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Vertical and horizontal assemblage patterns of bacterial communities in a eutrophic river receiving domestic wastewater in southeast China[☆]



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ABSTRACT

Bacterial communities in rivers receiving untreated domestic wastewater may show specific spatial assemblage patterns due to a wide range of physicochemical conditions created by periodic algal bloom. However, there are significant gaps in understanding environmental forces that drive changes in microbial assemblages in polluted rivers. In this study, we applied high-throughput sequencing of 16S rRNA gene amplicons to perform comprehensive spatio-temporal profiling of bacterial community structure in a local river segment receiving domestic wastewater discharge in southeast China. Multivariate statistics were then used to analyse links between bacterial community structure and environmental factors. Non-metric multidimensional scaling (NMDS) plots showed that the bacterial community structure was different between upstream and downstream sections of the river. While the upstream water contained a high proportion of bacteria degrading xenobiotic aromatic compounds, the downstream water experiencing stronger algal bloom had a more diverse bacterial community which included the genus *Aeromonas* comprising 14 species, most of which are human pathogens. Least discriminant analysis (LDA) effect size revealed that the surface water was mainly inhabited by aerobic microorganisms capable of degrading aromatic compounds, and also contained bacterial genera including pathogenic species. In contrast, in the bottom water we found, along with aromatic compound-degrading species, anaerobic denitrifiers and Fe³⁺-reducing and fermentative bacteria. Variance partitioning canonical correspondence analysis (VPA) showed that nutrient ratios had a stronger contribution to bacterial dissimilarities than other major physicochemical factors (temperature, pH, dissolved oxygen, total organic carbon, and chlorophyll *a*). These results show that microbial communities in rivers continuously receiving domestic wastewater have specific longitudinal and vertical assemblage patterns and may contain pathogenic species presenting a high threat to public health. These factors should be taken into consideration while developing pollution management strategies.

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1. Introduction

Wastewater generated by domestic activities is one of the major sources of natural water pollution, causing deterioration of the environmental conditions. Untreated or partially treated effluents may lead to eutrophication and promote proliferation of pathogens in natural water bodies (Akpor and Muchie, 2011), thus exposing water users to significant health risks. Inadequately treated wastewater discharged to rivers or reservoirs used for agricultural

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irrigation may also cause indirect adverse effects on human health (Drechsel et al., 2010). In addition, there are long-term problems created by refractory organic substances and heavy metals released with domestic wastewater.

Microorganisms are responsible for waterborne disease outbreaks, but also play an important role in decomposition of organic matter (OM) essential for carbon and nitrogen cycling in rivers (Besemer et al., 2009). Polluted rivers may contain recalcitrant organic compounds released in domestic wastewaters, which can resist biodegradation (Kim et al., 2008, 2013). However, functionally diverse microbial groups present in polluted waters (Zhang et al., 2016) and associated with OM degradation may still decompose refractory organic compounds through cooperative metabolic chains (Bianchi, 2011).

Rivers play an important role in the global cycling of carbon and nitrogen (Trimmer et al., 2012) as well as in transporting OM and spreading microbes from upstream to downstream regions. Rivers exhibit longitudinal gradients of physicochemical factors such as NH_4^+ , NO_3^- , dissolved oxygen (DO), pH, and carbon sources (Esteves et al., 2015), which may cause shifts in bacterial assemblages along the course of the river (Winter et al., 2007; Esteves et al., 2015; Read et al., 2015). Therefore, bacterial communities observed in different river segments present locally adapted microbial groups transported from the upstream regions. Accordingly, pathogenic bacteria may also show longitudinal distribution patterns that are shaped by the availability of nutrients, especially OM governing the growth of heterotrophic microbial pathogens (Vital et al., 2010).

However, there is a lack of knowledge regarding vertical distribution and diversity of bacterial communities, especially in the overlying water. In shallow rivers, the overlying water has been thought to have homogeneous bacterial composition; however, there is increasing evidence of microbial heterogeneity or even stratification, especially under the condition of frequent cyanobacterial bloom (Serafim et al., 2006; Gao et al., 2016). During algal growth, heat redistribution from the surface is reduced by light screening due to a dense algal layer and turbid water columns (Mazumder and Taylor, 1994; Weyhenmeyer, 1996; Diehl et al., 2002), causing thermal stratification (Wilhelm and Adrian, 2008) and limiting the downward oxygen transfer from the surface. Such temperature and oxygen gradients may lead to the formation of vertical gradients of nutrients (N/P) and dissolved organic carbon (DOC) (Gu and Stefan, 1995; Song et al., 2013), resulting in heterogeneity of bacterial communities in stratified water columns observed both in fresh water reservoirs and sea (Kim et al., 2011; Bougouffa et al., 2013; Yu et al., 2014; Walsh et al., 2016).

Understanding spatial distribution and diversity of bacterial communities could shed light on their ecological functions. The association of bacterial community structure with geographic space has been well studied in terrestrial ecosystems at the scale range from centimetre to kilometre (O'Brien et al., 2016). Heterogeneous microbial distribution in lakes and oceans has also received increasing attention. However, previous studies have focused mainly on longitudinal variations in bacterioplankton composition of rural rivers with low pollution loads. Consequently, little is known about the vertical changes in microbial populations and the underlying driving factors in eutrophic rivers continuously receiving domestic wastewaters. Reports consistently showed ecological succession of bacterial communities from upstream to downstream (Winter et al., 2007; Read et al., 2015; Savio et al., 2015), but an abrupt and clear shift was detected in river segments with intense phytoplankton blooms (Winter et al., 2007). García-Armisen et al. (2014) have studied seasonal variations and longitudinal distribution of bacterial populations in a sewage-polluted urban river, but only at the phylum level, and did not analyse dynamics of pathogenic microbes and OM-degrading

bacteria. None of the above studies performed vertical profiling of bacterial communities. However, comprehensive analysis of spatial distribution of microbial groups, including pathogens and OM-metabolizing species is vitally important for understanding the effects of environmental pollution.

We hypothesized that in the overlying water of polluted urban rivers characterized by a wide range of physicochemical conditions due to periodic algal bloom, bacterial communities may show both longitudinal and vertical variations and specific assemblage patterns. Therefore, in this study, we analysed seasonal and spatial (at the centimetre scale) microbial diversity in a river segment known for distinct gradients of physicochemical parameters (temperature, DO, pH, and nutrients) in the water column (Gao et al., 2016) using high-throughput sequencing of the 16S rRNA gene and multivariate statistics. The relationships between bacterial community structure and environmental factors were also investigated.

2. Materials and methods

2.1. Sampling sites

The study was conducted in a eutrophic river segment flowing through Jiangsu Academy of Agricultural Sciences (JAAS) near the foot of Zijin Mountain in the Zhongshan scenic area, Nanjing, China ($32^\circ 02' 21'' \text{N}$, $118^\circ 52' 37'' \text{E}$). The river has been receiving domestic wastewater and rainwater from the Zijin mountain area and is characterized by periodic proliferation and dominance of cyanobacteria. Total nitrogen (TN) and total phosphorus (TP) concentrations were 14 mg L^{-1} and 4 mg L^{-1} , respectively, in the influx to the JAAS river segment throughout the year. As Nanjing is located in the subtropical climate zone of southeast China and has four distinct seasons, water temperature varies from -5°C in winter up to 32°C in summer. The upstream and downstream sections of the river segment (total surface area $\sim 14,000 \text{ m}^2$, water depth 1.5–3.5 m) are connected by a narrow stream, and there is a sluice installed downstream to control water flow for irrigation of rice fields. Four sampling sites, each with triplicate sampling points, were selected along the segment (Fig. 1). To investigate vertical profiles of bacterial assemblages, three depth points including surface (0.1 m below the surface), middle (0.6 m), and bottom (0.05 m above the sediment) were examined at each sampling site.

2.2. Sample collection

Sampling was conducted in January, June, and August on sunny days when a gradual increase of algal density was observed after a week without rain. During sampling, water quality field meters (YSI Pro Plus, USA) were manually placed at the indicated depths for *in situ* monitoring of DO, pH, oxidation-reduction potential, and temperature. Water samples were collected using a portable automatic water sampler JC-8000A (Qingdao Qichuang Environmental Equipment Co., LTD, China), with the sampling latex tube manually located at the fixed depths. After collection, the samples were immediately transported to the laboratory and analysed for concentrations of chlorophyll *a* (Chl*a*), NO_3^- , NO_2^- , NH_4^+ , total dissolved nitrogen (TDN), PO_4^{3-} , total dissolved phosphorus (TDP) and total organic carbon (TOC) (Table S1). Water samples (500 mL) were filtered through $50 \text{ }\mu\text{m}$ -pore filter paper to remove visible impurities and then through $0.22\text{-}\mu\text{m}$ Millipore membranes using a vacuum air pump; the membranes were stored at -80°C until DNA extraction. Isolated genomic DNA was used for amplification and sequencing of 16S rRNA genes performed by Novogene Corporation (Beijing, China) using the MiSeq system (Illumina, Inc., San Diego, CA, USA).

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