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Integrating microbial biomass, composition and function to discern the level of anthropogenic activity in a river ecosystem

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ABSTRACT

Anthropogenic activities (e.g., wastewater discharge and pesticide and fertilizer use) have considerable impact on the biotic properties of natural aquatic ecosystems, especially the microbial community and function. Microbes can respond to anthropogenic activities and are thus potential indicators of activity levels. Several reports have documented the impacts of anthropogenic activities on the variations in the microbial community, but the direct use of microbial community indices to discern anthropogenic activity levels remains limited. Here, we integrated flow cytometry, 16S rRNA sequencing, and natural organic matter metabolism determination to investigate microbial biomass, composition, and function in three areas along a gradient of anthropogenic disturbance (less-disturbed mountainous area, wastewater-discharge urban area, and pesticide and fertilizer used agricultural area) in a river ecosystem. Multiple statistical methods were used to explore the causal relationships between changes in environmental factors and microbial variation. Results showed that anthropogenic activities (e.g., wastewater discharge, pesticide and fertilizer use) facilitated bacterial production, affected dominant species distribution, and accelerated natural organic matter (NOM) metabolic rate by microbes. After screening the possible factors influencing the microbial community, we determined that cyanobacterial concentration could be a diagnostic indicator of nutrient levels. We also developed a NOM metabolic index to quantitatively reflect the holistic influence of nutrients and xenobiotics.

1. Introduction

With economic development, freshwater ecosystems have become increasingly threatened (Foley et al., 2008; Malmqvist and Rundle, 2002; Sibanda et al., 2015). River ecosystems are greatly affected by human activities, including municipal drainage and agricultural irrigation, which release diverse chemical pollutants into river systems (Malmqvist and Rundle, 2002). There are clear evidences that anthropogenic pollutants can adversely affect aquatic ecosystems (Englert et al., 2013; Schallenberg and Armstrong, 2004), including ecosystem destruction, habitat modification, water chemistry alteration, and direct addition or removal of species (Baer et al., 2000; Caracciolo et al., 2015; Malmqvist and Rundle, 2002).

Microbes are very sensitive to the ambient environment, and thus the effects of anthropogenic activities on natural aquatic ecosystems may be reflected by changes in the aquatic microbial community. Recently, researchers have examined how anthropogenic activity has impacted microbial bio-geographical properties by describing the spatial and temporal distribution patterns of microbes, including the fluctuation of diversity, patterns of dispersal, and levels of species interactions in different environments (Hamilton et al., 2011; Montuelle et al., 1996; Schneider and Topalova, 2009). For example, wastewater discharge can increase microbial phylogenetic diversity and microbial metabolic capacity (Blunt et al., 2018). A recent study reported abundant photoautotrophic cyanobacteria and diatoms in less-disturbed regions compared to near-complete absence in disturbed areas (Lindsay et al., 2017), suggesting the existence of a measurable anthropogenic footprint on aquatic ecosystems (Adams, 2008). Similarly, microorganisms can react to anthropogenic activities, e.g., evolutionary adaption to the environment (Lupo et al., 2012) and biodegradation of xenobiotic compounds through horizontal gene transfer (Springael and Top, 2004). Hence, interactions between microbial communities and anthropogenic activities provide the opportunity to use bacterial community composition and function to establish the levels of

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anthropogenic activity in an aquatic ecosystem (Clements and Rohr, 2009; Garrido et al., 2014b). How bacterial populations respond to external disturbance could be a key indicator of the effects of pollution (Sharuddin et al., 2018). However, most studies have not yet provided a quantitative correlation between microbiological changes and human impacts on aquatic ecosystems (e.g., impact of nutrients and xenobiotics discharge).

Hence, we selected a typical anthropogenically-disturbed river (Chaobai River) that showed distinct land use partitioning. Integrating flow cytometry (FCM) for biomass determination, high-throughput sequencing for microbial composition, and the Biolog™ EcoPlate system for natural organic matter (NOM) metabolism determination, we investigated how anthropogenic activities affected the composition and metabolism of the microbial communities and examined the factors correlated with activity degree. We further evaluated whether these factors could be used as indicators for predicting levels of anthropogenic activity. Our ultimate goal was to establish a causal relationship between microbiological changes and anthropogenic activities and test the hypothesis that quantification of microbiological changes can more sensitively reflect the levels of anthropogenic activities than the physicochemical characteristics of water (Muela et al., 2011; Sharuddin et al., 2018).

2. Material and methods

2.1. Site description

Based on its distinct land use types and gradient of human activity, the Chaobai River in China was studied. The river originates from north of Yanshan Mountain and first flows through a less-disturbed mountainous area (MA, 5530 km^2). Afterwards, it flows through an urban area (UA, 695 km^2) where treated wastewater becomes the main source of river water. Yearly runoff from Chaobai River is approximately $2000 \times 10^4\ m^3$, and average annual flow from the UA wastewater treatment plant to Chaobai River is $919 \times 10^4\ m^3$. The river finally flows through agricultural farmland (AA, 4624 km^2) before merging with the waters of Bohai Bay. The amount of nitrogen and phosphate fertilizer used in the AA is approximately 89,900 and 29,900 tons per year, respectively. Although the collected estuary samples (A12, A13, and A14) were affected by seawater, and therefore had higher conductivity than the other samples, they were placed in AA as human activity was the focus in this study. Increasing exploitation of the limited water resources of Chaobai River for agriculture and growing urban agglomerations has resulted in considerable changes in water quality and biodiversity (Li et al., 2013; Li et al., 2016; Xiong et al., 2017). Detailed description of the sampling areas and disturbance levels, such as population density and rainfall, are provided in the Supplementary Information (SI).

A total of 34 sampling sites were selected, with distances between adjacent sites of ~10 km (Xiong et al., 2017). The sites were in the main stream and tributaries of Chaobai River, and included 13, 7 and 14 sites in MA, UA and AA, respectively (Fig. 1). In December, due to the freezing of water, we only collected 27 samples. And in June, because of channel cutoff, we only collected 32 samples. Thus, we have 93 samples in total.

2.2. Water sample collection and field measurements

Sampling was undertaken in 34 sites along Chaobai River (39°–40° N to 116°–117° E) in December 2016, March 2017 and June 2017. We chose these three months because in these months, the water temperature exhibited an obvious escalating trend (Fig. S1f). Water temperature (Temp), conductivity (COND), oxidation-reduction potential (ORP), total dissolved solids (TDS), and pH were recorded at each site in the field with a multi-parameter water quality sonde (MYRON Co., USA). Dissolved oxygen (DO) and chlorophyll A (Chl-a) were measured in situ with a portable dissolved oxygen meter (HACH Co., USA) and handheld fluorometer (Turner Designs, USA), respectively.

Surface water (10*L*) was collected from each site using an iron drum, with 250 mL of water then transferred to glass bottles with screw caps and acidified to pH < 2 with sulfuric acid for chemical analysis. We then loaded 1 mL and 15 mL of water into centrifuge tubes for microbial biomass and function detection, respectively. In addition, 700–900 mL of surface water was filtered using 0.45-μm filter membranes in the field with a pump followed by the addition of a LifeGuard™ preservation solution (MoBio Laboratories Inc., Carlsbad, CA, USA) for DNA sequencing. All samples were transported to the laboratory on ice and stored at 4 °C prior to physiochemical analysis or − 80 °C prior to flow cytometry and DNA analyses.

2.3. Chemical analyses

Water samples were filtered through 0.45-μm filter membranes for the measurements of soluble reactive phosphorus (SRP), nitrate nitrogen (NO_3-N), dissolved organic carbon (DOC), and ammonia nitrogen (NH_4-N). Total nitrogen (TN), NH_4-N, and NO_3-N were determined using alkaline potassium persulfate digestion UV spectrophotometry, Nessler's reagent spectrophotometry, and ultraviolet spectrophotometry, respectively; Total phosphorus (TP) and SRP were measured by ammonium molybdate spectrophotometry (State Environmental Protection Administration of China, 2002). The chemical index results were the means of triplicate tests.

2.4. Bacterial biomass

Bacterial biomass was determined as per (Nescerecka et al., 2016) in quadruplicate, with slight modification. Briefly, the staining solution was prepared using SYBR® Green I (10,000 × stock, Sigma-Aldrich Trading Co., Ltd., USA) diluted (100×) in anhydrous dimethyl sulfoxide (DMSO). The solution (10 μL) was then added to the water samples (100 μL) and incubated for 15 min at room temperature in the dark. All samples were diluted (10×) in TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) solution. Fluorescent beads in TruCount absolute count tubes (Becton, Dickinson, and Company) with known concentrations were spiked to the samples as a standard to determine cell concentration. Samples were analyzed using a flow cytometer (FACSCalibur™, Becton, Dickinson, and Company).

2.5. Bacterial community composition

The filtered water samples were subjected to DNA extraction using a PowerWater® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions.

The V4 region of the 16S rRNA gene was amplified using barcoded primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). For PCR, Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA) was used as per the manufacturer's instructions. Cycling conditions were 95 °C for 3 min followed by 30 cycles of 95 °C for 45 s, 56 °C for 45 s, and 72 °C for 45 s, followed by an elongation step of 72 °C for 10 min. The PCR products were then purified with an AxyPrep DNA Gel Extraction Kit (Axygen, USA) in accordance with the manufacturer's protocols. The DNA concentration of the purified PCR products was measured using a TBS-380 Fluorometer (Turner Biosystems, CA, USA). Sequencing was performed on an Illumina Hiseq 2500 sequencing platform at the Beijing Genomics Institute (BGI, Shenzhen, China). Duplicate DNA aliquots were combined into a single DNA pool for sequencing. The raw sequence reads were initially filtered to remove low quality reads and barcode primers. The remaining high-quality paired-end reads were connected to tags based on overlaps. A total of 3,250,720 high-quality sequences from 93 samples were obtained after pre-filtration of ambiguous sequences, ranging from 31,129 to 38,187 per sample. The connected tags were

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