LIMNOLOGY and OCEANOGRAPHY

Bacterial communities in cascade reservoirs along a large river

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Abstract

Dam construction is widespread, changing the hydrological and biogeochemical conditions and thereby the bacterial communities in the rivers of the earth. To date, knowledge is lacking about bacterial communities in cascade reservoirs. Here, we investigated the bacterial communities and potential functions of nine cascade hydropower reservoirs in 1290 km of the upper Mekong River (Lancang River in China). Along the reservoir cascade, the water temperature, rather than the presence of dams, was the main cause for the geographical patterns of bacterial community composition. Within a reservoir, significant spatial differences in sediment bacterial communities were observed between the tail, middle, and head of a reservoir. The differences in sediment properties resulted by flow velocity-sieved sedimentation from the tail to head of a reservoir caused the spatial variation in sediment bacteria communities, forming potential hotspots for biogeochemical cycling in the middle of the reservoir. In contrast, unlike in deep lakes and deep single reservoirs, the bacterioplankton community composition had no distinctly layered features in the deep cascade reservoirs, because density-induced underwater currents and convection resulting from hydropower production reduced the vertical hydro-environmental gradients. This study provides a novel perspective on the processes affecting the distribution and function of bacterial communities in river-reservoir cascades, and is a first step toward forecasting the consequences of microbially mediated biogeochemical cycling in existing and future reservoirs worldwide.

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Rivers are the vessels on the earth, transporting water and substance from land to lakes and seas, and providing rich social-economic services (Seitzinger et al. 2010). Along the flow path, biogeochemical cycling of nutrients takes place, affecting the water quality and ecosystem in the river corridor and downstream (Shi et al. 2020). To meet the globally increasing energy demand and to improve water supply as well as flood management, over 70,000 large dams (more than 15 m in dam height or more than 3,000,000 m^3 in storage capacity) have been built worldwide, and 3700 large dams are planned for construction within 15 yr (Grill et al. 2015; Maavara et al. 2015). The development of hydropower provides human populations not only with clean energy, but also with other social-economic benefits such as poverty alleviation in remote rural areas (Chen et al. 2020a). However, river damming disrupts the continuity of a river and alters the

hydrological as well as hydrodynamic regimes, and thus the nutrient conditions and riverine ecosystem (Maavara et al. 2020).

Microbial communities play a key role in the material cycling and energy transfer of aquatic ecosystems, and are meanwhile affected by hydro-environmental conditions. Great efforts have been made to understand how the bacterial communities are shaped by geographical and environmental factors (Bier et al. 2015; Aldunate et al. 2018; Milaković et al. 2019). It has been reported that the riverine bacterial communities are affected by latitude (Segovia et al. 2016), elevation (Liu et al. 2018), water temperature (Xia et al. 2014), pH (Liu et al. 2015), land use (Gibbons et al. 2014), the surrounding landscape (Kolmakova et al. 2014), nutrient regime, and organic matter (Logue et al. 2012; Hu et al. 2014; Staley et al. 2016). Along a river, the upper reach had an uncrowded environment, favoring species like *Bacteroidetes* with high growth rates and low competitive ability (*r*-selection). With relatively higher water temperature and slower flow velocity downstream, species like *Actinobacteria* with low growth rates and highly competitive ability become dominant (*k*-selection) (Andrews and Harris 1986). The tributaries carry microbial communities from different habitats, mainly contributing the "mass effects" along a river (Lindström et al. 2005; Moitra and Leff 2015; Niño-García et al. 2016). However, local hydro-environmental conditions make specific species more competitive and dominate the local bacterial community, mainly contributing the "environmental selection," and resulting in the "species-sorting" (Székely et al. 2013; Staley et al. 2015).

The alteration of hydrological conditions and the interception of sediment with organic matters in reservoirs by dam construction can increase local hydro-environmental heterogeneity, enhancing "species-sorting" and thus affecting riverine microbial communities (Lindström and Bergström 2004; Niño-García et al. 2016). In many cases, a single deep reservoir holds similar hydro-environmental features of a deep lake. For example, they usually have thermal stratification seasonally, separating the water column into hypolimnion and epilimnion (Boehrer et al. 2008; Degermendzhy et al. 2010; Liu et al. 2019). The stratification of water temperature and dissolved oxygen (DO) can have significant impacts on the bacterioplankton community distribution, showing relevant difference in species composition vertically (Yu et al. 2014). The possible changes in bacteria communities by river damming could have significant impacts on biogeochemical cycling of materials (Chen et al. 2018; Chen et al. 2020b), particularly carbon (C) and nitrogen (N), whose biogeochemical processes are closely linked to bacterial composition, rich, and activity (Arrigo 2005).

Nowadays, cascades of reservoirs on a river are becoming increasingly common. Cascade dams in a river usually form a series of reservoirs with different features, such as small storage reservoirs with short hydraulic residence time (HRT) and vertically mixed water, as well as large storage reservoirs with long HRT and vertically stratified water (Ruiz-González et al. 2013). In a reservoir cascade, the hydro-environmental condition of downstream reservoir is affected by the discharge from the nearest upstream reservoir, which may cause the characteristics of bacteria community different from that of a single reservoir or lake. Currently, there is a great knowledge gap concerning bacterial communities in cascade reservoirs.

The aims of this study are: (1) to understand the spatial patterns of bacterial communities in water and sediment along cascade reservoirs; (2) to reveal the features as well as associated mechanism of bacterial communities in water and sediment within a reservoir of a cascade. We hypothesized that cascade dams isolate the bacterial community along a river, and deep cascade reservoirs hold relevant differences in bacterial communities longitudinally and vertically, with a consistent impact on riverine biogeochemical cycle of materials locally and globally.

Materials and methods

Study area

The Lancang-Mekong River, which originates from the Tibetan Plateau in China and discharges into the South China Sea, is an important transboundary river in Southeast Asia. It is the 12th longest river in the world, flowing a total length of 4880 km from north to south and falling more than 5000 m in elevation. It drains an area of 800,000 km^2, and has an average annual runoff of 475×10^9 m^3. A number of hydropower dams have been built, are under construction or are planned, to exploit the rich hydro-energy of the Lancang River. By the end of 2018, nine hydropower dams from upstream to downstream were operating in China, including Huangden, Dahua, Miaowei, Gongguoqiao, Xiaowan, Manwan, Dachaoshan, Nuozhadu, and Jinghong (Fig. 1). The Nujiang River, which is not dammed, has the same origin and elevation change and climatic zones as the upper Mekong River, and flows in parallel to the upper Mekong River, was investigated for comparison with the heavily dammed Lancang River.

Field survey

Sixty-nine sampling sites (Fig. 1a) were carefully selected according to the hydrological conditions along the river, the location and properties of the reservoirs, and information from previous studies (Shi et al. 2017). Vertical water samples were collected from surface to bottom at different depths and from the tail to head of the two deep reservoirs named Xiaowan and Nuozhadu, which have a water depth of over 150 m. Meanwhile, nine sampling sites were carefully selected along the Nujiang River roughly according to the locations of the nine reservoirs in the parallel Lancang River (Fig. 1a).

Sampling along the Lancang River was carried out at the 69 sites in September 2018, because bacterial communities tend to be more active in warm periods, and the study area has no distinct seasonal characteristics with respect to local
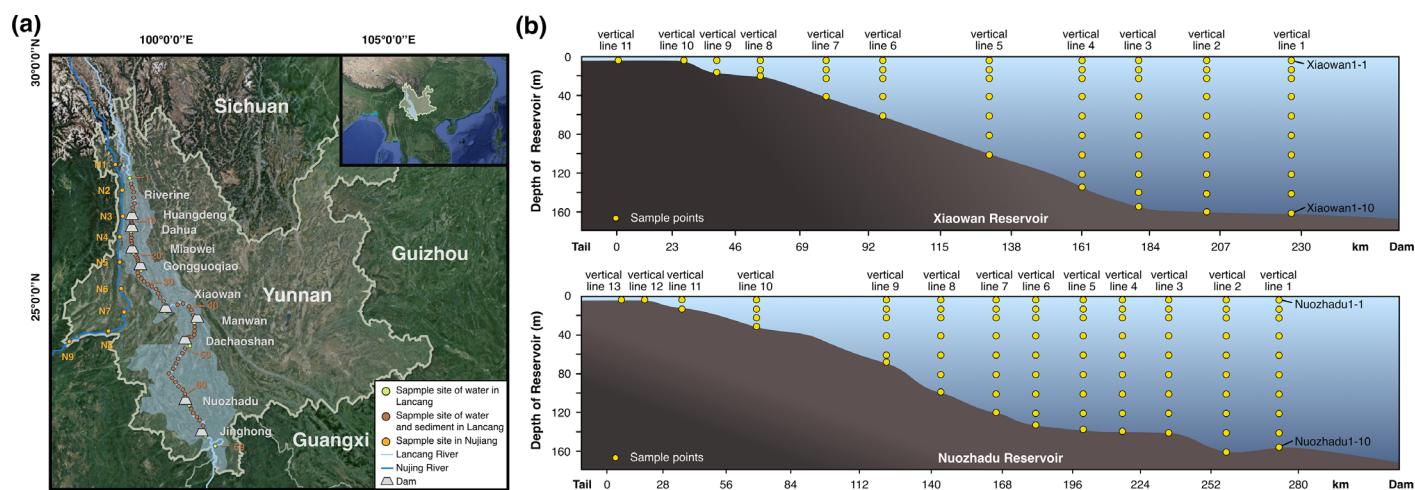
Fig. 1. Study area and sampling sites. (**a**) The 69 sample sites along the Lancang River and 9 sample sites along the Nujiang River; (**b**) vertical sampling lines in Xiaowan Reservoir (11 vertical profiles) and Nuozhadu Reservoir (13 vertical profiles).

temperature (Nadarajah et al. 2007; Arandia-Gorostidi et al. 2017). In total, 69 water samples and 58 sediment samples were collected for analyses of water quality and bacterial communities (Fig. 1a). Because of the high-water velocity immediately after the dams, and in the first as well as the last sites, no sediment was available to sample, which reduced the number of sediment samples in the Lancang River. In the same period, nine water samples and nine sediment samples were collected along the Nujiang River for the same analyses (Fig. 1a). The specific information on sampling sites and sampling times is provided in Tables S1 and S2. At each site along the river, surface water was collected from a depth of 0.5 m with a stainless-steel bucket. Surface sediment with depths ranging from 0.1 m in the river channel to 0.5 m in the reservoir were sampled with an Ekman grab sampler, transferred to a 15 mL sterile centrifuge tube and stored at $-80°C$ until further analysis.

Vertical profile sampling in the Xiaowan Reservoir and the Nuozhadu Reservoir (Fig. 1b) was carried out simultaneously, because stratification is more common in this period (Elçi 2008). The vertical profile sampling was conducted using an automatic deep-water sampler (Fig. S1). Sampling depths of each vertical line was set in advance according to the vertical temperature profile. Water samples were collected at 0.5, 3, 10, 20, 40, 60, 80, 100, 120, 140, and 160 m in sequence from the surface to bottom waters. In total, 62 and 85 vertical water samples were collected from the two deep reservoirs, respectively.

Sample collection and determination

At each site, triplicate samples were collected and mixed together to produce one sample for analysis, both for water and sediment. Water samples were filtered through Durapore membrane filters (pore size 0.22 μm, Millipore). Water temperature, DO, pH, and turbidity were measured in situ using a multisensor probe (YSI EXO2, Yellow Spring Instruments).

Sediment pH was determined at a 1 : 2.5 sediment to distilled water ratio (*w/w*). Concentrations of total nitrogen (TN) and total phosphorus (TP) were analyzed after acid hydrolysis at $340°C$. Nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), ammonium nitrogen (NH_4-N), and phosphate phosphorus (PO_4-P) in both water and sediment were determined spectrophotometrically according to standard methods (Ministry of Environmental Protection of China 2002). Total carbon content in the sediment samples was determined using an elemental analyzer (Vario MACRO cube, Elementar) after being freeze-dried by a freeze-dryer (ALPHA 2–4 LSC, Martin Christ). The denitrification potential of sediment samples was determined by the acetylene-inhibition method under the conditions of constant temperature at $20°C$ and light shielding, and shaking the culture at a rate of 70 r min^{-1} for 4 h. The N_2O gas produced was brought back to the laboratory under normal temperature and dark conditions, and determined by a gas chromatograph (7890B, Agilent) within 24 h. The N_2O gas production was calculated by the Weiss gas solubility formula (Weiss and Price 1980). The measurement was repeated three times for each sample. Sediment size d_{50} was determined by a Laser Particle Size Analyzer (Microtrac S3500). Elevation, latitude, and longitude were measured by a portable GPS map system (629sc, Garmin) at each sampling site. River-kilometer (Rodriguez-Iturbe et al. 2009) values between sampling sites were obtained by ArcGIS software (V10.2, ESR).

DNA extraction and sequencing

Bacterial DNA was extracted using a FastDNA SPIN Kit (Bio 101) following the manufacturer's instructions (Hu et al. 2014). The extracted DNA was diluted in 60 μL TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0), followed by concentration and purity analyses (Thermo Fisher Scientific NanoDrop-2000) and stored at $-20°C$ until use. The V4 region of the prokaryotic 16S rRNA gene was amplified using a universal primer pair 515F: 5'-GTGCCAGCMGCCGCGGTAA-3'/806R: 5'-GGACTACHVGGGTWTCT

AAT-3′ under the following conditions: initial denaturation for 5 min at 95°C, followed by 30 cycles of denaturation at 95°C for 45 s, annealing at 52°C for 45 s as well as elongation at 72°C for 1.5 min, and followed by a final elongation step at 72°C for 15 min (Youssef et al. 2009). Triplicate polymerase chain reactions (PCRs) were performed for each sample and electrophoresed on a 2% agarose gel and then recovered. The mixture of PCR products was purified with Qiagen Gel Extraction Kit (Qiagen). Sequencing libraries were generated using TruSeq DNA PCR-Free Sample Preparation Kit (Illumina) following the manufacturer's recommendations, and index codes were added. The high-throughput sequencing was run on the Illumina MiSeq platform (Illumina) using a paired-end (2 × 250 bp) approach.

qPCR for specific genes

The extracted sediment DNA was subjected to real-time quantitative polymerase chain reaction (qPCR) to quantify the N-cycling and C-cycling bacteria in sediment. Genes encoding catalytic enzymes responsible for ammonia-oxidation (AOA-*amoA*), nitrite reductase (*nirS*), and nitrous oxide reductase (*nosZ*) were used as

molecular markers to assess the abundances of crenarchaeal ammonia-oxidizers and denitrifiers in two key steps, respectively (Dhillon et al. 2005; Lam et al. 2009; Hafeez et al. 2012; Wang et al. 2015). C-cycling microbial communities were quantified using the alpha subunit of the methanobacterium methyl coenzyme M reductase (*mcrA*) as a molecular marker to assess the abundances of methanogens in sediment microbial communities (Luton et al. 2002; Henry et al. 2006). The qPCR assays were performed in triplicate on a BioRad CFX 96 PCR system (BioRad). All reactions were completed in a total volume of 20 μL containing 12.5 μL SYBR® Premix Ex TaqTM (Takara), 0.5 μL of each primer, 0.5 μL BSA (3 mg mL^{-1}, Sigma), 4 μL double-distilled water, and 2 μL of template DNA. The qPCR program is listed in Table S3. The results of qPCR efficiency and R^2 of the standard curve is showed in Fig. S2.

Statistical analysis

The 16S rRNA gene sequences were analyzed on the online platform of Majorbio I-Sanger Cloud Platform. The quality filtering of raw DNA sequences was analyzed using QIIME
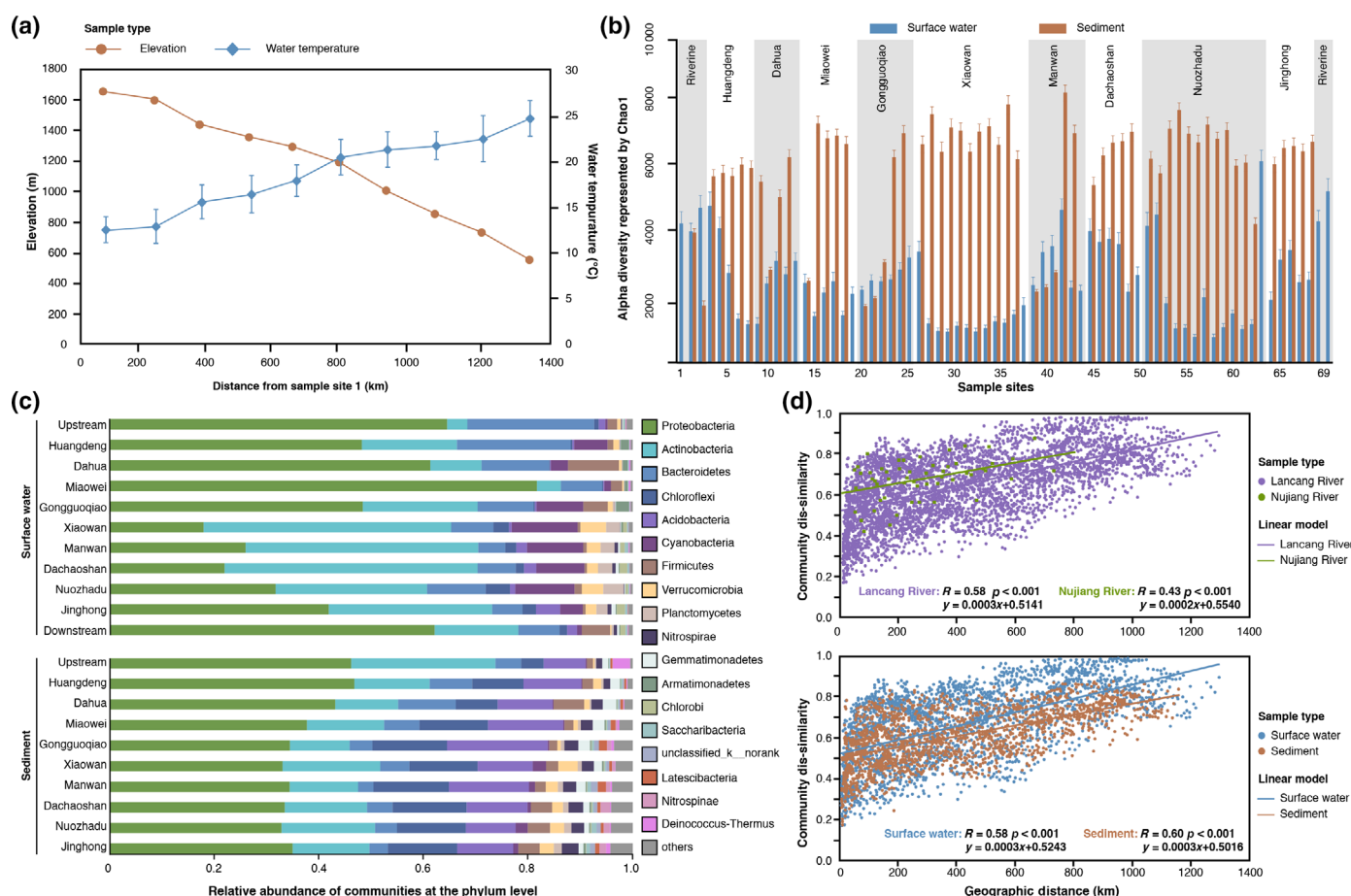

Fig. 2. The geographical features and bacterial community distribution along the Lancang-Mekong River with nine cascade reservoirs. (**a**) Elevation and water temperature along the Lancang River; (**b**) alpha diversity of bacteria in surface water and sediment along the Lancang River. (**c**) Relative abundance of bacteria at the phylum level categorized by surface water and sediment; (**d**) relationship between bacteria community dissimilarity and geographic distance in the Lancang River with cascade dams and the parallel Nujiang River with no dam.

(version 1.9.1). Nonrepetitive sequences were clustered to operational taxonomic units (OTUs) by Usearch (version 7.0) and RDP Classifier (version 2.11, default confidence threshold = 0.7) with 97% similarity in each taxonomy level. The singletons were removed, and sequence data were then normalized to the same sequence number to reduce bias. The Chao1 index was used to represent the alpha diversity of bacterial communities by estimating the number of OTUs contained in samples (Chao 1984). Nonmetric multidimensional scaling (NMDS) and the Mantel test were conducted using Bray–Curtis similarity in the *vegan* package and *ggplot2* package in R (version 3.6.1). Heatmap analysis was used to show the correlation between bacterial communities and environmental factors (water temperature, pH, sediment size d_{50}, turbidity, DO, TN, TP, NO_2-N, NO_3-N, NH_4-N, PO_4-P, TOC, and river-kilometer) by the *pheatmap* package in R software (version 3.6.1). Principal component analysis was applied to reduce the dimensions of the original variables by the *Facto MineR* package and the *ggplot2* package in R software (version 3.6.1). Analysis of similarities was used to examine whether there is a significant difference between two groups. Sample

enzyme gene abundances in predictive relative metabolic turnover (PRMT) analysis and heatmap for relative abundance of functions related to N-cycling and C-cycling were transformed by a weighted matrix of possible metabolic reactions collected from the Kyoto encyclopedia of genes and genomes (KEGG) database (Ogata et al. 1999; Dunlap et al. 2013).

Results

Structure and geographic patterns of bacterial communities along the river

A total of 13,588 bacterial OTUs were identified in all samples by high-throughput sequencing, which contained 11,133 OTUs in water, and 12,848 OTUs in sediment along the dammed Lancang River. The bacterial OTUs obtained from the current sequencing depth were sufficient to represent the bacterial community in all samples (Fig. S3). The lowest water temperature was 12.19°C in the upstream river channel, and the highest was 29.03°C in the downstream Nuozhadu Reservoir (Fig. 2a). The elevation of the highest sampling point was 1663 m and the lowest was 496 m (Fig. 2a).
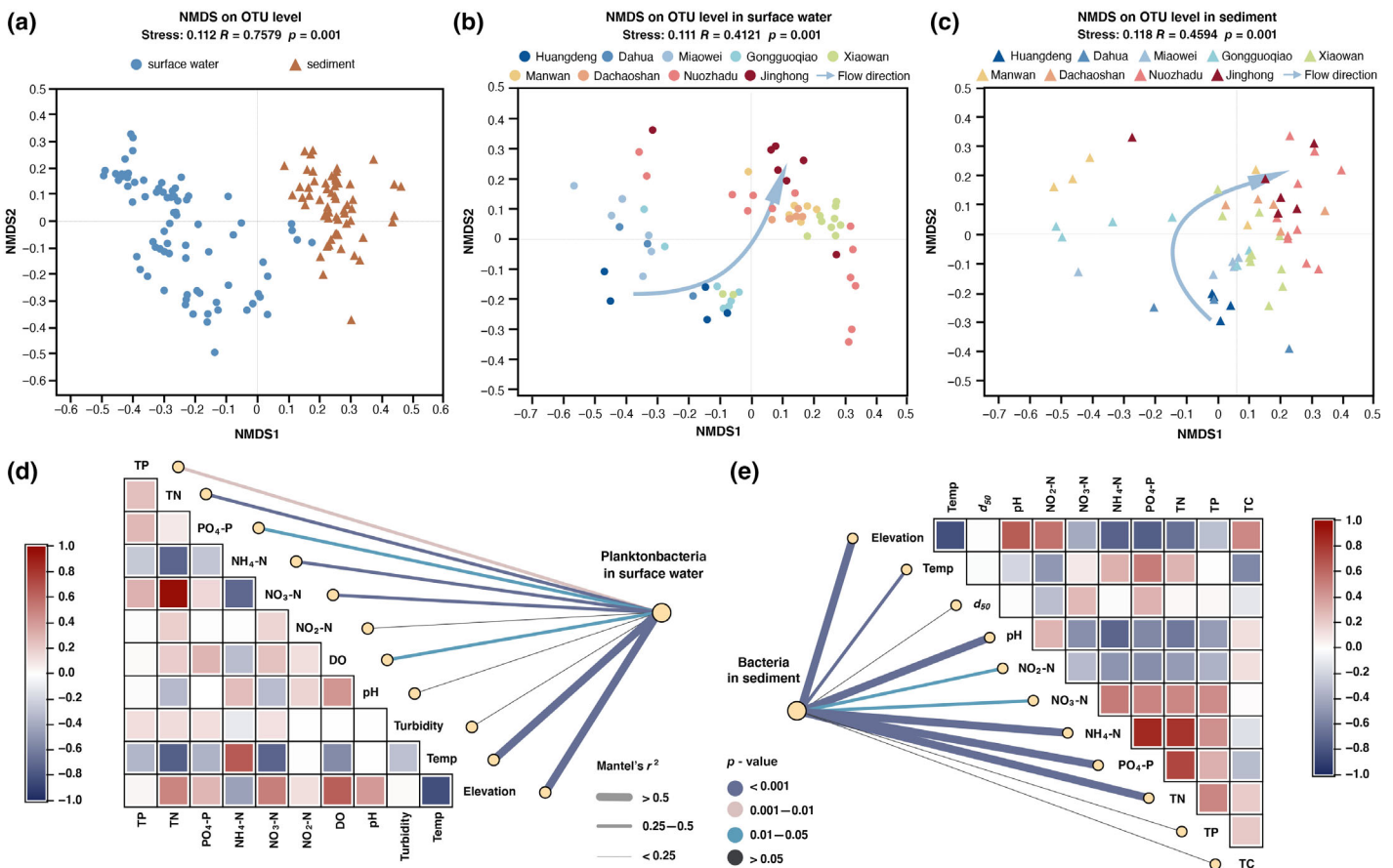

Fig. 3. NMDS ordination for bacteria along the Lancang-Mekong River and heatmap analysis with linear regression of correlation between bacterial community diversity and environmental factors. (**a**) Categorized by surface water and sediment in all samples; (**b**) categorized by nine reservoirs in surface water; (**c**) categorized by nine reservoirs in sediment; (**d**) heatmap of correlation between surface water bacterial OTUs and environmental factors; (**e**) heatmap of correlation between sediment bacterial OTUs and environmental factors.

The alpha diversity of bacteria in surface water and sediment was represented by the Chao1 index, which showed more sensitivity to rare species (Fig. 2b). At the catchment scale, there was no significant difference in diversity in either the surface water or sediment from upstream to downstream. Even immediately after a dam, there was no dramatic change in diversity in the surface water. However, the average diversity in sediment was 1.35 times higher than that in surface water, and particularly significant differences ($p < 0.01$) in diversity were observed between surface water and sediment in the deep reservoirs of Xiaowan and Nuozhadu.

At the phylum level, the bacterial communities were dominated by *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Choroflexi* in both surface water and sediment along the entire Lancang River (Fig. 2c). However, surface water bacteria had higher proportions of *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Verrucomicrobia*, and *Planctomycetes*, while sediment bacteria had higher proportions of *Chloroflexi*, *Acidobacteria*, *Firmicutes*, *Nitrospirae*, and *Gemmatinonadetes*. The proportion of *Proteobacteria* to total phyla was over 45%, with an average of 57%, from the upstream river channel to the Miaowei Reservoir. The proportion of *Proteobacteria* decreased to 27% on average from Gongguoqiao Reservoir to Jinghong Reservoir.

The geographic similarity of bacterial communities in the dammed Lancang River was compared to that in the parallel Nujiang River without dams (Fig. 2d). Since the flow velocity was not uniform because of the cascade dams, the mean dendritic stream length was not applicable here. Thus, river-kilometer was used as the geographic distance between the sampling sites in this study. Significant correlations were found between Bray–Curtis-based bacteria community dissimilarity, including sediment bacteria and bacterioplankton, and geographic distance in both the Nujiang River ($R = 0.43$,
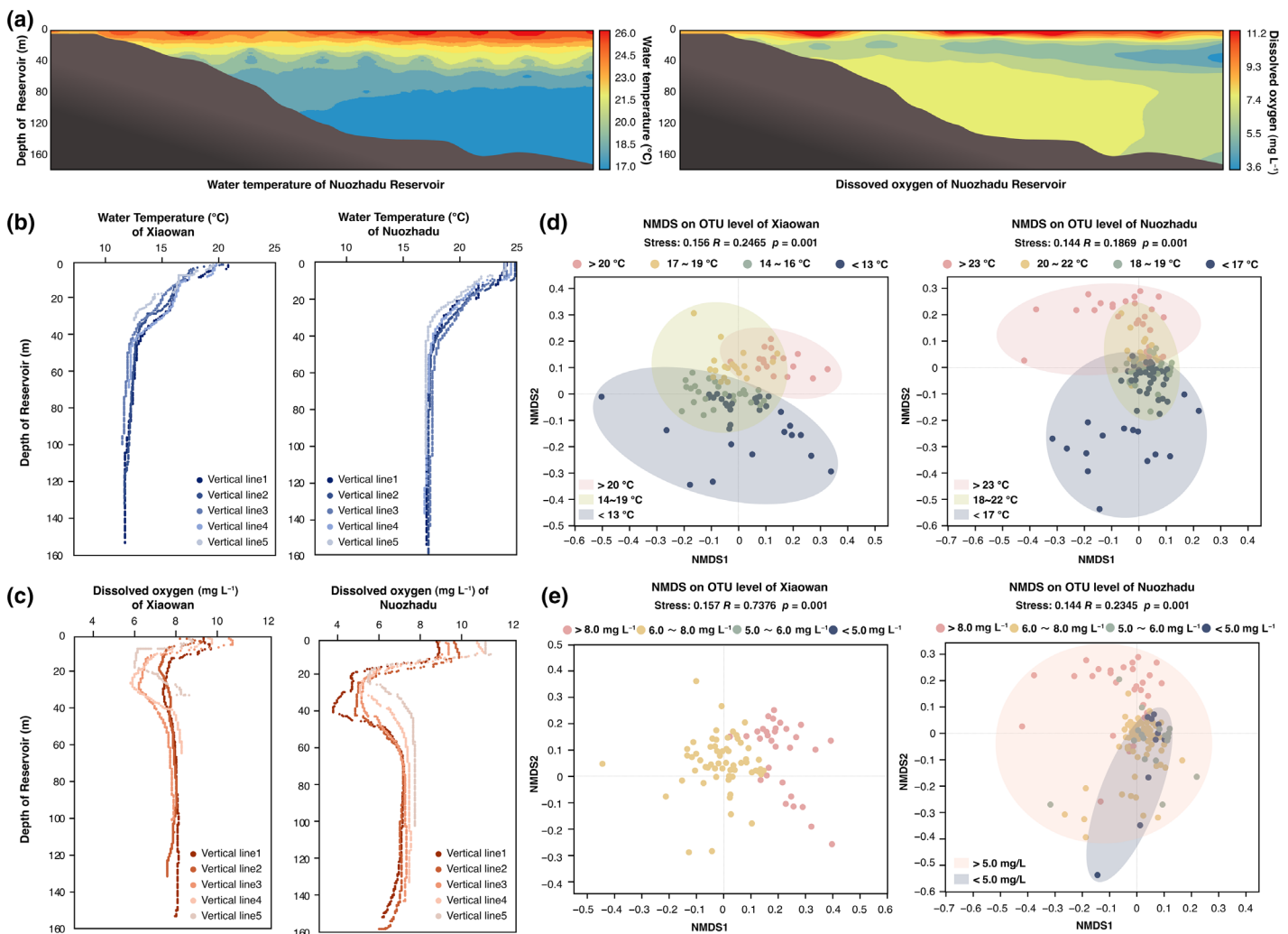

Fig. 4. Vertical distribution of water temperature, DO and bacterial community in Xiaowan Reservoir and Nuozhadu Reservoir. (**a**) Contour map of water temperature and DO; (**b**) vertical profile of water temperature; (**c**) vertical profile of DO concentration; (**d**) NMDS ordination of bacterioplankton community for different water temperatures; (**e**) NMDS ordination of bacterioplankton community for different DO concentrations.

$p < 0.001$) and the Lancang River ($R = 0.58$, $p < 0.001$). The slope of the trend was slightly lower in the Nujiang River without dams than in the dammed Lancang River. Significant correlations were also found between community dissimilarity and geographic distance in water ($R = 0.58$, $p < 0.001$) and sediment bacteria ($R = 0.60$, $p < 0.001$) in the Lancang River.

Our NMDS showed that the geographical distributions of bacteria were clearly divided between surface water (blue dots in Fig. 3a) and sediment (orange triangles in Fig. 3a). We therefore analyzed bacterial beta diversity in surface water and sediment separately. Similar geographic distribution patterns were found in both surface water and sediment, where in the ordinations of bacterial beta diversity in the cascade reservoirs followed the flow direction from upstream to downstream (Fig. 3b,c).

The heatmap of correlation between the bacterial beta diversity and the environmental factors in surface water and sediment (Fig. 3d,e) showed that water temperature, which is associated with elevation, was the major factor shaping bacterial communities in surface water ($R = 0.77$, $p < 0.001$). The nutrient conditions (TN, NH$_4$-N, PO$_4$-P) and pH had greater impacts on sediment bacteria ($R = 0.63$, 0.59, 0.62, 0.51, $p < 0.001$) than on bacterioplankton.

Vertical distribution of bacterioplankton community within reservoirs

Considering the possible stratification of temperature and oxygen in deep-water bodies, the vertical distributions of bacterioplankton and the related physical properties in the two deep reservoirs (Xiaowan and Nuozhadu) were analyzed. The vertical profiles of water temperature and oxygen in the two reservoirs are shown in Fig. 4a–c as well as in Tables S4 and S5. The NMDS analyses on the bacterioplankton community, with respect to water temperature and DO, are shown in Fig. 4d,e.
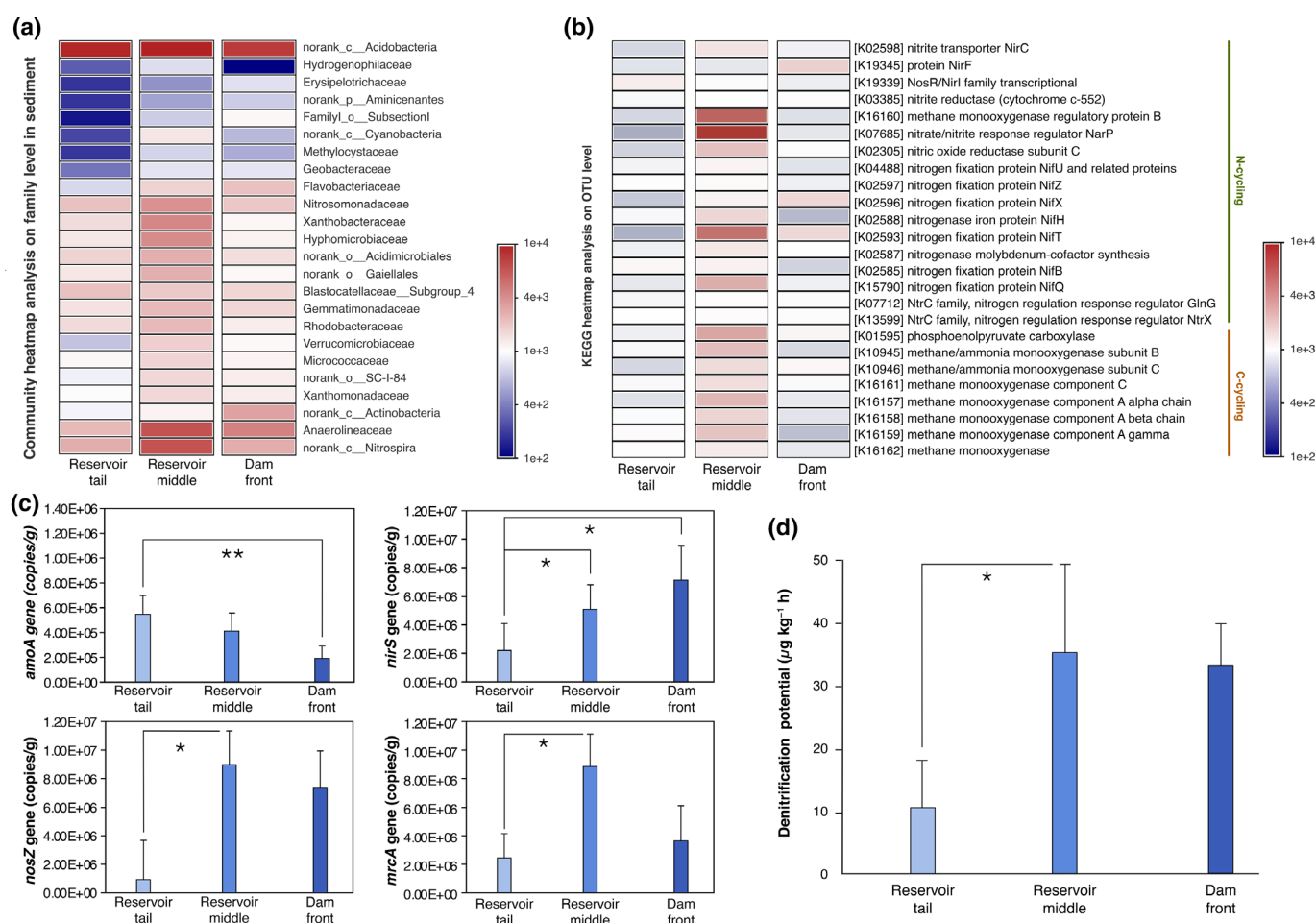

Fig. 5. Spatial distribution patterns of sediment bacterial community and functions. (**a**) Community heatmap at family level; (**b**) relative abundance heatmap of functional genes corresponding to KEGG pathway related to N-cycling and C-cycling. The relative abundance was displayed by a numerical matrix heatmap with color depth indicating the average ratio of each group; (**c**) abundance of catalytic enzymes gene responsible for N-cycling (*amoA*, *nirS*, and *nosZ*) and C-cycling (*mrcA*); (**d**) denitrification potentials. The error bars represent the standard deviation, and the *t*-test shows the significance of difference between the reservoir tail, reservoir middle and dam front (**p* < 0.05, ***p* < 0.01).

In both reservoirs, the water temperature decreased gradually from the surface to the depth of about 60 m and then kept almost constant, and no clear thermocline was observed (Fig. 4b). NMDS analysis with respect to water depth showed that no distinctly layered distribution of bacterioplankton was observed in the two deep hydropower reservoirs (Fig. S4). However, NMDS analysis with respect to water temperature (Fig. 4d) showed that in Xiaowan Reservoir, the bacterioplankton communities below 13°C at the hypolimnion were separated from the communities above 20°C in the surface layer; while in Nuozhadu Reservoir, the bacterioplankton communities below 17°C at the hypolimnion were separated from the communities above 23°C in the surface layer. Interestingly, there was no detectable difference in bacterioplankton community in the layer between 13°C and 20°C in Xiaowan, and in the layer between 17°C and 23°C in Nuozhadu. To further investigate temperature effects, the bacterial communities in the hypolimnion (17°C) in Nuozhadu, the epilimnion (17°C) in Xiaowan, and the hypolimnion (13°C) in Xiaowan were selected to compare their similarity. The results showed that the Bray–Curtis-based similarities of bacterial communities were similar in the same temperature layer (17°C) even in different reservoirs, while the bacterial communities were different between the epilimnion (17°C) and hypolimnion (13°C) even in the same reservoir (Fig. S5).

In both reservoirs, there was a sharp decline in DO from the surface to the depth of about 30 m. The DO dropped below 6.0 mg L^{-1} in Xiaowan Reservoir and below 4.0 mg L^{-1} in Nuozhadu Reservoir. However, the DO recovered back to 7.0–8.0 mg L^{-1} below the depth of 60 m (Fig. 4c). NMDS analysis on the bacterioplankton community (Fig. 4e) showed that there was no significant vertical difference ($p = 0.61$) in bacterial community between relatively high DO (> 5.0 mg L^{-1}) and low DO (< 5.0 mg L^{-1}) layers, even though there existed a thin layer with low DO in both reservoirs (Fig. 4c).

Longitudinal patterns of sediment bacteria communities within reservoirs

The sediment samples were divided into three groups according to their location in reservoirs: the reservoir tail (reservoir tail), the middle of the reservoir (reservoir middle) and in front of the dam (dam front) to analyze the spatial patterns of the sediment bacterial community. A heatmap (Fig. 5a) on the family level of the sediment community showed that bacteria related to nitrogen metabolism such as genera *Nitrospira*, *Nitrosomonadaceae*, and *Anaerolineaceae* were relatively more abundant in the reservoir middle and dam front than in the reservoir tail. The results of PRMT on the abundance of functional genes showed 32,130 predicted metabolic processes, associated
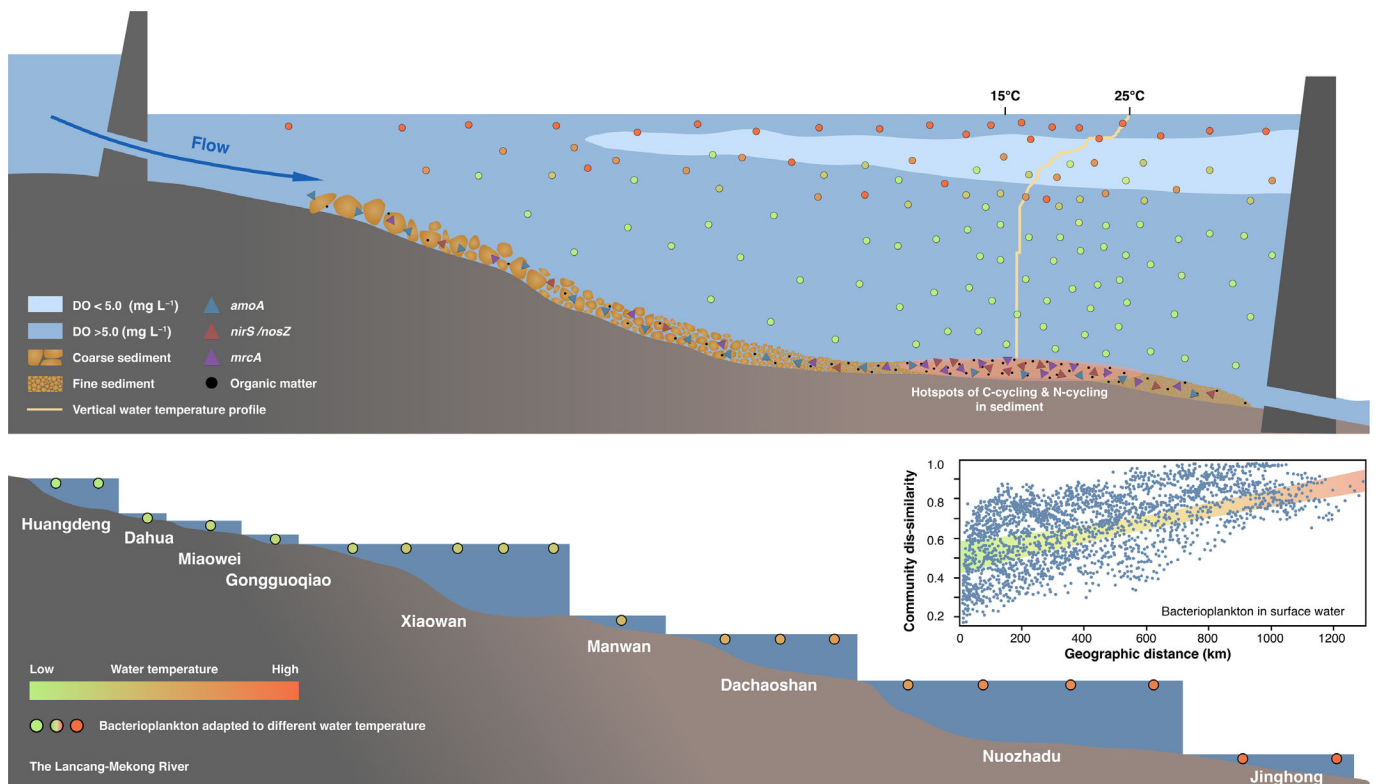

Fig. 6. Conceptual scheme for how cascade dams reshaping the bacteria community. The dots marked from green to orange corresponding to the distributions of bacterial communities from low water temperature to high water temperature are presented in a single reservoir and a cascade of reservoirs, respectively.

with 255 KEGG pathways. The abundance was highest at the reservoir middle, and lowest at the dam front (Fig. S6).

According to the relative abundance heatmap (Fig. 5b) of functional genes corresponding to the KEGG pathway, functional groups related to N-cycling and C-cycling, such as nitrate/nitrite response regulator *narP* (K07685), nitric oxide reductase subunit C (K02305), protein *nifE* (K02587), and the methane mono-oxygenase family, were mostly enriched at the reservoir middle. The qPCR results (Fig. 5c) of sediment bacteria showed that the abundance of AOA-*amoA* was the highest at the reservoir tail and gradually decreased from the reservoir middle to the dam front. Meanwhile, the abundances of *nirS*, *nosZ*, and *mrcA* were the lowest at the reservoir tail and gradually increased from the reservoir middle to the dam front. Although the nitrification process represented by AOA-*amoA* abundance in the reservoir middle was slightly lower than in the reservoir tail, the catalytic enzymes of denitrification represented by *nirS* and *nosZ* as well as the catalytic enzymes of methane production represented by *mrcA* had the highest abundance in the reservoir middle. The denitrification potential results showed the lowest values at the reservoir tail, and the highest values at the reservoir middle (Fig. 5d).

Discussion

Impacts of cascade dams on geographical distribution of bacterial community in rivers

River damming is believed to alter the habitat of bacteria and thus influence the richness, structure, and function of the bacterial community (Chen et al. 2018). However, the succession of bacterial community along the heavily dammed Lancang River followed the typical feature shift commonly found in natural rivers, saying from *r*-selection to *k*-selection (Andrews and Harris 1986; Wang et al. 2018). The bacterial community composition changed from *Proteobacteria* dominance in the upstream to *Actinobacteria* dominance downstream, accompanied by a decline in the proportion of *Bacteroidetes* along the river. The trend between geographical distance and community dissimilarity in the Lancang River with cascade dams was similar to that in the parallel Nujiang River with no dam, except that the dammed Lancang River had a slightly higher slope. These results revealed that the geographical patterns of bacterial community dissimilarity in the two rivers were not significantly different.

Although the bacterial communities in the cascade reservoirs were clearly separated into surface water and sediment (Fig. 3a), they are not clearly separated between cascade reservoirs, either in surface water (Fig. 3b) or in sediment (Fig. 3c), and the spatial ordination of bacterial beta diversity was consistent with the flow direction of the river. The Lancang River flows from north to south, and the sampling sites covered a distance of 1290 km with an elevation change of 1150 m. The temperature difference in surface water and sediment exceeded 10°C from upstream to downstream. Heatmap of correlation showed that temperature was the most important

environmental factor determining the bacterial community along the Lancang River. Temperature could regulate the way that bacteria take up nutrients, thereby determining the bacterial community composition on the whole (Vrede 2005), although bacterial growth is affected by nutrients as well. Since the water temperature gradient is primarily associated with the elevation change and flow direction along the river, the distribution of the bacterial community at the river scale was in fact attributed to the geographical pattern, regardless of the reservoir cascade. Therefore, geographical factors outweigh the presence of cascade dams in determining the bacterial community at the river scale.

Impacts of cascade dams on bacterial community in water and sediment in reservoirs

Thermal stratification is common in deep lakes and reservoirs, forming a low-temperature and anaerobic environment at the bottom layer (Liu et al. 2019), which causes significant differences in the vertical distribution of bacterioplankton (Xue et al. 2017). However, no significant vertical differences in bacterioplankton community were observed in the layer between 13°C and 20°C in Xiaowan Reservoir, and in the layer between 17°C and 23°C in Nuozhadu Reservoir, despite their great depth. The reason can be attributed to the lack of a stable thermocline in the two deep reservoirs (Walker and Lucke 2019). The discharge from the power station outlet of the upper reservoir generated density flow, which wedged into the water body of the next reservoir. The subsurface intrusion flow destabilized the thermocline and enhanced convection in the reservoir. Consequently, there were no distinct layers of bacterioplankton distribution with respect to water temperature in the two deep reservoirs. The DO maintained a rich state of 6.0–8.0 mg L^{-1}, except that there was a thin layer (at a depth from 20 to 40 m) with relatively low oxygen concentration (< 5.0 mg L^{-1}) in the two reservoirs. This low oxygen layer could be caused by the decay of dead algae from reservoir surface water (Chen et al. 2020*a*). However, the bacterial community composition in this layer was not different from the other layers. Overall, no distinct layers of bacterioplankton distribution with respect to DO were observed. These vertical features of the bacterial community in the hydropower reservoirs were greatly different from that in deep reservoirs and lakes with stable stratification (Nyirabuhoro et al. 2020).

Dams convert lotic river systems into lentic reservoirs and greatly alter the natural flow fields, enhancing the uneven distribution of nutrients in sediment, and promoting "environmental selection" on the bacterial community. Specific species that were more competitive in the corresponding niche dominated the local bacterial community by "species-sorting." The bacterial community in sediment was mainly determined by the properties of local sediment materials resulting from velocity-sieved sedimentation (Fig. S7). The rapid decrease in flow velocity in the reservoirs enhanced the spatial unevenness of sedimentation of particles with different physical and

chemical properties (Wang et al. 2018). Fine nutrient-rich sediment settled at the middle and dam front of the reservoir and thus formed "hotspots," where bacterial activity and bacteria-mediated biogeochemical cycles were relatively high (Ruiz-González et al. 2013). These changes made "mass effects" no longer the dominant influence on the bacterial community (Lindström and Bergström 2005; Read et al. 2015), while "species-sorting" took precedence over "mass effects" in shaping the bacterial community progressively at the reservoir scale, leading to spatial patterns in the bacterial community in sediment within a reservoir. Bacteria in water of natural rivers is largely transported laterally from tributaries and surrounding riparian land, which usually have a higher richness in their bacterial communities (Crump et al. 2012). The increase in HRT due to reservoir storage enhanced sedimentation (Lindström and Bergström 2004); thus, the suspended particles carrying bacterial communities in water settled down to the bottom, resulting in an increase in the diversity and spatial difference in sediment bacteria. Therefore, under the influence of uneven sedimentation due to heterogeneous flow velocity (Fig. S7), the longitudinal differences of bacterial community are significant.

Conceptualizing bacterial community and potential function in cascade reservoirs

Figure 6 schematized the bacterial community in cascade reservoirs along a river. Cascade reservoirs did not isolate the bacterial community, while the geographical effects remain the most important factor determining the geographical patterns of bacterial community along a dammed river. Unlike ordinary deep lakes, a stable thermocline is lacking in deep cascade hydropower reservoirs due to the density flow from the upper reservoir during hydroelectricity production. Thus, there are no distinct layers of vertical bacterioplankton distribution with respect to either water temperature or DO. However, when a river is dammed, particularly the canyon river, the lotic flow is altered to lentic flow. Fine sediments with higher content of organic matters and bacteria settle down in the middle of the reservoir. "Species-sorting" gradually overtakes "mass effects" and assumes the major role in shaping bacterial community composition within a reservoir. The most abundant function gene in sediment bacteria was different between the reservoir tail, reservoir middle, and dam front. The highest function gene abundance related to carbon and nitrogen cycling was found in sediment at the reservoir middle, which was consistent with the highest proportion of carbon and nitrogen cycle-related bacteria in the microbial community there. Further analyses showed that the abundance of denitrification-related genes (*nirS* and *nosZ*) was higher in sediment at the reservoir middle, which indicates high denitrification potential may exist (Shi et al. 2017, 2020). For the consumption of oxygen, the gene AOA-*amoA* related to nitrification was more abundant at the reservoir tail and reservoir middle than at the dam front. These findings implied

there could be a carbon and nitrogen cycle "hot zone" in the reservoir middle due to the special properties of sediments in this area (Yang et al. 2018; Zhang et al. 2020).

Conclusion

Along the cascade reservoirs in a large River, bacterial community was mainly determined by geographical effects of water temperature rather than the presence of dams. Vertical distribution of bacterioplankton does not show distinct layers in deep cascade reservoirs, due to the disruption of flow from hydropower production. There are significant differences in sediment bacteria between the tail and middle and head of a reservoir because of flow velocity-sieved sedimentation.

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Acknowledgments

Conflict of Interest

None declared.