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Research article

Development and validation of a bacteria-based index of biotic integrity for assessing the ecological status of urban rivers: A case study of Qinhuai River basin in Nanjing, China

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A B S T R A C T

With the increasing human disturbance to urban rivers, the extinction and biodiversity losses of some macroorganism species decreased the accuracy of bioassessment. In this study, a novel index of biotic integrity based on bacteria (Ba-IBI) was first developed for Qinhuai River in Nanjing city, China. Thirty-two biofilm samples were collected along the river bank and the bacterial communities were identified by high-throughput sequencing. By the range, responsive, and redundancy tests, four core metrics were selected from the dataset of 78 candidate metrics, including Pielou's evenness index, proportion of *Paenibacillus*, proportion of OTUs tolerant to organic pollution and proportion of *Nitrosomonas.* The results showed that the Ba-IBI was able to effectively discriminate different impaired site groups, and had a good correlation with the index of water quality ($r = 0.79$, $p < 0.01$) and the qualitative habitat evaluation index ($r = 0.51$, $p < 0.01$). Moreover, the Ba-IBI was negatively correlated with the number of population within a 1 km buffer ($r = -0.71$, $p < 0.01$). Application of the index showed that most of the sites were in the poor or bad class in the river. Our study revealed that the Ba-IBI is an effective and reliable approach for assessing the ecological status of Qinhuai River basin, which can complement the existing ecological assessment approaches for urban rivers. Meanwhile, repeted surveys and field validations are still needed to further improve the applicability of the index in future studies.

1. Introduction

Rivers play a crucial role in regional sustainable development, providing a wide range of ecosystem functions, such as irrigation, drinking water supply, fisheries, transport, and recreation (Jia and Chen, 2013). Unfortunately, with the rapid development of urbanization, a large quantity of wastewater and sewage containing abundant pollutants was discharged into rivers, which highly degraded the river ecosystem functions, freshwater security and river biodiversity (Pan et al., 2015; Vorosmarty et al., 2010).

Many approaches have been developed to assess the aquatic ecosystem health, which further served for the maintenance and recovery of river ecosystem functions (Borja et al., 2008; Dos Santos et al., 2011). Among them, the index of biotic integrity (IBI) first introduced by Karr (1981) has been widely used in the process of bioassessment, restoration, and conservation of aquatic ecosystems (Ruaro and Gubiani, 2013). Since the IBI represents various structural and functional attributes of an ecosystem and responds differently to different stressors, it can provide a reliable measurement of overall ecological status in a comprehensive manner, which is difficult to assess by traditional physical and chemical methods (Wu et al., 2012). Besides, the IBI can provide a quantitative assessment of ecological status and respond sensitively to anthropogenic pressure, making it useful for conservation and environmental management (Hallett et al., 2012).

Nowadays, many groups of macroorganism have been widely used as indicators for assessing ecological health. For example, fish (Jia et al., 2013; Krause et al., 2013), benthic invertebrates (Couceiro et al., 2012; Huang et al., 2015), diatom (Tan et al., 2015; Zalack et al., 2010) and macrophyte (Beck et al., 2010) were commonly

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used in the development of IBI. Despite the above organisms which are highly sensitive to environmental change and considered to be good indicators, there still remain many limitations. Particularly, in some degraded ecosystem, such as urban rivers, some species were facing extinction and biodiversity losses (McKinney, 2002), that would largely confine the application of these bioindicators and decrease the accuracy of bioassessment.

Bacterial communities within stream biofilms have been proved to be a more sensitive indicator of ecological health than macroinvertebrates within highly impacted streams (Lear et al., 2009). Successful monitoring requires the ability to describe ecological change accurately using quantitative indicators (Ryder and Miller, 2005). With the development of molecular techniques that offers the possibilities to develop quantitative assessment systems based on bacterial communities. Lau et al. (2015) developed a bacterial community index (BCI) to assess the stream health using a partial least squares regression algorithm against the macroinvertebrate community index. However, the BCI was not an independent index, and not correlated to the taxonomic composition and functional attributes of bacterial communities, which may cause it not robust and effective. Thus, it is necessary to develop a novel index based on bacteria to make up for the above existing limitations.

Therefore, the objectives of this study were to develop a bacteria-based index of biotic integrity (Ba-IBI) that was responsive to urbanization gradients, and validate the sensitivity and reliability of the index for the ecological assessment of urban rivers. The Qinhuai River, a representative urban river in Yangze River Delta, was selected as the study area. High-throughput sequencing that can identify the whole taxonomic composition of bacterial communities, was used to identify the bacterial communities in biofilm samples.

2. Materials and methods

2.1. Study region

Qinhuai River, standing in the downstream of Yangze River, locates in Nanjing City, China. The total length is 110 km, and the watershed area is 2631 km^2. Both Lishui River and Jurong River are the two sources of the Qinhuai River. The topography of Qinhuai River is a fan-shaped tectonic basin, surrounded by hills and mountains. Meteorologically, the basin belongs to the subtropical semi-humid monsoon climate. The annual average rainfall is 1047.8 mm and average annual runoff is 6.95×10^8 m^3 (Ji et al., 2014). In recent decades, the dramatic urbanization has caused high impairment to the aquatic ecosystem of the Qinhuai River.

2.2. Sampling and identification

Thirty-two sampling sites were selected along the Qinhuai River (Fig. 1). The detailed descriptions of sampling sites were listed in Table S1. The biofilm samples attached on the river banks were collected in November 2015. In order to minimize the influence of the spatial fluctuation, a composite biofilm sample was made with three replicates within a fifty meter distance at each site. Biofilm samples were kept in sterile tubes (50 ml) respectively and stored at −80 °C until DNA extractions. Values of pH, temperature (T), dissolved oxygen (DO), conductivity (Cond) and turbidity (Tur) were measured in situ by portable meters (HACH HQ40d). Whilst water samples were collected using a polypropylene bottle (1 L) for further laboratory analysis to determine total nitrogen (TN), ammonia nitrogen (NH_4-N), total phosphorus (TP), chemical oxygen demand in manganese (COD_{Mn}), and total dissolved solids (TDS). All these variables were measured according to the standard from the State Environmental Protection Administration of China (2002b).

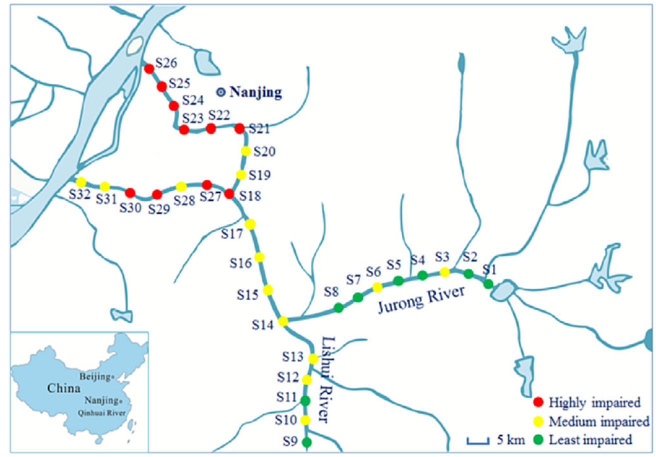

Fig. 1. Locations of the sampling sites along the Qinhuai River.

Total DNA were extracted from the samples using E.Z.N.A® a soil DNA kit (Omega Bio-Tek Inc., USA) according to the manufacturer's instructions. All the DNA samples were sequenced by Illumina Miseq Sequencing at Shanghai Majorbio Bio-pharm Technology Co., Ltd, China. The obtained sequence reads were processed as previous studies (Niu et al., 2015). Raw sequences that were shorter than 150 bp or had even a single base bias were removed from consideration. The operational taxa units (OTUs) were defined by 97% sequence similarity cut-off. The dataset was deposited into the NCBI short reads archive database (Accession Number: SRX2347277, SRX2347304).

2.3. Development of Ba-IBI

2.3.1. Sites classification

In order to select appropriate reference sites for the development of Ba-IBI, a prior classification was performed to classify the sampling sites into least and highly impaired sites based on the level of water-quality impairment. For this, a composite index of water quality (IWQ) was developed to classify all the sampling sites into least and highly impaired sites. The primary parameters for the IWQ were derived from principal component analysis (PCA) of measured physiochemical variables. The scoring system was developed based on the Environmental Quality Standard for Surface Water in China (2002a). Each primary parameter was scored based on the five classes of water quality of the standard (Table 1), and summed to obtain the value of IWQ. The sampling sites were finally categorized into three groups by taking into account the 25th and 75th percentiles of the ranges of IWQ: least impaired, medium impaired and highly impaired.

Table 1
Scoring system for water quality variables.

Assigned individual value	DO (mg L^{-1})	TP (mg L^{-1})	Cond (μs cm^{-1})	COD_{Mn} (mg L^{-1})
0	<2	>0.4	>600	>15
1	2–3	0.3–0.4	500–600	10–15
2	3–5	0.2–0.3	400–500	6–10
3	5–6	0.1–0.2	300–400	4–6
4	6–7.5	0.02–0.1	200–300	2–4
5	>7.5	<0.02	<200	<2

The scoring criteria of DO, TP, and COD_{Mn} were based on the Environmental Quality Standard for Surface Water in China (GB3838-2002). The scoring criteria of Cond refer to the 5, 25, 50, 75, and 95% percentile of the dataset in study area.