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Microbial diversity accumulates in a downstream direction in the Three Gorges Reservoir

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ABSTRACT

Organic and inorganic materials migrate downstream and have important roles in regulating environmental health in the river networks. However, it remains unclear whether and how a mixture of materials (i.e., microbial species) from various upstream habitats contribute to microbial community coalescence upstream of a dam. Here we track the spatial variation in microbial abundance and diversity in the Three Gorges Reservoir based on quantitative PCR and 16S rRNA gene high-throughput sequencing data. We further quantitatively assess the relative contributions of microbial species from mainstem, its tributaries, and the surrounding riverbank soils to the area immediately upstream of the Three Gorges Dam (TGD). We found an increase of microbial diversity and the convergent microbial distribution pattern in areas immediately upstream of TGD, suggesting this area become a new confluence for microbial diversity immigrating from upstream. Indeed, the number of shared species increased from upstream to TGD but unique species decreased, indicating immigration of various sources of microbial species overwhelms local environmental conditions in structuring microbial community close to TGD. By quantifying the sources of microbial species close to TGD, we found little contribution from soils as compared to tributaries, especially for sites closer to TGD, suggesting tributary microbes have greater influence on microbial diversity and environmental health in the Three Gorges Reservoir. Collectively, our results suggest that tracking microbial geographic origin and evaluating accumulating ef-

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fects of microbial diversity shed light on the ecological processes in microbial communities and provide information for regulating aquatic ecological health.

Introduction

Dam construction has provided great economic value, but has also caused severe ecological consequences (Grill et al., 2015). The influences of changes in flow regime and sedimentation regime on microbial community structure have been previously demonstrated (Besemer et al., 2007; Wu et al., 2013; Liu et al., 2018). For instance, microcosm experiments have demonstrated that flow velocity can shape river biofilm architecture and drive diverging microbial successional trajectories during the early growth stage (Besemer et al., 2007). Another study reported that changes in sediment regimes altered soil properties (i.e., soil nutrients, soil texture and moisture) which subsequently caused pronounced shifts in bacterial community structure and microbial biomass carbon (Wu et al., 2013). Recently, an abrupt decrease in microbial diversity was observed at sites immediately downstream of a dam compared to sites upstream due to riverbed scouring (Liu et al., 2018). These findings supported deterministic community assembly (species sorting) (Li et al., 2019), but an increasing number of studies have shown the important roles of stochastic processes (i.e., dispersal) in aquatic community assembly (Zhou et al., 2014; Zhou and Ning, 2017; Chen et al., 2019; Fodelianakis et al., 2019).

In aquatic ecosystems, there is a passive dispersal transfer of material downstream. A growing number of studies have shown that freshwater communities can be transported to saline estuaries with large river discharges (Crump et al., 2004; Mason et al., 2016). It has been inferred that upstream bacterial diversity acts as seed-bank for the downstream microbial community (de Oliveira and Margis, 2015). The detection of typical freshwater species has been considered as a biomarker of freshwater intrusion (Braun et al., 2016; Mason et al., 2016). Taken together, these suggest that the upstream microbial community has a large effect on the downstream community. This influence could be enhanced with damming, generating a retaining effect on material dispersed from upstream sites (Chen et al., 2013; Wolf et al., 2013; Zhou et al., 2013). It has been reported that potential organic pollution (Chen et al., 2013; Wolf et al., 2013), as high as 85% of the sediment (Zhou et al., 2013) and nutrients loads such as phosphorus and ammonium nitrogen (Zhao et al., 2013; Zhou et al., 2013) and dissolved organic carbon (DOC) (Yang et al., 2013a) accumulated immediately upstream of Three Gorges Dam (TGD). Species diversity of fish community has been observed to accumulate in a downstream direction at the tributary confluences (Fernandes et al., 2004). However, little is known if microbial species accumulates in a downstream direction in the reservoir.

Microbial community coalescence, a newly proposed concept to describe the mixing events of microbial assemblages from various moving pieces of environments (Rillig et al., 2015), is common in river ecosystems (Mansour et al., 2018). Various sources could contribute to microbial species downstream along the river course, such as tributary, upstream mainstem and terrestrial input. For example, a previous study reported that ammonia-oxidizing microbes (AOA/AOB) at sites close to dam were transported from areas further upstream (Huang et al., 2016). Organic geochemistry and environmental DNA evidences have demonstrated the terrestrial input into river water (Yang et al., 2013b; Deiner et al., 2016), especially at the area immediately upstream of the dam where higher terrestrial input was detected than sites further upstream (Yang et al., 2013b). Accordingly, we hypothesized that along with transfer of material, microbial species could be dispersed passively via the flow of water and be retained by damming. If so, a higher microbial diversity and abundance should be observed at areas immediately upstream of the dam. There is evidence that the effects of source microbes on downstream community varies with hydrological dynamics (i.e., water resident time), with more pronounced effects observed when there is long water resident time (Lindstrom and Bergstrom, 2004). As water resident time is more prolonged in area immediately upstream of the dam, we hypothesized that the sites closer to dam would make a larger contribution to microbial community immediately upstream of dam.

TGD has attracted worldwide attention throughout its construction and up to the present day. It is one of the most controversial projects in China, with great economic benefits, but also huge environmental risks (Yang and Lu, 2013). The growing log of evidences have shown the environmental, ecological and social issues associated with TGD, especially after the first impoundment to 135 m in 2003 and the ultimate impoundment to 175 m in 2009 (Wang et al., 2012; Maren et al., 2013; Xu et al., 2013; Yang et al., 2013c; Zhao et al., 2013; Zhou et al., 2013; Bao et al., 2014; Qi et al., 2014; Yan et al., 2015; Huang et al., 2016; Li et al., 2017, 2019; Liu et al., 2018). To understand whether TGD has an accumulating effect on microbiota, we examined the microbial abundance, diversity and community structure by using quantitative polymerase chain reaction (qPCR) and 16S rRNA gene based high-throughput sequencing. The sampling area is mainly located at the lacustrine zone (Wushan-Maoping section, ˜110 km) of the Three Gorges Reservoir (TGR). Our main objectives were to answer the following questions: (1) whether an accumulating effect exists in microbial abundance and diversity; (2) whether the reservoir acts more as a conveyer belt, transporting the upstream microbial species to the dam site (shared species) or if the microbial community was mainly shaped by local environmental filtering (unique species); (3) what are the relative contributions of main river, tributary and soil communities to TGD microbial community.

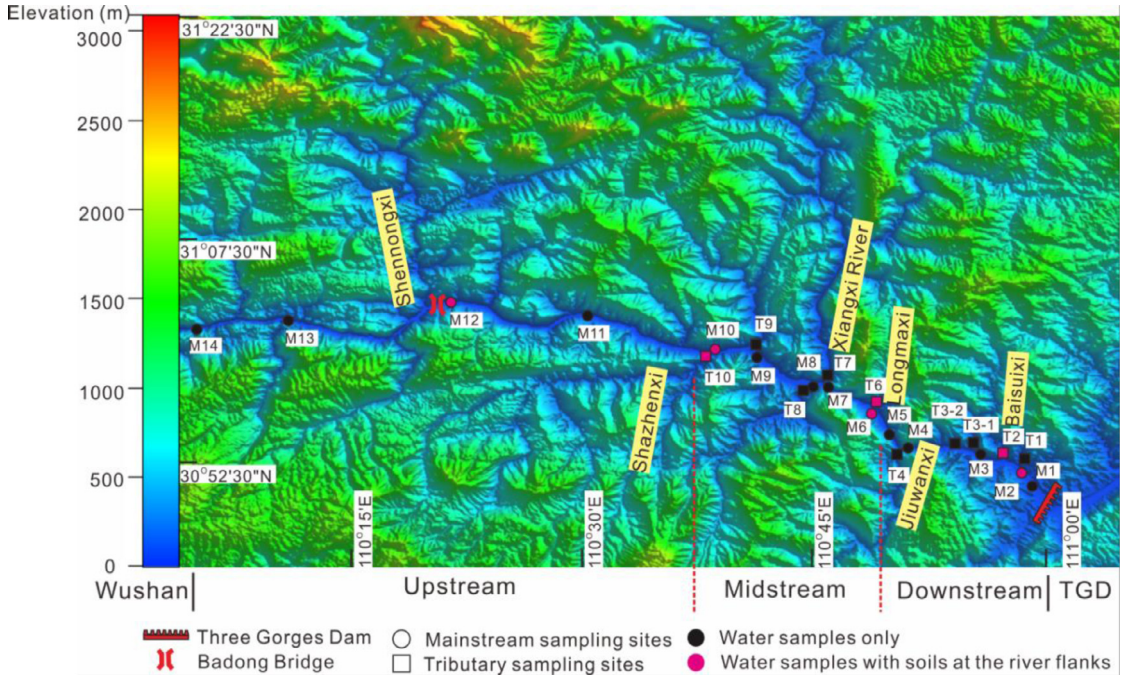

Fig. 1 – Sampling sites distribution in a downstream direction from Wushan to Three Gorges Dam (TGD) in the reservoir. M denotes mainstem site and T denotes tributary site.

1. Material and methods

1.1. *Field measurements and sample collection*

Damming has caused severe hydrological regime shifts. According to geographic gradients, the Three Gorges Reservoir could be divided into three longitudinal zones: riverine, transitional and lacustrine zones. The lacustrine zone was formed due to reducing flow velocity and increasing water retention time (Tang et al., 2018). Our sampling sites are mainly distributed in the lacustrine zone. Samples were collected from the lacustrine zone of the Three Gorges Reservoir (Wushan-Maoping section, ~110 km) in August 2010, including mainstem water samples ($n = 14$), tributary water samples ($n = 9$) and surrounding riverbank soils ($n = 7$) (Fig. 1). A routine water level fluctuation on an annual basis is observed due to strict operational scheme, water impoundment during the dry season (up to 175 m), followed by water release during the rainy season (as low as 145 m) (Tang et al., 2018). The water depth during our sampling period is 145–150 m, corresponding to the low water level operational scheme due to water release. Before we decide the water depth for collecting water from, we measured vertical profiles of water physico-chemical parameters using a multiple-probe set Horiba (U20D, Japan), including depth, temperature, conductivity (COND), pH, total dissolved solids (TDS), dissolved oxygen (DO), turbidity (TURB), oxidation–reduction potential (ORP) from surface to 25 m. No significant change was found from 0 to 5 m water depth in terms of the measured parameters. In order to minimize the influence of human activity (i.e., plastic trash) on the surface water, we decided to sample water from 5 m depth, except for some tributaries where water depths were shallower. In order to minimize the influence of mainstem backwater on tributary water, we use conductivity as an indicator to determine the positions where to collect tributary water. We measured the conductivity from the confluences of mainstem and tributaries to the further upstream sites in the tributaries and sampled where the conductivity values are extremely lower than mainstem water. Water samples for microbial abundance analysis were collected by filtering 15–60 L of water through 0.7 μm pore-size filters, sequentially followed by filtering 4–12 L of water through 0.2 μm filters in triplicate (polycarbonate, 142 mm diameter, Whatman, USA). Pore-size of 0.7 μm was used to separate the large particles from free-living microorganisms (Feng et al., 2009). Soil samples from the mainstem or tributary riverbanks were collected in triplicate with sterile spatulas and spoons. Filters and soils for microbiological analyses were immediately frozen in liquid nitrogen and transported on dry ice. In the lab, samples for DNA analyses were stored at −80°C until further processing.

Additional 0.22 μm filtered water were collected for laboratory measurements of inorganic nutrients including ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), silicate (SiO_3^{2-}), phosphate (PO_4^{3-}). Samples for dissolved organic carbon (DOC) were filtered through Whatman GF/F filter, collected in 40-mL bottles and acidified to pH = 2. For dissolved inorganic carbon (DIC) analysis, water was collected into 40-mL bottles and 50 μL concentrated $HgCl_2$ solution added to stop further microbial activity. The details for geochemical analyses have been described previously (Huang et al., 2016). The sampling transect from Wushan to TGD could be roughly divided into three segments (Fig. 1, upstream segment: >50 km to TGD; middlestream segment: 50–25 km to TGD and down-

stream segment: <25 km to TGD) based on water geochemistry (Huang et al., 2016). Notably, water turbidity and total dissolved solid (TDS) decreased from upstream to TGD, representative of the relatively longer water resident time near TGD. In contrast, nutrient concentrations, such as DOC, SiO_3^{2-} and NO_3^- increased near TGD, consistent with previous studies that demonstrated the accumulating effect of TGD on nutrient loads (Yang et al., 2013a; Zhao et al., 2013; Zhou et al., 2013).

1.2. *DNA extraction, polymerase chain reaction, pyrosequencing and quantitative polymerase chain reaction (qPCR)*

Filters containing biomass and soils were subject to DNA extraction by using the FastDNA SPIN Kit for Soil (MP Biomedical, OH, USA). We mixed the extracted DNA from different fractionated bacterioplankton (free-living and particle-attached) for the same site as a composite sample, which was used to evaluate the bacterioplankton diversity and composition at a specific site. The V4-V8 region of the 16S rRNA gene was amplified using primer set 515F (5′-GTGCCAGCMGCCGCGGTAA-3′)−1391R (5′-GACGGGCGGTGWGTRCA-3′) with 8-bp distinguishable barcode sequence for each sample. PCR reactions were performed in triplicate 25 μL mixture containing 2.5 μL 10× PCR buffer, 2.0 μL of 2.5 mmol/L dNTP, 1.0 μL of each primer (10 mmol/L), 1.0 μL bovine serum albumin, 0.25 μL of DNA polymerase (TaKaRa rTaq, Dalian, China) and 1.0 μL of genomic DNA (20–50 ng) as a template. Amplicons were extracted by 1.5% agarose gels and purified with the Agarose Gel DNA Purification Kit Ver2.0 (TaKaRa bio, Dalian, China). The concentration was quantified by using Qubit fluorimeter (Invitrogen, USA). Equal molar of barcoded PCR products for each sample were mixed and sent for pyrosequencing on a 454 GS FLX platform in Chinese National Human Genome Center in Shanghai.

Quantitative PCR of total bacteria and total archaea were performed for different fractionated bacterioplankton. Primer pairs Eub338F / Eub518R and Arch349f / Arch806R were employed to amplify bacterial and archaeal 16S rRNA gene, respectively. Each 20 μL PCR reaction contained 10 μL Power SYBR Green PCR Master Mix (Applied Biosystems, USA), 0.5 μL of each primer (10 mmol/L; Sangon, Shanghai, China), 1.0 μL of bovine serum albumin (TaKaRa bio, Dalian, China), 7 μL of H_2O, and 1.0 μL of template DNA (20–50 ng). Plasmid standards were serially diluted from 10^9 to 10^2. The procedure of qPCR was as following: 30 sec at 95°C for initial denaturing, followed by 40 cycles of 95°C for 30 sec, a specific annealing temperature for 30 sec (bacteria 53°C and archaea 57°C), and 72°C for 1 min. A dissociation step was added after amplification for the formation of primer-dimers. The amplicons were run on a 1.5% agarose gel to further confirm the specificity. All qPCR were run on a real-time PCR detection system (ABI 7500, Applied Biosystems, USA). The final results of gene copy numbers are presented as copy number per liter of water.

1.3. *Data processing and statistical analyses*

Sequence demultiplexing and quality control were performed in MOTHUR (Schloss et al., 2009) and QIIME (Caporaso et al., 2010). Quality control was done in MOTHUR with the following criteria: (1) removing reads with average quality scores < 25, or ambiguous bases/errors existing in barcodes, or more than two mismatches in primer sequences; (2) those reads with more than 6 homopolymers or length shorter than 120 bp were discarded. The following steps were subsequently completed by using QIIME. The remaining sequences were subject to chimeric checking and then clustered at 97% sequence identity level. Taxonomy was assigned using the ribosome database project (RDP) classifier algorithm. Alpha diversity (within samples) and beta diversity (among samples) were calculated using species-level operational taxonomic units (OTUs) (at the 97% identity level) in QIIME. Data used for alpha diversity calculations (Chao1, Shannon, Simpson, dominance, evenness) were normalized by randomly resampling to 1000 sequences in each sample. Despite this sequence depth is relatively shallow, previous study has shown that robust patterns are already discovered with 1000 reads per sample for human microbiome (Hamady and Knight, 2009). Furthermore, according to previous study on which sequencing depth is sufficient to describe patterns in bacterial alpha and beta diversity, we noticed that for stream water, 1000 sequences are sufficient to capture more than 80% the trends in microbial diversity (Shanon, Chao1 and pielou's evenness) (Lundin et al., 2012). On the basis of this, resampling to the same sequencing depth of 1000 reads could capture most of the alpha diversity.

The non-metric dimensional scaling (NMDS) ordination based on the Bray-Curtis dissimilarity matrix was built to depict pattern in community structure. The BIO-ENV procedure (Clarke and Ainsworth, 1993) was used to identify the best subset of predictors of changes in community structure (Bray-Curtis dissimilarity). Both Bray-Curtis dissimilarity and Jaccard distance were used in Mantel test to decipher the correlation between microbial community and environmental factors, because species incidence data (i.e., Jaccard) were very sensitive to undersampling, while abundance-based data (i.e., Bray-Curtis) focusing on the most common taxa were particularly robust (Beck et al., 2013). To examine the environmental associations to microbial abundance and diversity (Chao1 and Shannon) across individual samples, quadratic fitted models were compared to linear fit and the best model was determined by lower AIC values and higher correlation coefficient. All the statistical analyses were done in the R package 'vegan' (Oksanen et al., 2007). The pyrosequencing reads were deposited to the Short Read Archive database at NCBI (accession no. PRJNA598282).

1.4. *Estimating microbial immigration from upstream to downstream*

Three methods were used to evaluate the impacts of immigration from upstream to downstream in TGR. The first approach simply defined the source as where maximum relative abundance of each abundant OTU (relative abundance > 0.1% in at least one sample) in pooled sequences was found. The source location was determined by both distance to TGD (upstream segment, middlestream, or downstream) and system attribute (mainstem, tributary or soil). For example, if an OTU was most abundant in the downstream mainstem, then we defined it as a Main_down OTU. Finally, we

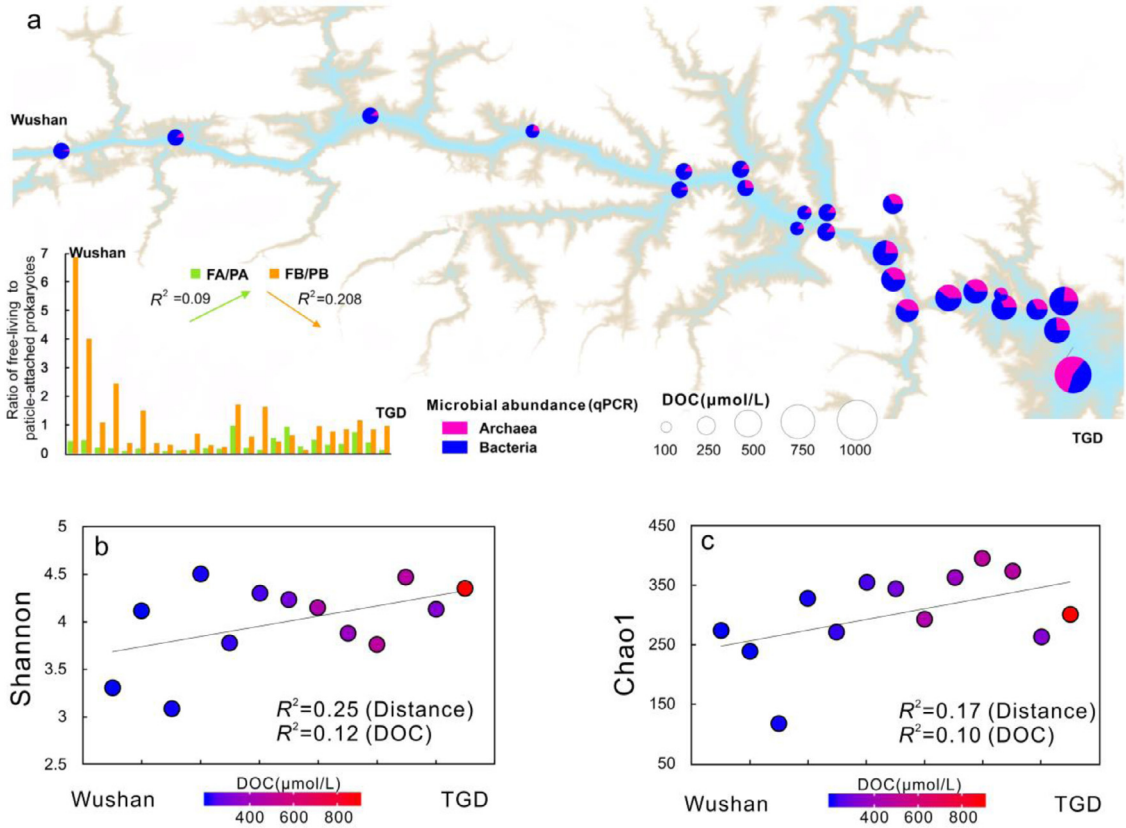

Fig. 2 – Microbial diversity accumulates in a downstream direction with differential changes in bacterial and archaeal abundances. (a) Bacterial abundance (copies/L) decreases in a downstream direction, whereas archaea increases, concurrent with increasing in dissolved organic carbon (DOC) concentration. The inset shows spatial variations in ratios of free-living to particle-attached archaea (FA/PA) and bacteria (FB/PB); (b, c) Microbial diversity and richness accumulate in a downstream direction.

qualitatively classified the OTUs into eight different groups: Main_down, Main_middle, Main_up, Tributary_down, Tributary_middle, Soil_down, Soil_middle, Soil_up and Rare. Rare was defined as the sum of taxa with abundance less than 0.1% in any sample. This method has been used to reveal the spatial variation in community structure in aqueous environments, including rivers and oceans (Fortunato et al., 2012). The second approach considered the furthest site as microbial source pool using presence/absence data and recorded the first occurrence of an OTU from the furthest site (M14) to near TGD site (M1). In this way, we could classify OTUs into shared (common OTUs transported from the furthest site M14) and unique (first observed in a given sample) categories. The third approach utilized a microbial source tracking procedure (Knights et al., 2011), which estimates the proportions of contribution from source environments to the sink community.

2. Results and discussion

2.1. *Accumulation of microbial diversity but reduction of microbial abundance from upstream to TGD*

Rivers have been a conduit for both materials and biodiversity, inflows from different sources intermixing at confluences and disperse to further downstream sites (Altermatt, 2013). Species diversity increases at tributary confluences (Fernandes et al., 2004; Grant et al., 2007). However, with damming, the connectivity is cut and area immediately upstream of dam could serve as a new mainstem confluence. Thus, higher diversity should be observed. This hypothesis was proven by our results, microbial diversity increasing from the furthest upstream site to TGD (Fig. 2b and c), and is concurrent with the increase in DOC concentration, but distance to TGD had higher correlations with microbial diversity. This finding illustrated that similar to fish community (Fernandes et al., 2004), there is accumulation effect in microbial diversity in a downstream direction.

Along the downstream direction (Wushan to TGD), the abundance of archaea increased from Wushan (4.76×10^8 copies/L) to TGD (5.04×10^9 copies/L), whereas bacteria decreased (from 1.21×10^{10} to 4.25×10^9 copies/L). The enrichment of archaea at near TGD sites was possibly promoted by the high level of DOC concentration, as revealed by the significant positive correlation to DOC (Fig. 3c). However, for bacteria, temperature (linear regression $r = 0.65$, Fig. 3a) played a more important role than DOC ($r = -0.48$, Fig. 3c). The positive correlation of bacterial abundance with temperature could be partially explained by the metabolic

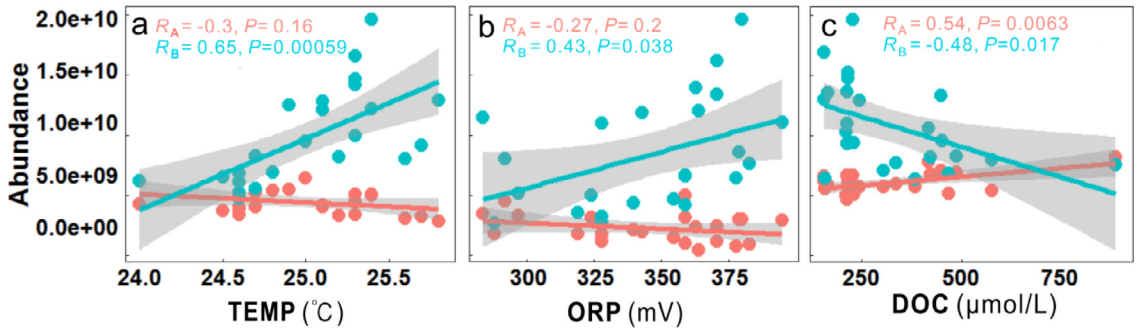

Fig. 3 – Significant linear relationships between microbial abundances (archaea, red; bacteria, green) and various controlling environmental factors. (a) TEMP: temperature; (b) ORP: oxidation–reduction potential; (c) DOC: dissolved organic carbon.

theory of ecology (MTE) which predicts that higher temperatures lead to higher metabolic rate, speciation rate and generation turnover (Brown et al., 2004). Thus, we observed more bacterial biomass with higher temperature. It should be noted that the effects of temperature on bacterial abundance could be also exerted indirectly through dissolved oxygen and total dissolved solids, because these variables were strongly correlated with temperature (Spearman's $\rho^2 > 0.7$) (Appendix A Fig. S2).

In contrast to microbial diversity, a decreasing trend in total microbial abundance was detected from upstream to TGD (Appendix A Fig. S1). This reduction was mainly due to loss of bacteria (Fig. 2a). The decrease of microbial abundance in the downstream direction is contract with our hypothesis. The basis of our hypothesis is that the movement of microorganisms is determined by the direction and flow of water. However, the strong correlation of microbial abundances with water physico-chemical variables indicated that not like microbial diversity, microbial abundances were more responsive to environmental changes.

A similar spatial trend of variation was observed in the ratio of free-living to particle-attached archaea (FA/PA) and bacteria (FB/PB) (Fig. 2a inset). Differential responses of fractionated microbes to various environmental parameters were observed (Appendix A Fig. S3). For example, DOC was identified as the major driving forces of the particle-attached archaea (Appendix A Fig. S3d), followed by ammonium which is marginally significant (Appendix A Fig. S3e), whereas ORP, DOC and temperature had great influence on free-living archaea (Appendix A Fig. S3b, c and d). The discrepancy patterns between free-living and particle-attached microbes has been reported in other studies (Liu et al., 2019; Wang et al., 2020), indicating their different habitat preferences, likely determined by physiological capabilities and distinct community assembly mechanisms (Smith et al., 2013; Wang et al., 2020). In addition, we found from midstream to downstream, microbial abundances in tributaries were lower than or similar to those in the adjacent downstream mainstem samples. In contrast, in the closest tributary (T2) to TGD, the microbial abundances were higher than those in its corresponding downstream mainstem water sample (M2) (Appendix A Fig. S4). This higher abundance may ensure the probability that more species immigrate from tributaries into the mainstem sites.

2.2. *Spatial variations in microbial community structure and major taxa*

Previous studies in rivers and streams have reported that microbial community composition were predominately comprised of Proteobacteria (i.e., Beta, Delta, and Alpha) and Actinobacteria (Crump, 2005; Hullar et al., 2006; Crump et al., 2009; Bucci et al., 2014). Typical freshwater microbial taxa were detected across the sampling transect (Fig. 4). We found a "Y" shaped spatial distribution in microbial community, with mainstem and tributary communities converging near TGD site M2 (Appendix A Fig. S5), indicating the similarity between tributary and mainstem assemblages increased at site near TGD. On the other hand, this pattern revealed that communities from sites near TGD were determined by both mainstem and tributary communities due to the import of species from upstream. BIO-ENV identified turbidity and SiO_3 as the best predictors for driving variation in microbial community structure (Appendix A Table S1). To further assess the associations with examined environmental factors, we performed partial Mantel tests which confirmed that microbial community structure was mainly controlled by turbidity (r_M=0.394) and SiO_3 (r_M=0.339), followed by DIC concentration (r_M=0.239) (Appendix A Table S2). Turbidity is the only factor influencing both abundance-based (Bray-Curtis) and presence/absence-based (Jaccard) microbial community structure. This was well in accordance with previous studies in stream (LeBrun et al., 2018) and estuary ecosystem (Easson and Lopez, 2018). Turbidity could be taken as an alternative measurement for water resident time. Therefore, microbial community structure was fundamentally influenced by variation in water resident time.

The abundances of some taxa gradually increased from upstream to near TGD sites (Fig. 4), including Actinomycetales (mainly ACK-M1 at family level, also named acI-A (Warnecke et al., 2004)), Acidimicrobiales (mainly CL500–29 at family level, also named acIV-A (Warnecke et al., 2004)) and Synechococcales (mainly genus *Prochlorococcus*). Actinobacteria members of ACK-M1 and CL500–29 are commonly found in freshwater lakes (Lindstrom et al., 2005) and river-intrusion groundwater (Braun et al., 2016). We should note that sites close to TGD were characterized by low ammonium and high DOC contents. Thus, the proliferation of Actinobacteria ACK-M1 and *Prochlorococcus* at these sites is in contrast to the commonly held view that members from ACK-M1 prefer

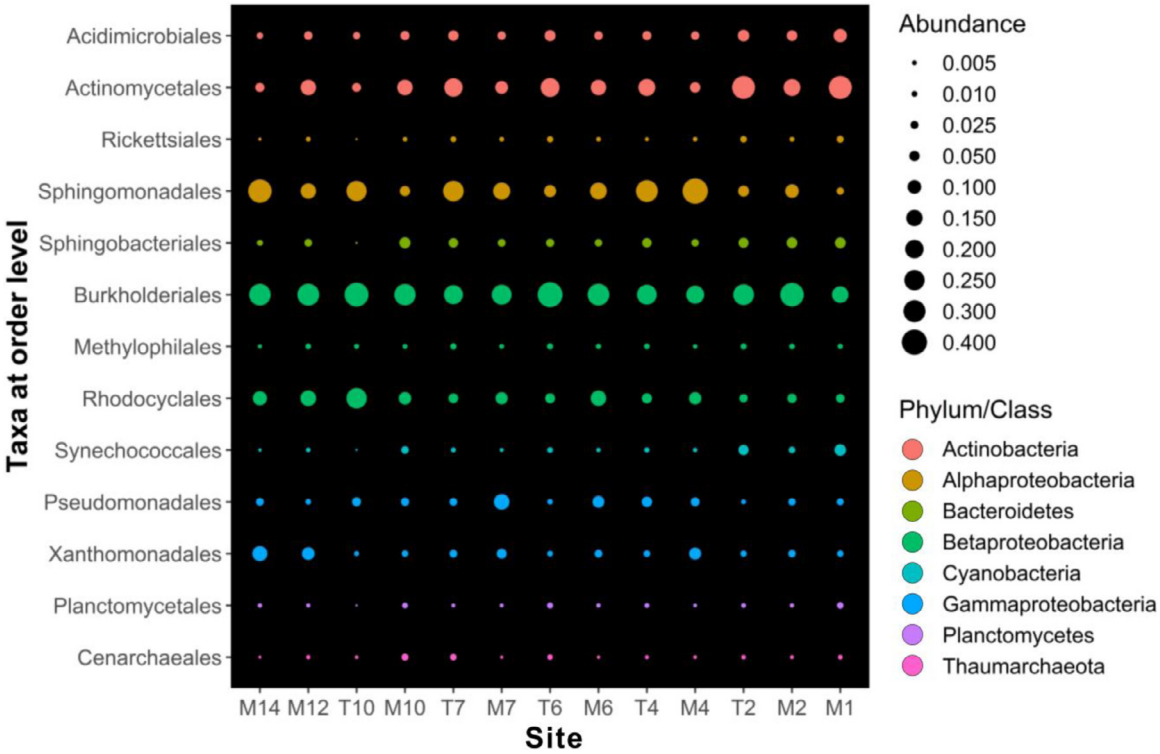

Fig. 4 – Spatial variations in major taxa at order level. The size of the dots was proportional to their relative abundances and colors indicate various phyla (Proteobacteria at class level). Only taxa with relative abundance > 0.1% in at least one sample are included.

relatively high ammonium or N-rich compounds in aquatic environments (Dennis et al., 2013; Ghylin et al., 2014) and that *Prochlorococcus* is at a competitive disadvantage in nutrient rich environments (Partensky et al., 1999). One possible explanation for this discrepancy is that these species were transported from upstream sites where the environmental conditions were more favorable and enriched immediately upstream of TGD. This result agrees well with a previous study that the relationship between *Prochlorococcus* and nutrient availability is more complex than previously thought (Flombaum et al., 2013). Furthermore, interaction with other species may aid in coping with unsuitable conditions. Indeed, a strong positive correlation was observed between relative abundances of Actinobacteria (mainly ACK-M1) and Cyanobacteria (mainly *Prochlorrococcus*) (Appendix A Fig. S6), indicating that their growth are highly dependent on their combined presence. This interaction has been previously demonstrated in both culture and field samples (Li et al., 2015; Zheng et al., 2018). Meanwhile, microautoradiography and fluorescence in situ hybridization (MAR-FISH) showed that Actinobacterial acI was able to actively assimilate low-molecular-weight organic compounds, which was suggested to be phytoplankton exudates (Salcher et al., 2013).

2.3. *Shared and unique microbial species from upstream to downstream*

The presence of a microbial seed-bank was demonstrated in the river ecosystem, therefore, while the occurrence of microbial taxa throughout the river course is not determined by the environment parameters, these parameters do affect the changes in relative abundance of microbial taxa (Staley et al., 2013). Furthermore, the headwater is an important seed-bank, which means microbial diversity in the river source spreads with water flow (de Oliveira and Margis, 2015; Huang et al., 2016) and even migrates into marine environment (Mason et al., 2016). It is summarized that at small spatial scale, differences in communities are likely structural rather than compositional due to rarely dispersal-limited distribution (Martiny et al., 2006). According to partial Mantel test, we indeed found microbial community differences in reservoir water were structural (Bray-Curtis) rather than compositional (Jaccard) (Appendix A Table S2).

Deciphering the variation trends in shared and unique OTUs along the flow direction can shed light on the ecological processes that underlying the microbial assembly in the reservoirs. In this study, we tracked the immigration path of each OTU from the furthest site (M14) to TGD (M1) based on presence/absence data (Fig. 5a). Interestingly, the shared species gradually increased from M14 to M1, whereas the unique species decreased, indicting microbes at near TGD sites were more likely transported from sites further upstream. Site-specific OTUs highlighted a degree of endemism due to local environmental differentiation; however, the decreasing trends in site-specific OTUs indicated less effect of environmental conditions in structuring microbial community closer to TGD. This finding represents the general case in aquatic ecosystems where microorganisms can disperse passively via the flow of

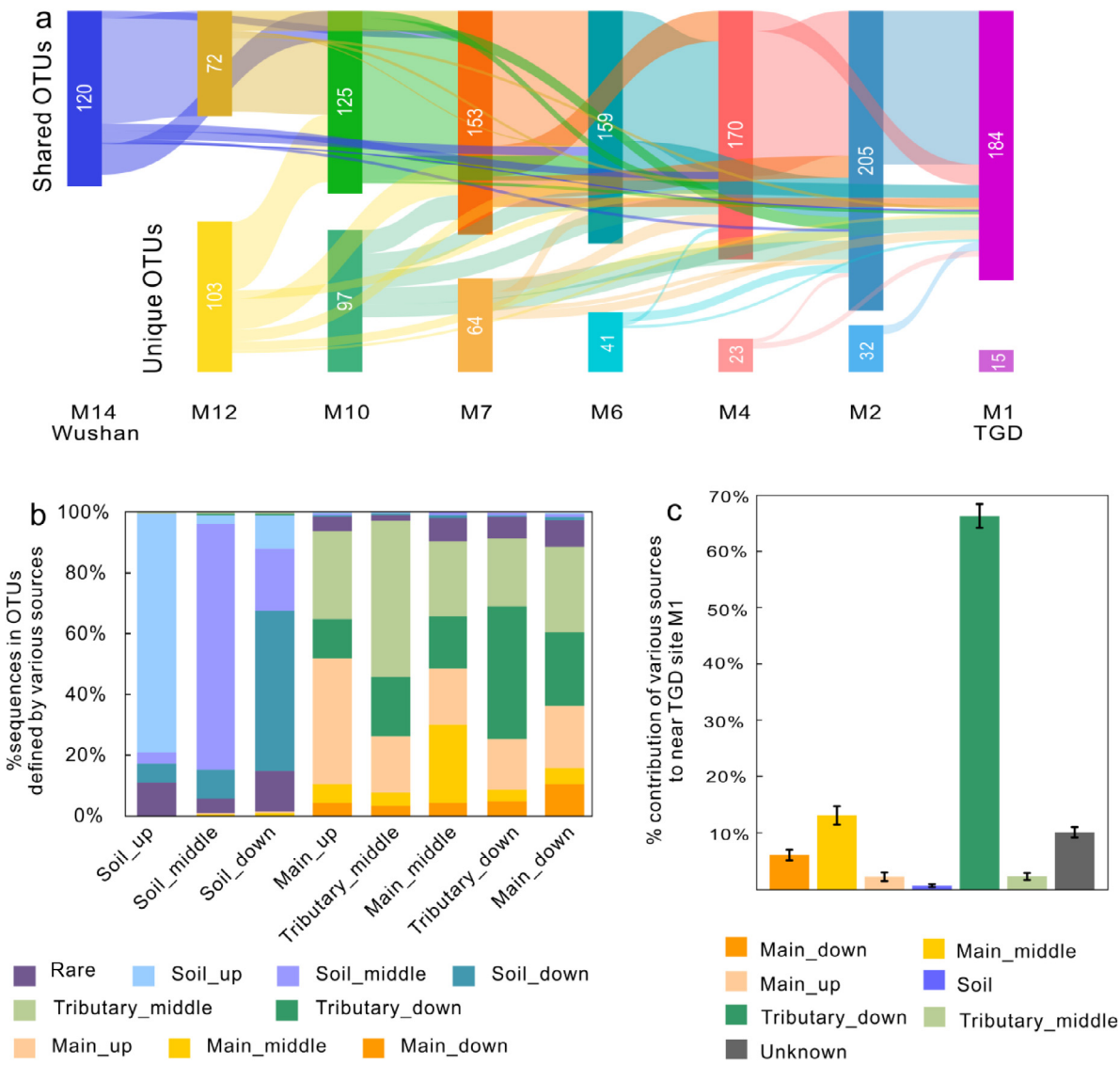

Fig. 5 – Tracking species immigration from upstream to downstream. (a) The shared and unique number of OTUs in main river along the stream wise direction from upstream (M14) to downstream (M1); (b) Relative abundances of OTUs defined by locations (up, middle and down) and sample attributes (mainstem, tributary and soil); (c) The relative contributions of various sources to community M1 immediately upstream of dam. Up, middle and down represent locations at upstream, middlestream and downstream segment, respectively on Fig. 1.

water (Chen et al., 2019), suggesting metacommunity is neutrally assembled (Liu et al., 2018). We have acknowledged that the flow rate is reduced due to TGD construction in Yangtze River, especially in the reservoir where ecosystem shifts to relatively lentic from lotic state, which may consequently enhance the selective strength of local environments. Previous study demonstrated that the average microbial immigration rate (*m* in sloan's neutral model) in water is in the range of 1.8–2 over the Yangtze River course (Liu et al., 2018), and the reservoir could define the lower limits of the immigration due to reduced flow rate. However, a recent study manifest that dispersal homogenized community even with low immigration rate, highlighting the role of dispersal in lentic environments (Fodelianakis et al., 2019).

2.4. *The relative contributions of main river, tributary and soils communities to near TGD community*

The geographic origin of species in aquatic ecosystems has long been discussed (Crump and Hobbie, 2005; Kisand et al., 2005; Crump et al., 2012; Huang et al., 2016). Rivers have been considered as conveyer belts for biodiversity across terrestrial and aquatic biomes (Deiner et al., 2016). Because of this property, microbial community at a given site is in dynamic equilibrium, constantly immigrating from upstream and emigrating to downstream. Thus, taxa identified in the downstream sites could contain "older" species immigrating from upstream waters (main river vs. tributary), or species scoured into the water from soils, or species indigenous to the local

sites. In this study, we found various the relative contributions of microbial communities from upstream mainstem, tributary and soils to sites immediately upstream of dam along the flowing direction, which coincided with a recent study that microbial coalescence may result in dominance of one community over another (Castledine et al., 2020).

By considering the relative abundance of each OTU, we define the source of the each OTU as the site where greatest abundance occurred. Some OTUs may occur at an upstream site with low relative abundance, but increases at a downstream site where environmental conditions favor its growth. Therefore, we observed that most of microbial OTUs were shared among all three transect sections. Also, mainstem and tributary microbial community often shared microbial OTUs, likely because these two systems were hydraulically connected (Fig. 5b). Each system possessed its own unique compliment of microbes which were less abundant in other systems, except for the down_main community where > 70% of the OTUs were composed of up_main, middle-tributary, and down-tributary communities, while its own unique OTUs only accounted for about 10% (Fig. 5b), suggesting the transport of upstream microbial species to the sites nearest to the TGD. This observation was further confirmed by microbial source tracking results which showed that downstream tributary communities contributed most to the near TGD M1 community (Fig. 5c), partially supporting the hypothesis that sites closer to TGD contribute more to the near dam community. This finding is consistent with those found in fish community that tributaries other than upstream mainstem sites contributes most to the immediately downstream mainstem sites (Fernandes et al., 2004).

A few potential explanations could be proposed to interpret this tributary effect. First, higher microbial abundance in the tributary (T2) closest to TGD than its corresponding downstream mainstem water sample (M2) may ensure the probability that more species immigrate from tributaries into the mainstem sites. Second, backwater effects due to differences in flow volume and water levels between mainstem and tributaries are more obvious in area closer to TGD, and this backwater effect tends to reduce current speed, which might directly increase the microbial diversity in the confluence. Third, tributaries may carry nutrients and organic matter from terrestrial sources and thus provide resources for microbial growth and indirectly increase microbial diversity in the confluence.

For the reservoir, especially after impoundment, soil erosion became a growing problem (Cui et al., 2011), which could import more soil species into the water system. Furthermore, organic geochemistry demonstrated that higher terrestrial input was detected immediately upstream of the dam than sites further upstream (Yang et al., 2013b). Based on this information, we sampled the soil along the corresponding riverbank and expected to see more contribution from soil communities. Although the percentage of soil OTUs occurring in mainstem community rose by 1% from upstream to downstream, soil microorganisms were primarily unique for their respective locations (Fig. 5b) and contributed little to community M1 near TGD (Fig. 5c), which suggested even with a greater chance being scoured into the river after impoundment, few of soil microbes could be detected in the aquatic ecosystems, probably due to the dilution effect from high discharge. On the other hand, we should acknowledge that the little contribution of soils may attribute to the limits of detection, because species with large abundance are prone to picking up by shallow sequencing efforts. However, our previous study regarding to ammonia-oxidizing microorganisms found similar phenomenon of less contribution from soils (Huang et al., 2016). Besides, there is evidence that patterns of α- and β-diversity are highly reproducible when samples are sequenced with 454 (rarefied to 1000 sequences) and Illumina Miseq (rarefied to 40,000 sequences) (Kellogg et al., 2014). In addition, increasing sequencing depth probably provides higher probability to detect more rare species (Gibbons et al., 2013), which would not change pattern that water and soil samples harbored distinctly different community compositions.

A few potential limitations should be considered in this study. First, only using one pore-size to retain particle-attached community may bias the observed results. A previous study of microbial diversity on a continuum of particulate sizes has demonstrated that microbial diversity enrich on larger particulate size (Mestre et al., 2017). Typically, particle sizes of sedimentation in the reservoir become smaller from upstream to dam, which may influence the microbial diversity and community structure. Despite of this, we still observed the accumulation of microbial diversity in a downstream direction. Second, only collecting water samples from one depth may not comprehensively reflect the microbial diversity and community. Increasing evidences have shown aquatic microbial diversity and community structure shift with water depth (Easson and Lopez, 2018; Liu et al., 2019). In this study, we collected surface water samples in August (rainy season), corresponding to the low water level period in the TGR. At this time, water flow speed is faster and discharge capacity reaches to the maximum, resulting in enhanced vertical mixing, with the vertical mixing intensity in deeper water much higher than surface (Gao et al., 2017). That's why we didn't observe obvious changes in physico-chemical parameters from surface to depth of 25 m. Although we focused on the spatial changes along water flow direction in this study, we could not rule out of the probability that both environmental conditions and microbial community change greatly in water deeper than 25 m. Therefore, future study should consider both the existence of a continuum of particulate sizes and vertical structures of hydrographic conditions.

3. Conclusions

In summary, by using qPCR and high throughput sequencing, we tracked the immigration of microbial species from upstream to TGD and have several novel findings. First, we demonstrated that microbial diversity accumulates in a downstream direction despite microbial abundance decreasing and microbial community structure was convergent at sites close to TGD; second, shared species increased in a downstream direction with unique species decreasing, indicating dispersal overwhelm local environmental conditions in the reservoir; third, we assessed the relative contributions of microbial species from mainstem, tributary and soils to community immediately upstream of dam, illustrating that there is little contribution from soils compared to tributaries, espe-

cially those closer to TGD. These findings highlighted the accumulating effect of dam on microbial diversity and community composition. Collectively, the mixture of different sources of microorganisms maintained the high level of microbial diversity in the areas immediately upstream of TGD and consequently assured the reservoir ecosystem function.

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Appendix A. Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.08.006.

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