

Bacterial diversity and community structure in nitrate-contaminated shallow groundwater in the Poyang Lake basin, China

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Abstract. The Poyang Lake basin in the Jiangxi province of China has been exposed to nitrate pollution caused by irrigation practices, leading to high groundwater nitrate concentration. Eight groundwater samples were collected from shallow wells for hydrochemical, bacterial diversity, and community structure analysis in November 2017. Shallow groundwaters of the basin are weakly acid and in an oxidizing state, with EC ranging from 87.6 to 279.5 $\mu\text{S}/\text{cm}$ and TDS varying between 53 to 344 mg/L and averaging of 164 mg/L. The $\text{NO}_3\text{-N}$ form is the dominant nitrogen species in groundwater, with concentrations of $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$ ranging between 2.5 to 164 mg/L, 0.01 to 0.10 mg/L, <0.01 to 0.08 mg/L, respectively. Groundwater communities are dominated by actinobacteria, alphaproteobacteria, gammaproteobacteria and betaproteobacteria both in high- and low-nitrate groundwaters. The results of a 16S rRNA gene clone library indicate that the bacterial community structure of the high-nitrate groundwater is different from that of the low-nitrate groundwater. The bacterial populations *Denitratisoma* and *Sulfuritalea* detected in low-nitrate groundwater suggest that these bacteria are capable of denitrification in anaerobic groundwater environment. Bacterial populations *Flavobacteria* and *Cytophagia* in high-nitrate groundwater are common in the wetlands examined and likely capable of nitrification.

1 Introduction

Nitrogen contamination in groundwater, mainly nitrate, is a safe-drinking water problem globally in over 110 countries and regions [1]. The largest fresh water natural reservoir in China, the Poyang Lake in the basin of the same name located in Jiangxi province, is exposed to nitrate pollution caused by intense and large-scale irrigation practices. Agricultural activities in this area also lead to nitrate enrichment of the surface

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water and groundwater. Nitrate concentration in shallow groundwater reaches up to 206 mg/L [2], exceeding the WHO recommended value of 11.29 mg/L for drinking water.

Previous studies indicated that nitrate migration, transformation, and enrichment in groundwater are integrated results of series of microbially mediated reactions and geochemical processes. As one kind of key geological mediators, microorganisms can affect the geochemical behaviour of nitrate in groundwater. Therefore, it is worthy of better understanding of the microbially mediated mechanism of nitrate-contaminated groundwater.

This work investigates the hydrochemical characteristics, bacterial diversity and community structure in nitrate-polluted shallow groundwater in the Poyang Lake basin. This research aims at better understanding the nitrate enrichment mechanisms with the contribution of microorganisms in the aquifer system of the Poyang Lake basin.

2 Methodology

2.1 Study area

Poyang Lake basin is located in Jiangxi province, southeast China, with an area of 16.2×10^4 km². The area of Poyang Lake varies in different seasons, covering up to 4,125 km² in flood season, while as low as 500 km² in a dry season. It belongs to the subtropical monsoon climate with an average annual temperature of 17.5 °C and average annual precipitation of 1400-2400 mm.

Groundwater in the Poyang Lake basin is distributed in loose-rock pores, carbonate karst fissures, clastic karst fissures, and bedrock fissures. Groundwater is primarily recharged by local precipitation, and surface runoff in a wet season, but discharges to rivers in a dry season.

2.2 Sampling and analysis

Eight groundwater samples were collected from wells with the depth between 4.6 and 12.1 m in November 2017 for hydrochemical, bacterial diversity, and community structure analysis. Hydrochemical parameters including temperature (T), pH, electrical conductivity (EC), oxidation-reduction potential (ORP) and dissolved oxygen (DO) were measured *in situ* using a portable Analyzer (Hach, HQ40d). Groundwater samples for NO₃-N, NO₂-N, and NH₄-N measurement were collected by a syringe and filtered (<0.45 μm) into prepared colorimetric reagent solutions and then determined *in situ* by portable spectrophotometry (Hach, DR2800).

All the 8 samples were treated with the following method. 2 L of each groundwater sample was filtered on site through a 0.22-μm cellulose acetate membrane. The membrane filters were frozen in a portable refrigerator at -20 °C until DNA extraction. DNA extractions were carried out using Power Water DNA Isolation (MoBio, Carlsbad, CA 92010, USA). Primer pair 338F (5' -ACTCCTACGGGAGGCAGCAG-3') and 806R (5' -GGACTACHVGGGTWTCTATT-3') were used to amplify bacterial 16S rRNA gene sequences. The purified amplification was quantified using a QuantiFluor™ -ST (Promega, Madison, USA) and then pooled together. Samples were sequenced on MiSeq System (Illumina, USA) in Majorbio (Shanghai).

3 Results and discussion

3.1 Groundwater hydrochemistry

The main hydrochemical characteristics of 8 groundwater samples are listed in Table 1. These groundwaters have moderate solute contents, with total dissolved solids (TDS) contents of 53 to 344 mg/L and an average TDS value of 164 mg/L, electrical conductivity values varying between 88 to 279 μ S/cm, and are weakly acidic with pH values ranging from 4.1 to 6.8. Their oxidation-reduction potential (ORP) is between 56 to 278 mV, indicating an oxidizing groundwater environment. The groundwater sample with a pH value of 4.1 contains no HCO_3^- . Concentrations of $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$ range between 2.5 to 164 mg/L, 0.01 to 0.10 mg/L, <0.01 to 0.08 mg/L, respectively. $\text{NO}_3\text{-N}$ is the dominant nitrogen species in groundwater, consistent with previous studies [3].

Table 1. A summary of groundwater hydrochemistry.

	T (°C)	pH	EC (μ S/cm)	ORP (mV)	TDS (mg/L)	DO (mg/L)	HCO_3^- (mg/L)	$\text{NO}_3\text{-N}$ (mg/L)	$\text{NO}_2\text{-N}$ (mg/L)	$\text{NH}_4\text{-N}$ (mg/L)
Maximum	22.3	6.77	605	277.7	344	6.58	95.5	164.0	0.10	0.08
Minimum	19.0	4.1	87.6	56.2	53.0	3.1	0.0	2.5	0.01	<0.01
Mean	20.8	5.8	279.5	207.5	164.3	4.5	45.4	42.7	0.03	0.04

3.2 Bacterial diversity and community structure in groundwater

As illustrated in Figure 1, bacterial communities in all groundwater samples were dominated by actinobacteria (4.12 to 55.1%), alphaproteobacterial (3.2 to 39.2%), gammaproteobacteria (1.7 to 21.6%) and betaproteobacteria (3.9 to 22.0%)

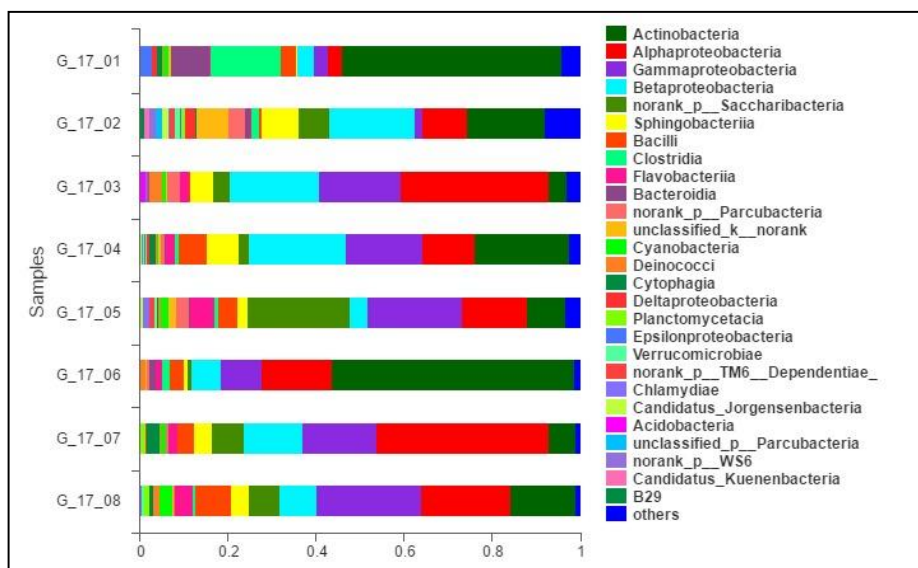


Fig. 1. The bacterial community structure and relative abundance of bacterial classes based on 16S rRNA sequence extracted from groundwaters of the Poyang Lake basin.

Results of a 16S rRNA gene clone library indicate that the community structure of high-nitrate groundwater is different from that of low-nitrate groundwater (Fig.2.). Besides

bacterial populations actinobacteria, alphaproteobacterial, gammaproteobacterial and betaproteobacteria, sphingobacteria is also a dominant population in low nitrate groundwater, sample G-17-02 with NO₃-N of 2.5 mg/L. Bacterial populations *Denitratisoma* and *Sulfuritalea* were found in low nitrate groundwater with a relative abundance of 6.88 and 2.49%, respectively, indicating it is capable of denitrification in aerobic groundwater environment. Bacterial populations including *Planctomycetacia*, *Cytophagia*, *Bacilli*, *Sphingobacteria*, cyanobacteria, and flavobacteria are also present in high-nitrate groundwater sample G-17-07, which has a NO₃-N of 164.0 mg/L. Bacterial populations of *Cytophagia* and flavobacteria are common in wetlands and capable of nitrification [4]. The genus *Limnohabitans* is of importance in water eutrophication [5].

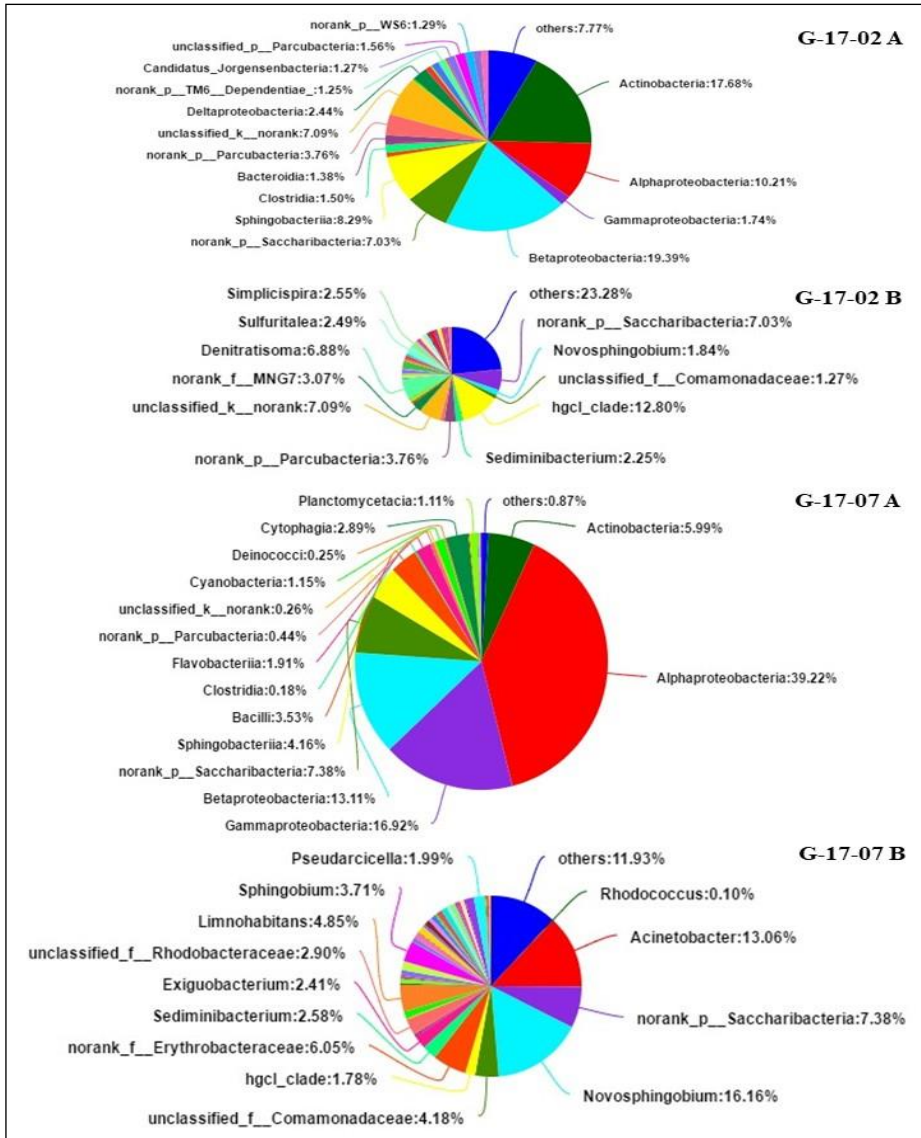


Fig. 2. Comparison of bacterial community structure, based upon the 16S rRNA gene, between groundwater sample G-17-02 with a NO₃-N concentration of 2.5 mg/L and sample G-17-07 with NO₃-N concentration of 164.0 mg/L, (A) classification on a class level; (B) classification on genus level.

The difference in nitrate concentrations and microbial populations between high- and low-nitrate groundwaters suggests that two possible causes for changing concentrations are microbially-mediated denitrification and nitrification.

4 Conclusion

Shallow groundwaters in the Poyang Lake basin are an oxidizing and weakly acid environment where the NO₃-N form dominates the speciation of nitrogen, and actinobacteria, alphaproteobacterial, gammaproteobacteria and betaproteobacteria dominate the microbial communities. Importantly, the results of 16S rRNA gene clone library show the community structure of high-nitrate groundwater is different from that of low-nitrate groundwater, suggesting that different microbially-mediated mechanisms may control the nitrogen enrichment in groundwater.

Acknowledgments. This study was supported by China National Foundation of Natural Sciences (No. 51861145308) and Science and Technology Project Fund of Jiangxi Provincial Education Department (GJJ170443). The study of inorganic nitrogen species was supported by the Russian Science Foundation (RSF), Project No 17-77-10017.

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