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Characteristics of aquatic bacterial community and the influencing factors in an urban river

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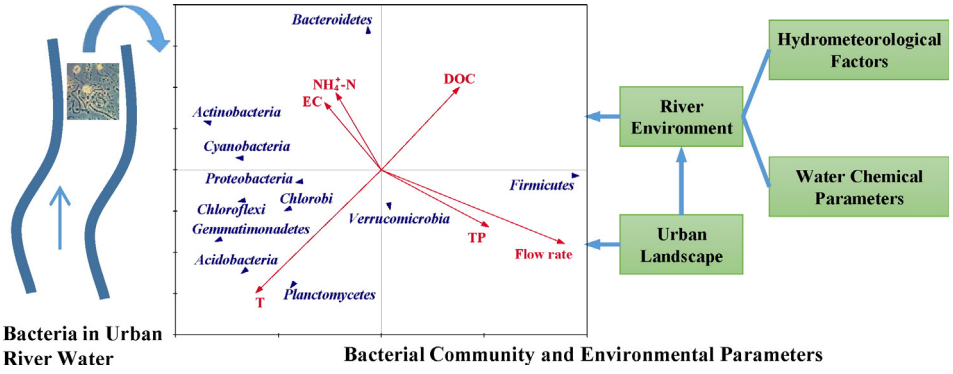
HIGHLIGHTS

- *Actinobacteria* and *Proteobacteria* are the dominant phyla in river water.
- *Firmicutes* (mostly genus *Lactococcus*) is the dominant phylum during flooding.
- Temperature and runoff are the main influencing factors of bacterial community.
- The influence of urban landscape on the bacterial community is about 17%–34%.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacteria play a critical role in environmental and ecological processes in river ecosystems. We studied the bacterial community in the Ganjiang River, a major tributary of the Yangtze River, as it flowed through Nanchang, the largest city in the Ganjiang River basin. Water was sampled at five sites monthly during the wet season, and the bacterial community was characterized using Illumina high-throughput sequencing. A total of 811 operational taxonomic units (OTUs) were observed for all samples, ranging from 321 to 519 for each sample. The bacterial communities were maintained by a core of OTUs that persisted longitudinally and monthly. *Actinobacteria* (41.17% of total sequences) and *Proteobacteria* (31.80%) were the dominant phyla, while *Firmicutes* (mostly genus *Lactococcus*) became most abundant during flooding. Temperature and flow rate, rather than water chemistry, were the main factors influencing the bacterial community in river water. Temperature was the best individual parameter explaining the variations in OTU abundance, while flow rate was the best individual parameter explaining the variations in phylum abundance. Except for *Proteobacteria*, the relative abundance of bacterial phyla did not differ significantly between sites, and the degrees of influence of urban landscape on the bacterial community were estimated to be 17%–34%.

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1. Introduction

Bacteria carry out key processes in river nutrient cycles and are responsible for a large part of organic matter breakdown (Findlay, 2010), which strongly influences water quality. Understanding the bacterial community composition and its influencing factors is helpful in evaluating river water quality and clarifying nutrition cycle mechanisms (Anderson et al., 2002). Due to its ability for deep sequencing of bacterial communities and identification of rare populations in low abundance, high-throughput sequencing technology has become a popular tool for examining bacterial communities (Caporaso et al., 2011; Glenn, 2011). The most abundant bacterial phyla in river water have been found to be *Proteobacteria* (mostly *Betaproteobacteria*), *Actinobacteria* and *Bacteroidetes*, which is similar to the results observed in lake water (Newton et al., 2011; Tamames et al., 2010).

Factors such as nutrient concentrations (Liu et al., 2012), temperature (Staley et al., 2015), water residence time (Read et al., 2015) and other biotic and abiotic factors (Kolmakova et al., 2014) have been found to alter bacterial communities in river water. However, the specific factors that drive temporal and spatial variations in bacterial community structure are not well understood because different influencing factors are observed in different studies. Moreover, most studies of bacterial communities in river water are based on one-time sampling (Gladyshev et al., 2015; Liu et al., 2012; Read et al., 2015; Xia et al., 2014), so it is difficult to analyze the influence of changes in hydrometeorological factors such as temperature, rainfall and flow rate. In a study based on water samples collected along the Upper Mississippi River at three different times, Staley et al. (2015) found that temperature and rainfall had the greatest influence on microbial communities. Sun et al. (2014) studied the dynamics of microbial communities in water in a reservoir of the Yangtze River based on monthly sampling from February to October, and found that air temperature had the strongest effect on the microbial community structure.

Rivers supply water for domestic and industrial demands in most urban areas, and the output from urban landscapes such as wastewater effluent may result in variations in physico-chemical parameters of river water, which would affect bacterial communities (Paul and Meyer, 2008), as well as the direct inflow of sewage bacteria (Newton et al., 2013). These bacterial genera usually include waterborne pathogens which are a danger to human health (Pandey et al., 2014; Rochelle-Newall et al., 2015).

Nanchang, the capital of Jiangxi Province, is located on the Ganjiang River — a major tributary of the Yangtze River, immediately before it flows into the Poyang Lake, which is the largest freshwater lake in China. Although the Ganjiang River is the only water resource of Nanchang and provides about half of the water that flows into Poyang Lake, the water quality of the river is deteriorating because of increased agricultural and industrial wastewater discharge (Ji et al., 2014; Li et al., 2015), and the aquatic bacterial community in the Ganjiang River has not yet been investigated. In this study, we analyzed the characteristics of the bacterial community in the Nanchang section of the Ganjiang River during the wet season based on monthly sampling (April to August). We hypothesize that the bacterial community is influenced by the river environment, including water chemical factors and hydrometeorological factors, and that the urban landscape influences the bacterial community in river water by changing the river environment or direct input of bacteria. We specifically investigated: (1) the characteristics of the bacterial community in river water; (2) the dominant environmental variables influencing the bacterial community in the river water; (3) to what extent the urban landscape influences the bacterial community in river water.

2. Materials and methods

2.1. Study area, sample collection and physicochemical analysis

The Ganjiang River flows into Poyang Lake before it joins the Yangtze River (Fig. 1). The length of the main river channel is approximately 823 km, and the basin area is 8.28×10^4 km^2, 98.45% of which is in Jiangxi Province. The basin is situated in the monsoon zone of East Asia, and the average annual precipitation is 1580 mm. The wet season runs from April to August, during which time 62.2% of the annual precipitation falls. The area has an average annual air temperature of 18.3 °C, and the maximum and minimum temperatures are usually observed in July and January, respectively.

Before flowing into Poyang Lake, the Ganjiang River is divided into three branches in Nanchang, the south branch, the middle branch and the north branch (Fig. 1). The south branch surrounds the main urban area and then goes through suburban farmland, the middle branch mainly flows through suburban farmland, and the north branch goes through some new urban areas and then suburban farmland.

A single water sample was collected from each site (G1–G5) from a depth of approximately 50 cm in the centre of the river channel. G1 lays on the Ganjiang River before entering the urban region, G2 in the centre of the city, and G3–G5 on the three branches leaving the urban region, respectively. Water was collected in the middle of April, May, June, July and August 2015. The sampling period corresponds with the wet season in the study area, when temperature and rainfall are high, which benefits bacterial growth. The flow rate of the Ganjiang River on the sampling day was obtained from Waizhou gauge station (Fig. 1), and the average air temperature of the sampling day was recorded. The electric conductivity (EC) was measured in situ using a HI 98360 probe (Hanna Instruments Ltd., Italy), and the probe was calibrated before measurement. NH_4^+-N and total phosphorus (TP) were measured using a Smartchem 200 Discrete Analyzer (Brookfield, USA), and the dissolved organic carbon (DOC) was measured using a TOC analyzer (Shimadzu TOC-L CPH, Japan). Water samples for NH_4^+-N and DOC analysis were filtered through a 0.45 μm Durapore membrane filter (Xinya, China), while those for TP analysis were not filtered. Water samples (3 L) for bacterial analysis were pre-filtered through a 5 μm Durapore membrane filter (Xinya, China) to reduce particulate and algal biomass, then through a 0.22 μm Durapore membrane filter (Xinya, China) to collect microbial cells. Filters were stored at −80 °C for later analysis.

2.2. DNA extraction and sequencing

Microbial DNA was extracted from water using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA) according to the manufacturer's protocols. The V4–V5 regions of the bacteria 16S RNA gene were amplified by PCR (95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min) using primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Dennis et al., 2013). PCR reactions were performed in triplicate using 20 μL mixtures containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase, and 10 ng of template DNA. A negative control containing all reagents except the template DNA was included with each set of reaction mixtures. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, USA). All PCR products were sequenced using the Illumina MiSeq platform by the Shanghai Majorbio Bio-pharm Technology Co., Ltd., China. The raw reads were deposited into the NCBI Sequence Read Archive database (Accession Number: SRP074362).

Raw fastq files were demultiplexed, then quality-filtered using QIIME (version 1.30) with the following criteria: (i) 250 bp reads