Is core bacterial community more vulnerable to environmental changes in dammed river?

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Abstract. It is well known that dam construction has a potential to impact water quality, resulting other related changes on bacterial community structure and diversity. Yet, effects of dam construction on planktonic and sediment bacterial structure are much more fragmentary. Through sampling of water and sediments along a dammed river between winter and summer, bacterial community were investigated using Illumina high-throughput sequencing. Core bacterial community, including Proteobacteria (55.29%), Firmicutes (25.29%), Bacteroidetes (17.22%), Verrucomicrobia (1.27%) and Gemmatimonadetes (0.93%), were maintained among water and sediments and between seasons. Mantel test showed the core bacterial community was less sensitive to environmental variable. However, a few of dominant microbe, such as class Alphaproteobacteria and family Sphingomonadaceae in core community were still tightly correlated with physiochemical properties according to correlation analysis. Physiochemical characteristics in water and sediment were mainly affected by season fluctuations. Changes in sediment texture has been attributed to the dam construction. This study suggests a potential to detect possible biological indicators of human and natural pressures in riverine system.

1. Introduction

‘Core microbial community’, which are persisted monthly and longitudinally, refers to a group of operational taxonomic units (OTUs) founds in all samples [1,2]. A recent high-throughput sequencing study showed that species sorting (i.e., bacterial growth competition) potentially impacts core bacterial community, while mass effects (i.e., anthropogenic disturbance and runoff) are highly responsive to changes in the majority of non-core bacterial community (non-shared OTUs) [2]. The majority of sequence reads at each sampling site are usually assigned to the core bacterial community throughout the study area [2,3], thus they are ecologically important.

Several studies have described the impacts of hydropower stations on upstream and downstream water quality [4,5] and other related changes on microbial community structure and diversity [6-8]. These changes can help us identify the variations of ecosystem functioning, such as biogeochemical cycling and biodegradation of pollutants [9]. However, effects of hydropower stations on planktonic and sediment bacterial assemblages are much more fragmentary, given the spatial heterogeneity of bacterial community in rivers or streams ecosystems. The high habitat heterogeneity has suggested a large difference between bacterial communities in sediments and the water column [10,11], but a large dispersal potential in flowing water systems has led to a displacement by core bacterial community as the river moved downstream [3]. Those core bacterial communities are repeatedly detected in rivers around the world [1-3,12,13].

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Current understanding of core bacterial community is limited by scope, typically focused only on planktonic bacterial community, with limited integration of ambient conditions, such as the construction of hydropower station. In this study, we collected both sediments and water samples before and after the hydropower station. The microbial community structure was further detected by the Illumina next-generation sequencing method. Specifically, this study aimed to address the following questions: (i) How does hydropower station affect core and non-core bacterial community? (ii) How does environmental variables affect core and non-core bacterial community? We speculate that core bacterial community was persistent within both water and sediment, and non-core bacterial community was more sensitive to environmental changes. A comprehensive investigation of core and non-core bacterial community in dammed river would contribute valuable information for effective monitoring practices in aquatic environments.

2. Methods and Materials

2.1. Study area and environmental samples collection

The Gongguoqiao hydropower station is located in the middle reach of the Lancang River in China. Sediment (10 g) and surface water (1 L) samples were collected from each of the four study sites in duplicate (January, 2017) and triplicate (July, 2017) (Figure 1). Sediment samples (top 10 cm) in the reservoir (U) and clayey bank sediments (top 10 cm) at riverine sites (D, M, T) were collected with horizontal sediment sampler (inner diameter (ID) = 10.1 cm; tube length (L) = 45 cm) and soil core sampler (ID = 0.38 cm; L = 50 cm), respectively. Surface water samples (top 20 cm) were harvested using a 0.2 μm micropore membrane filter. Sediments and filters were stored in dry ice and transported to the laboratory in a few days.

Water temperature (T), pH, dissolved oxygen (DO), conductivity (COND), turbidity (TURB), total dissolved solids (TDS) and oxidation-reduction potential (ORP) were measured with HORIBA-U52 multiparameters water analysis instrument (HORIBA Corporation, Japan). Samples for total nitrogen (TN) and total phosphorus (TP) measurements were filtered with 0.2 μm membrane filter and stored in sterile polythene bottles. Water samples for bacterial abundance were fixed with 0.2 μm prefiltered formaldehyde to a final concentration of 1% vol/vol before stored in sterile polythene bottles. Sediments for water content (WC), organic matter (OM) content, TN, TP, microbial activity (MA), and bacterial abundance (BA) were collected with sterile plastic bags. All samples above were returned to the laboratory at 0-4 °C in dark within 48 hr.

Figure 1. Location of four sampling sites along the Lancang River.
2.2. Physiochemical, microbial activity and bacterial abundance analysis

TN and TP in water samples were measured by alkaline potassium persulfate oxidation method [14] and an ascorbic acid method after persulfate digestion [15], respectively. TN and TP in sediment samples were determined using simultaneous digestion method as described by Luo et al. [4]. Organic matter content (OM) of the sediment samples was determined by subtracting the sediment weight after combustion at 550 °C for 5 h from the oven-dry sediment weight (105 °C for 4 h) (WC). Sediment MA was measured by modified spectrophotometric determination of the hydrolysis of fluorescein diacetate (FDA) [6]. BA in water was analyzed using acridine orange (AO) according to standard protocols [16]. Sediments were pretreated with Tween-80 to detach bacteria. The bacterial cells were then quantified using AO according to the standard protocol.

2.3. Water and sediment DNA extraction, PCR amplification and Illumina sequencing

Both water DNA (retained on filters) and sediment DNA (0.2 g fresh sediment) were extracted using E.Z.N.A. soil DNA kit (Omega Biorek Norcross, GA, USA), according to the manufacturer’s protocol. The V3-V4 regions of the bacteria 16S RNA gene were amplified using bacterial 16S universal primers 341F: CCTACGGGNGGCWGCAG and 806R: GGAATTCNVGGTGATCTAAT. PCRs were performed in triplicate with 50 μL mixtures containing 1 μL of Taq enzyme, 1 μL of template DNA, 3 μL of primer mixture, 5 μL of dNTP mixture, 5 μL of PCR reaction buffer, and 35 μL of PCR-grade water. Thermal cycling conditions were as follows: 95 °C for 10 min (initial denaturation), 30 cycles of 30s at 95 °C (denaturation), 56 °C for 30 s (annealing), and 72 °C for 40 s (extension), followed by 10 min at 72 °C. PCR products were pooled together from triplicate and then purified using AMPure XP beads. Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina platform according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession number: PRJNA704754).

Paired end clean reads were merged as raw tags using FLSAH (v 1.2.11) with a minimum overlap of 10 bp and mismatch error rates of 2%. OUTs were filtered with a similarity threshold of 97% using UPARSE pipeline (v 7.1). Chimeras were also removed using UCHIME and sequences were aligned against the latest SILVA database.

2.4. Statistical analysis

Significant tests between any two compared objects were analyzed by one-way ANOVA with Tukey’s post hoc test. The relationship between bacterial assemblages and water or sediment physicochemical properties were assessed using Spearman rank correlation analysis. The core bacterial community in this study was considered as the OTUs shared by all sediment and water samples in two seasons, while the remaining OTUs were non-core bacterial community. Non-metric multidimensional scaling (NMDS) was performed to group the bacterial communities of water and sediment samples. 999 permutations of permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis dissimilarity was used to determine beta diversity differences. Mantel test was performed to determine the relationship between environmental variables and bacterial community composition. All statistical analyses described above were analyzed with either the vegan package in R software (v 1.1.4463) or SPSS (v 20).

3. Results

3.1. Physiochemical properties of water and sediment along the dammed river

Physicochemical properties of water and sediment in this study are summarized in table S1 in the supplemental materials. The one-way ANOVA with Tukey’s post hoc test indicated that physicochemical characteristics in water differed significantly between winter and summer seasons, with an exception of turbidity. The turbidity and TP varied greatly between sampling sites (p < 0.05) ranging from 86.7 to 10627.5 NTU and 0.06 to 0.24 mg/L, respectively. Significant differences in sediment TN, TP, N:P, MA and BA between two seasons were observed (p < 0.001). The WC in sediment ranged from 50.8 to 79.4% with the significantly low content in U. The Spearman correlation test revealed that TDS and TURB in water were positively correlated during winter (r = 0.82, p = 0.01)
and summer \((r = 1, p < 0.001)\), whereas N:P and TP were negatively correlated during two seasons \((p \leq 0.01)\). Moreover, water content in sediments showed strong negative correlations with TP during winter \((r = -0.76, p = 0.03)\) and summer \((r = -0.89, p < 0.001)\).

3.2. **Bacterial community characterization and comparison**

3.2.1. **Richness and diversity of core and non-core bacterial community.** After quality filtering for all 40 samples, a total of 1,575,280 high quality reads were obtained (rarified to 39,382 reads per sample), and were clustered into 42,736 OTUs at the cutoff of 97% sequence similarity. The core (OTU shared by all samples) and non-core (the remaining OTUs) bacterial community were estimated to be 28 and 42,708 OTUs, respectively. However, the percentage of core bacterial community \((0.066\%)\) accounted for 25.65% of the total sequence reads.

The diversity indices were listed in table 1. According to the \(\alpha\)-diversity analysis, considerably lower Shannon diversity index was observed for core bacterial community in water \((p = 0.04)\) between two seasons. One-way ANOVA indicated that Shannon and Simpson diversity index for core, non-core and whole bacterial community in sediment were greatly high in U, but they did not differ significantly between downstream sites (i.e., D, T, M) \((p > 0.5)\). Shannon diversity index for both non-core and whole bacterial community in water differed significantly between two seasons \((p < 0.05)\).

![Table 1. Diversity indices among all samples.](image)

3.2.2. **Bacterial community structure and composition of core and non-core bacterial community.** In the core bacterial community, classified OTUs belonged to 5 phyla, including **Proteobacteria** (55.29%), **Firmicutes** (25.29%), **Bacteroidetes** (17.22%), **Verrucomicrobia** (1.27%) and
Gemmatimonadetes (0.93%) (Figure 2a). As shown in Figure 2a, bacterial phylum Bacteroidetes was much more abundant in water samples for the core bacterial community ($p < 0.001$). Proteobacteria (32.39%) and Actinobacteria (5.86%) were the most abundant phyla for the non-core bacterial community (Figure 2a). Gammaproteobacteria and Betaproteobacteria were the most abundant class of Proteobacteria in both water and sediment for the core and non-core bacterial community, respectively (Figure 2b). When listed the top 10 abundant phyla across all sediment and water samples, we found Proteobacteria (46.82%), Bacteroidetes (21.10%) and Firmicutes (7.51%) were the dominant phyla in water, whereas Proteobacteria (40.13%) was the most abundant phylum in sediment, followed by Acidobacteria (13.10%) and Planctomycetes (11.05%). Moreover, the relative abundance of Bacteroidetes decreased from upstream to downstream of the hydroelectric dam.

![Figure 2. Community composition of core and non-core bacteria at the level of phylum (a) and class (b) in water and sediment samples.](image)

NMDS based on Bray-Curtis distance was performed to compare the microbial community structure among all water and sediment samples (Figure 3). It indicated that sediment and water samples grouped separately for core (Figure 3a), non-core (Figure 3b) and whole (Figure 3c) bacterial community. The non-core bacterial community was greatly concordant with that of the whole bacterial community. The seasonal effects for core, non-core and whole bacterial community in sediments were relatively weaker than those in water. Significant differences were observed among bacterial communities for all comparisons (PERMANOVA, $p < 0.05$). PERMANOVA test also showed the core community explained a large proportion of the variance in sediments and water during two seasons.

3.3. Influential factors governing core and non-core bacterial community

As analyzed by Mantel analysis, different environmental variables between different seasons were responsible for the differences in core community (table S2). For instance, the core bacterial community was significantly correlated with ORP, COND, TP and MA in summer, but TDS and N:P played a more important role on core community of water in winter. In sediments, core bacterial community was only related with sediment properties in summer. It seems that core community was less sensitive to environmental changes than non-core community. In contrast, environmental factors, such as COND, TURB and TP, contributed significantly on the variance of non-core and whole bacterial community in water between
Figure 3. Core (a), non-core (b) and total (c) community composition structure as indicated by non-metric multidimensional scaling (NMDS) plots. Replicate or duplicate sampled collected from the same site were marked with the same color.

two seasons. DO showed the highest correlation value with the non-core bacterial community in winter ($r = 0.70, p < 0.001$). A Pearson’s correlation analysis further showed physicochemical parameters in water were greatly correlated with the most abundant phyla of the core and non-core bacterial community. For instance, COND ($r = 0.74, p < 0.001$), DO ($r = 0.77, p < 0.001$), N:P ($r = 0.83, p < 0.001$), TN ($r = 0.71, p < 0.001$), TP ($r = -0.65, p = 0.002$), TDS ($r = 0.76, p < 0.001$) and BA ($r = 0.62, p = 0.004$) had significant influence on Bacteroidetes at the level of phylum for core bacterial community in water. Among Bacteroidetes, the class Flavobacteriia also showed a strong correlation with the majority of environmental parameters. In addition to Flavobacteriia, the class Alphaproteobacteria among Proteobacteria was found to be positively correlated with TDS ($r = 0.88, p < 0.001$), TN ($r = 0.83, p < 0.001$), DO ($r = 0.84, p < 0.001$), COND ($r = 0.89, p < 0.001$), N:P ($r = 0.90, p < 0.001$), pH ($r = 0.55, p = 0.011$) and BA ($r = 0.59, p = 0.006$), whereas the class Gemmatimonadetes was significantly negatively correlated with a majority of above environmental parameters. Similarly, for the non-core bacterial community, the classes Gammaproteobacteria (phylum Proteobacteria), Flavobacteriia (phylum Bacteroidetes), and Alphaproteobacteria (phylum Proteobacteria) were 3 most abundant classes identified from all samples, they were all strongly correlated with the measured environmental variables in water. Flavobacteriaceae (phylum Bacteroidetes) were highly represented in core and non-core bacterial community, however, only certain taxa in the non-core community, such as family Flavobacteriaceae and species Flavobacterium sp THG—DN619, were associated with physiochemical properties in water. In contrast to water, the most abundant phyla of core bacterial community in sediment, such as Proteobacteria, Firmicutes and Verrucomicrobia, were significantly associated with WC. Moreover, WC was also closely related to the dominant taxa on the class and family level (Figure 4). Whereas MA, N:P and TP were strongly correlated with the dominant phyla, classes and families of the non-core bacterial community.
this, observations among properties. Studies before that higher bacteria were accounted for and reported few species. Mantel's competitive community suggests a core study, were similarly bacterial physiochemical (32.74%) to the water. A few bacterial compounds, highly Bacteroidetes were converted and polysaccharides) was abundant related to the sediment. Planktonic and bacterial and microbial damming have suggested to contribute more independent and to operation areas. Winter. (such as) core and sediment non-core to bacterial tests to expected, Notably, for other widespread conditions but are still contributed to results the study community, core of non-core impacted by untreated wastewater and microbial historical variables, provides the operation area, and microbial flow were simpler and homogenized. As expected, Mantel test suggested that the core bacterial community in water and sediment was not highly dependent on environmental variables, especially for sediment samples in winter. However, a few dominant taxa of core and non-core microbial community at the...
level of phylum, class (Figure 4a and b) and family were highly influenced by a number of water variables (such as DO, TN, TDS, T, ORP, BA, pH, COND). Water chemistry from upstream to downstream of the river did not differ significantly during either winter or summer, which can be partially explained by the environmental homogeneity imposed by dam operations as mentioned above. However, water chemistry showed great seasonal variations. Greatly seasonal fluctuations in water chemistry are often attributed to the introduction of surface runoff and precipitation during raining season (i.e., summer) [2,21], and this directly influences some core microbial communities in water (Figure 4a). On the contrary, the most abundant taxa of core bacterial community in sediment were only considerably related with WC (Figure 4c), while main compositions of non-core community in sediment (such as Alphaproteobacteria, Deltaproteobacteria and Acidobacteria) were significantly correlated with both sediment nutrient levels like TP, N:P and MA and sediment texture like OM (Figure 4d). Seasonal changes in the nutrient levels (N:P, TP and TN) of the sediment were significant, which may account for the differences of non-core bacterial community between seasons [23]. Our results also indicated that WC was influenced by sampling sites instead of seasons. Several studies also hold the point that dam changes the sediment regime, thus inducing large alterations on WC, OM and particle size of sediment [24].

5. Conclusions
In conclusion, core bacterial community, which may not always the most abundant but ubiquitous among all sediment and water samples, was observed in our study. Dam construction and operation have changed the sediment texture (e.g., WC and OM content), resulting in variations on dominant taxa of non-core bacterial community. Seasonal fluctuations provided the greatest contributions to water chemistry and sediment nutrients. Moreover, sediment nutrients and water chemistry were tightly correlated with the most abundant taxa from class to family in core and non-core bacterial community, suggesting physiochemical properties still played an important role in shaping the dominant taxa in core and non-core community. In general, the core community was less sensitive to environmental changes than non-core bacterial community.

Conflict of Interest
The authors declare no conflict of interest.

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