 2010) and in the Santa Ana River in the USA (Ibekwe et al., 2016). Changes of pH in sediments create favorable conditions for specific microbial metabolic functions and taxonomic groups (Jordaan and Bezuidenhout,

2016) which can be sorted and dominate the community with their adaptive abilities (Zeglin, 2015). Elevation also influenced the variation of sediment community diversity and composition in the Yarlung Tsangpo River. The elevation variations are often accompanied by climatic parameter change, including temperature and solar irradiation, which play critical part in bacterial physiological activities (Zeglin, 2015). However, in the plain regions, no significant correlation was observed between elevation and bacterial community composition (Fierer et al., 2011; Y. Wang et al., 2015), suggesting that elevation is the endemic driver shaping the sediment bacterial community in the plateau river.

Moreover, TOC was also the considerable environmental driver for bacterial community composition in our study, which is in good agreement with results in the Dianchi Lake in China (Bai et al., 2012), in the Columbia River in the USA (Fortunato et al., 2013) and in the Siberian continental margin (Sparks, 2003). Sediment bacteria are usually considered as the primary catalysts of organic matter recycling and are potentially contributors of organic carbon to sediments (Zhu et al., 2017), implying that TOC, in turn, may also be an important driver of bacterial community composition variation in sediments. In addition, sediment grain size also contribute to variation of bacterial community composition in our study and in previous studies (Sessitsch et al., 2001). The sandy sediment contains lower organic carbon concentrations while the silty sediment typically contains higher concentration of organic matters (Sparks, 2003), which can be confirmed by the synchronous pattern of silt proportion and TOC concentration in our study. In consequence, the variation of grain size distribution would result in the availability of organic carbon in sediments and eventually shape the bacterial community composition.

Interestingly, different from community diversity and composition, the environmental drivers shaping sediment bacterial community functions were EC, BSi and $\text{NO}_x\text{-N}$. Since EC was also closely correlated with ion concentrations, it is suggested that ion and nutrient condition was significantly associated with functional variation of sediment bacterial community in the Yarlung Tsangpo River. Similarly, previous studies proposed that functional variation was correlated with nutrient concentrations while community composition was closely linked with habitat type (Eid et al., 2017). Nitrogen, silicon and ions are all considered as available essential nutrients for bacterial growth, and in turn, variation of nutrient concentrations would influence bacterial functions of biogeo-chemical cycling (Zwart et al., 2002; Bier et al., 2015), such as the dominance of Amino acid metabolism and Carbohydrate metabolism functions observed in our study. In addition, according to the existence of functional redundancy, bacteria species are considered to develop strategies to survive under a range of nutrient concentrations (Khatri et al., 2017). The species which exhibit contrasting nutrient affinities often co-exist, but with lower activity under suboptimal conditions (Fan et al., 2015), indicating the important role of nutrients in functional variation rather than in composition variation.

In order to investigate internal interactions of bacterial community, network analysis was conducted in our study to present the co-occurrence of OTUs in different bacterial taxo-

nomic groups. It was expected that the OTUs may potentially be ecologically interacted with each other. However, the 9 bacteria phyla were not highly connected and featured non-synchronized dynamics. Only some OTUs of Acidobacteria were observed to co-occur with other phyla, which was consistent with previous results that Acidobacteria tended to co-occur more than expected by chance (Johannes Rousk et al., 2010), suggesting that they share an unknown specific niche in aquatic ecosystems. In our study, few OTU was detected to possess associations in our study, indicating that there existed no 'hubs' in the sediment bacteria in spatial gradients along the Yarlung Tsangpo River. As a result, temporal distribution of sediment bacterial community would also be investigated in our future studies, including the collection of sediment samples in different layers and the seasonal sampling along the Yarlung Tsangpo River.

5. Conclusions

In summary, river damming influenced sediment physical properties and retained biogeochemical nutrients of carbon, silica and nitrogen in the reservoir, but phosphorus was more sensitive to wastewater discharge. Besides, wastewater discharge resulted in asynchronous change of bacterial biomass and diversity while they were both inhibited by the construction of dam. Moreover, significant alterations in the community composition were observed in dam-associated sites, but no remarkable functional variation was detected due to functional redundancy. Elevation was the endemic driver on the Tibetan Plateau shaping the sediment bacterial community diversity and composition along with pH, while nutrient concentrations were responsible for functional variation. Furthermore, little synchronized dynamics of co-occurrence was observed in the sediment bacteria in the Yarlung Tsangpo River. In conclusion, anthropogenic disturbances influenced the sediment bacterial community at the compositional level rather than the functional level in the typical high-elevation river. Therefore, our study provides potential evidence that diversity, composition and function of sediment bacterial community should all be taken into consideration in the assessment of microbial ecology in the Tibetan Plateau.

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