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Research article

Reduction of bacterial integrity associated with dam construction: A quantitative assessment using an index of biotic integrity improved by stability analysis

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ABSTRACT

Rivers are extensively regulated by damming, yet the effects of such interruption on bacterial communities have not been assessed quantitatively. To fill this gap, we proposed a bacteria-based index of biotic integrity (Ba-IBI) by using bacterial community dataset collected from the Three Gorges Reservoir and its upper reaches. Stability analysis based on bacterial resistance (*RS*) and resilience (*RL*) to external disturbance was conducted to improve the performance of the index. Four core metrics, i.e. the ratio of Bacilli, Bacteroidetes and Clostridia to Alphaproteobacteria (BBC/A), Oxalobacteraceae, Methanotrophs and Thermophiles were selected after range, responsive and redundancy tests. The improved Ba-IBI, ranging from 1.04 to 4.10, was better at distinguishing sites with or without direct dam effects compared with the unimproved one. The index values maintained high in the riverine sites while reducing in the reservoir, demonstrating the negative influence of dam construction on bacterial integrity. Based on the assessment results, 23.1%, 46.2% and 30.8% sampling sites were large, moderately and little affected by damming, respectively. A Random Forest (RF) regression model was trained and tested, offering a valid prediction of the input Ba-IBI and environmental parameters. Sensitivity analysis revealed the significant contributions of flow velocity towards the predicting process performance, indicating the importance of hydrodynamic conditions on determining the spatial variability of bacterial communities. This study provides not only a first quantitative insight for assessing bacterial response to damming, but also a guideline for applying the improved index in the dam regulation and ecological protection.

1. Introduction

The world's rivers are extensively dammed and regulated for many reasons including water supply, flood control, security assurance, clean energy generation and economic development. China has an extensive history of water resource development and possesses the largest number of dams and the largest amount of hydropower generation of the world. As the longest river in Asia and the third longest river in the world, the Yangtze River flows 6397 km eastward to the East China Sea, draining an area of approximately 1,800,000 km^2 and supporting 450 million people in China (Yang et al., 2014). More than 50,000 dams have been constructed throughout the Yangtze's watershed, including the largest hydropower project of the world, i.e. the Three Gorges Dam (TGD) (Yang et al., 2011).

The massive water projects have brought tremendous benefits accompanied by significant environmental and ecological changes. Due to the dams construction, rivers have been transformed from a riverine system into a lacustrine system, changing along with specific attributes of reservoirs including short water retention times and longitudinal heterogeneity (Moitra and Leff, 2015). These changes may result in the variations of river dynamics (Poff et al., 2007), material cycles (Scott et al., 2009), river discharge patterns (Azevedo et al., 2010) and biotic habitats (Poff and Olden, 2017). Considering the significant role of Yangtze River, effects of the TGD on the river processes and their associated ecosystems have been the focus of various debates. The increased water depth due to damming could limit the vertical mix of water and result in the enrichment of nutrients in the sediments (Gao et al., 2016). The TGD construction also changed water quality, riverine self-purification ability, aquatic and terrestrial biodiversity in the Three Gorges Reservoir (TGR) (Kaifeng Li et al., 2013). The physically, chemically and biologically altered environments could, in turn, significantly influence sediment bacterial communities, which exert

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important roles in biogeochemical processes including the biodegradation of organic matters, the cycling of nutrients and the biostabilization of sediment deposits (Fang et al., 2017).

Given the importance of bacterial processes to aquatic ecosystem and bacterial potential to offer an early indication of shifts in the ecosystem before macro-organisms respond (Lau et al., 2015), it is critical to evaluate the influence of damming on riverine systems with consideration of bacterial communities. The existing studies mainly focused on the composition of bacterioplankton in the reservoir systems (Wang et al., 2012a; Yan et al., 2015). Sediment bacterial community structure and diversity changes along the TGR have been investigated before (Sekiguchi et al., 2002) and after (Chen et al., 2013) the construction of the TGD, providing a reference for future changes of bacterial communities. However, our understanding of bacterial communities affected by damming is largely restricted to the spatial distribution along the environmental gradients, while the quantitative analysis based on multiple bacterial data has not been conducted. Therefore, a systematic and quantitative tool needs to be established for investigating bacterial response to dam construction.

The index of biotic integrity (IBI), first introduced by Karr (1981), is an essential tool in the process of environmental assessment, restoration and conservation of aquatic ecosystems (Ruaro and Gubiani, 2013). Indicators used to assess the biodiversity of freshwater ecosystems have largely been confined to macro-organisms including benthic macroinvertebrates, fishes, birds, amphibians, periphyton and plants (Marzin et al., 2012). Until recently, bacterial communities have been included in the IBI indexes and the sensitivity and reliability of the index for the ecological assessment were validated by Li et al. (2017a). In the development of IBI, the biotic metrics are susceptible to the external disturbances and could directly influence the reliability of assessments. Thus the accurate and precise selection of metrics has become a significant challenge. Ecological stability is an essential tool to reflect a community's responses to disturbances (Pimm, 1984). The responses can be quantified in terms of two components: resistance, the ability of bacterial properties to remain unchanged during disturbance, and resilience, the ability of these properties to recover following the end of disturbance (Thion and Prosser, 2014). Integrating the indices presented by Orwin and Wardle (2004) in the development of IBI might help us determine the difference in stability among the metrics and thus enhance the reliability of the assessment results.

Therefore, the main objective of this study was to develop a bacteria-based index of biotic integrity (Ba-IBI) and improve the index by integrating stability analysis in its development process. Then, the improved index was applied to quantitatively assess the impact of TGD construction on bacterial communities based on sediment samples collected in the TGR and its upper reaches in three different periods. By integrating the stability analysis in the development of IBI, our approach embraced a wider range of bacterial data and could exclude the impoundment disturbance simultaneously. This study not only filled the gaps of previous indexes, but also provided a quantitative and comprehensive assessment method for conservation and management of dams and other hydropower generation.

2. Materials and methods

2.1. Study area and sample collection

The Three Gorges Dam, 190 m high and 2 km wide, is located in the middle reaches of the Yangtze River. The Three Gorges Reservoir formed by the TGD, lies in a 663 km valley from Yichang to upstream Chongqing (105°50′-111°40′ E, 28°31′-31°44′ N), is the largest artificial reservoir in the world (Gao et al., 2016). With the TGD fully constructing, the water level of the Yangtze River raised to the highest value of 175 m, and fluctuated between 145 m and 175 m in the TGR.

In this study, 13 sampling sites were selected both in the TGR (S3 to S13) and its upper reaches (S1 and S2) as illustrated in Fig. 1. At each site, water and sediment samples were collected in triplicate in September 2016, December 2016 and April 2017, respectively. Water samples were collected using pre-cleaned polyethylene bottles and stored at 4 °C. The surface sediment samples were taken from the top of the streambed (0–10 cm) using a hand-held coring device (Wildlife Supply Company, Saginaw, MI). All sediment samples were homogenized and subsampled, and subsequently frozen at −80 °C for DNA isolation.

2.2. Physicochemical and molecular analysis

Water temperature (T), dissolved oxygen (DO) and the pH value (pH) were measured in situ using a HACH HQ30d portable meter (HACH Company, Loveland, CO, USA). Flow velocity was determined using a portable Doppler flow meter (DPL-LS12). Total nitrogen (TN), total phosphorus (TP), ammonium nitrogen (NH_3—N), biochemical dissolved oxygen demand (BOD_5), chemical oxygen demand (COD), suspended solids (SS) and *Escherichia coli* (*E.coli*) were determined following the Environmental Quality Standards for Surface Water in China. The devices and their manufacturers used in the physicochemical analysis were listed in Table S1.

Genomic DNA was extracted from each sediment sample using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, United States) following the manufacturer's recommended protocol. Polymerase chain reaction (PCR) amplification of the 16S rRNA gene was carried out in triplicate following the protocol: 95 °C for 3 min, 27 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s and 72 °C for 10 min (Cai et al., 2017). The extracted DNA was sent to Shanghai Majorbio for Illumina MiSeq sequencing. The 16S rDNA sequences were clustered into operational taxonomic units (OTUs) based on a 97% similarity cutoff.

2.3. Metrics selection and stability analysis

A dataset of candidate metrics characterizing the impact of the dam construction was selected based on the published literature (Chen et al., 2014; Li et al., 2017a). These metrics belonged to four categories that described aspects of diversity, composition, tolerance and function. Diversity metrics were calculated at phylum and genus OTU levels using the vegan package in R (v.3.12, http://www.r-project.org/). Composition metrics were selected based on the abundance of individual main bacterial taxa. The proportional variables were arcsine and square root transformed to increase reliability and availability of the metrics (Jia et al., 2013). Moreover, the sums or ratios of representative bacterial groups (Garrido et al., 2014) were also included in the metrics. Tolerance metrics were selected following the process of Li et al. (2017b). Function metrics were selected using METAGENassist (http://www.metagenassist.ca) to probe bacterial phenotypes, metabolism and functional pathways (Arndt et al., 2012).

In order to test the response of these metrics to the water level fluctuation caused by dam impoundment, the stability analysis was conducted using the indices proposed by Orwin and Wardle (2004). Resistance (*RS*) was calculated using Eq. (1):

$$RS = 1 - \frac{2|D_0|}{(C_0 + |D_0|)} \quad (1)$$

where C_0 is the scores of metric value before the dam impoundment (i.e. September 2016), and D_0 is the difference between the control (C_0) and metric value at the end of the impoundment disturbance (i.e. December 2016). The obtained index increases monotonically with resistance. It is bounded by −1 and +1, with a value of +1 indicating a maximal resistance to the disturbance, and lower values showing less resistance (i.e. the impoundment disturbance had significant effect on certain candidate metric). An index value of 0 indicates a 100% change compared to the control and a value < 0 indicates a more than 100% change.

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