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# Diversity, abundance and community structure of ammonia-oxidizing archaea and bacteria in riparian sediment of Zhenjiang ancient canal

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ABSTRACT

Ammonia-oxidizing microorganisms play an important role in the nitrification process. In this study, the abundance, diversity and community structure of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in riparian sediment of Zhenjiang ancient canal were investigated using *amoA* gene as a molecular biomarker. Clone libraries and qPCR (quantitative polymerase chain reaction) results indicated both abundance and diversity of AOB were higher than that of AOA, suggesting that AOB may play a more important role than AOA in nitrification process. The abundances, diversity and community structure of AOA and AOB had an obvious spatial variation in six different sampling sites, which may be attributed to the differences in sediment physico-chemical characteristics. 66.7%, 26.5% and 6.8% of the total 117 AOA gene sequences fell into the *Nitrososphaera*, *Nitrosopumilus* and *Nitrosotalea* cluster, whereas, 52.2% and 47.8% of the total 134 AOB *amoA* gene sequences belonged to the *Nitrosomonas* and *Nitrosospira* cluster. There was a strong positive relationship between the potential nitrification rates (PNRs) without ampicillin and bacterial *amoA* gene copy numbers, whereas no significant correlation between the archaeal *amoA* gene copy numbers and the PNRS without ampicillin. In addition, when ampicillin was added into the cultured systems in order to inhibit the bacteria, the values of PNRs decreased by 21.71%, 39.62%, 46.04%, 27.06%, 39.26% and 7.24%, respectively, for the C1, C2, C3, C4, G5 and G6 samples sites, indicating that PNRs were apparently affected by ammonia-oxidizing bacteria. These results further indicate that nitrification is more related to AOB than AOA in the riparian sediment of Zhenjiang ancient canal.



## 1. Introduction

Excessive input of nitrogen (N) and phosphorus (P) by human activities has been reported to result in water quality declining in river ecosystems (Bu et al., 2011; Funkey et al., 2014; Glibert et al., 2014; Guo, 2007; Wu and Chen, 2013; Zhang et al., 2015). Therefore, N and P are the major target for restoring river ecosystems (Garland et al., 2004; Smith et al., 1998). At present, extensive studies are involved in nutrients cycle and verification of microorganisms in water or sediments, which may have key ecological functions in the N biogeochemical cycling (Li et al., 2013; Sun et al., 2014; Liu et al., 2011). As the first and rate-limiting step of nitrification, ammonia oxidation, the microbial oxidation process of ammonia to nitrate via nitrite, plays a critical role in global N cycle (Kowalchuk and Stephen, 2001). This process is carried out by two groups of microorganisms, ammonia-oxidizing archaea (AOA) (Könneke et al., 2005) and ammonia-oxidizing bacteria (AOB) (Koops et al., 2003).

Numerous studies have demonstrated AOA and AOB often coexist and widely distributed in various habitats (Cao et al., 2011a,b; Norman and Barrett, 2014; O'Sullivan et al., 2013; Sakami, 2012; Shen et al., 2014; Wang et al., 2011). However, their relative contribution to ammonia oxidation remains controversial (Cao et al., 2011a,b; Prosser and Nicol, 2008; Wang et al., 2014). AOA usually outnumber AOB in different ecosystems (Leininger et al., 2006; Mincer et al., 2007; Nakagawa et al., 2007; Wuchter et al., 2006), such as marine and soil habitats (Francis et al., 2007; Könneke et al., 2005; Schleper and Nicol, 2010; Venter et al., 2004), terrestrial environments (Leininger et al., 2006), river water (Liu et al., 2011), drinking water bioreactor (Kasuga et al., 2010) and

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macrophyte rhizosphere (Herrmann et al., 2008), implying the importance AOA in nitrification (Chen et al., 2013a), whereas some studies reported that AOB may be dominant in agricultural soils, lakes and rivers at a higher N loading level (Jia and Conrad, 2009; Mosier and Francis, 2008; Pester et al., 2012). The difference in environmental conditions may explain the conflicting reports in literature. Previous studies indicated that the abundance, distribution, activity and community structure of AOA and AOB were affected by environmental conditions, such as ammonium concentration (Wuchter et al., 2006), N availability (Di et al., 2009, 2010; He et al., 2007; Shen et al., 2008), temperature (Avrahami and Conrad, 2003; Tourna et al., 2008), moisture (Chen et al., 2013b; Horz et al., 2004; Gleeson et al., 2010), salinity (Bernhard et al., 2010; Santoro et al., 2008), pH (He et al., 2007; Nicol et al., 2008), amount of soil organic matter (Chen et al., 2008; Leininger et al., 2006) and plant species (Herrmann et al., 2009). In general, AOA may dominate ammonia oxidation in ecosystems with low ammonium concentration (Martens-Habbena et al., 2009; Stopnišek et al., 2010), low pH and rich in sulfide (Erguder et al., 2009), while AOB become more competitive in the environment with a higher ammonium concentration (French et al., 2012). Apparently, AOA and AOB are widely distributed and regulated by physiochemical characteristics of the environment (Li et al., 2011).

Although previous studies have demonstrated the ammonia-oxidizing microorganism could play an important role in removing excessive N from aquatic systems (Hou et al., 2013), to date, little information is available regarding these prokaryotes in rivers riparian sediments, especially urban rivers. Compared to other river ecosystems, N biogeochemistry in urban river is more influenced by anthropogenic activities, such as impermeable riparian, wastewater outfall, and river course alterations (Grimm et al., 2008a,b; Zhang et al., 2015).

The Zhenjiang ancient canal (Guyun river), originates from Jianshan Lake (receiving water from the Yangtze River), flows through downtown of Zhenjiang and finally converges into the Beijing–Hangzhou Grand Canal (the longest artificial river in the world). The ancient canal is the largest and oldest artificial river in Zhenjiang, China, with a length of approximately 17 km, a watershed area of 81 $km^2$, and an average water depth of 4–6 m. Owing to rapid economic growth and enhanced anthropogenic activities along the ancient canal, water quality has declined and the eco-environment has degraded in recent years. This change has influenced river landscape, ecosystem health, and community living standards. Therefore, it is crucial to know ammonia-oxidizing microorganism community distribution, diversity, abundance, and their role in the oxidation of ammonia in the riparian sediment of the ancient canal, which could provide some new insights and expands our knowledge regarding the attributes of ammonia-oxidizing archaea and bacteria in riparian sediment for better understanding of the N cycling in the urban river ecosystems. The objectives of the present study were to: (1) describe whether and how change of AOA and AOB abundance, diversity, richness and community composition at different locations of the ancient canal; (2) determine the major environmental variables that affect the relative abundance, diversity, richness of AOA and AOB and (3) to analyze the relative contributions of AOA and AOB to the ammonia oxidation in riparian sediment of ancient canal in Zhenjiang, China.

## 2. Materials and methods

### 2.1. Study region and sampling sites

This Zhenjiang ancient canal region has the north subtropical monsoon climate, with a mean annual temperature of 15.6 °C and four obvious seasons: spring (March–May), summer (June–August), autumn (September–November) and winter (December–February). The annual average rainfall is 1088.2 mm, most of which occurs in the summer and autumn season. However, the river flow did not change with the seasonal rainfall variation, as the water level was controlled by two dams in the upstream and downstream of the canal. As a result, the hydrodynamic conditions were stable all the year around for each of the sampling sites.

The ancient canal mainly received pollutants from the overflow of sewer and sewage pumping station, and runoff water. The major contaminants include organic matter, nutrients (N and P), and the discharged wastewater contained these contaminants above the critical level for Class V according to the guideline of surface water environmental quality standard of China (GB 3838-2002). Zhenjiang ancient canal had been present more than 700 years, but its sediment was dredged a few years ago, and consequently the differentiation of sediment profile was minimal. Six sampling sites from upstream to downstream along the ancient canal were selected by accounting for the polluted water discharge, shoreline morphology and nutrient gradients in the overlying water. All the six sites were located in the permeable riparian without aquatic plants growing near the sampling sites. Of the six sites, four were located in the mainstream (G1, G2, G4 and G5), other two were in the Tuanjie river tributary (G3) and Yudai river tributary (G6), respectively (Fig. 1). Detailed information of the sampling sites was listed in Table S1.

### 2.2. Collection of sediment samples

Riparian surface sediment samples (0–25 cm, land/inland ecotones) in triplicate were collected using a core sampler from the selected sampling sites along the riverside of the ancient canal in March 2013. The sediment samples from the same site were mixed thoroughly and immediately put into sterile plastic bags on an ice bath and transported to the laboratory. In laboratory, each sediment sample was divided into two parts. One was freeze dried with a freeze dryer (LGJ-12, Songyuan, China), powdered, sieved through a 100-mesh nylon sieve and stored at 4 °C for the analyses of sediment physico-chemical properties, the other was stored at −20 °C for molecular and potential nitrification rates (PNRs) analyses

### 2.3. Physicochemical analyses

Sediment pH was measured using a PHS-3C digital pH meter (INESA Scientific Instrument Co., Ltd., China) at the water to sediment ratio of 2.5:1 (Lu, 1999). Total N (TN) was determined using semi-micro Kjeldahl method (Bao, 1981). Ammonium ($NH_4^+$–N) and nitrate ($NO_3^-$–N) were extracted with 2 M KCl and then measured using indophenol blue and phenol disulfonic acid methods (Bao, 1981), respectively. Organic matter (O.M) was measured with the dichromate oxidation method (Lu, 1999). Total phosphorus (TP) was colorimetrically measured through ascorbic acid-molybdate blue method (Lu, 1999). Triplicates were performed for each parameter.

### 2.4. Molecular analyses

Total DNA was extracted using FastDNA® SPIN Kit for Soil (MP Biomedicals, LLC, USA) in 0.5 g of sediment sample according to the manufacturer's instruction. Three replicates were performed for each sample, and DNA solutions were homogeneously mixed. The archaeal *amoA* gene fragments and bacterial *amoA* gene fragments were amplified with the primer pair Arch-*amoA*F and Arch-*amoA*R (Francis et al., 2005) and *amoA*-1F and *amoA*-2R (Rotthauwe et al., 1997), respectively. Amplification was performed in 25 μl reaction mixture: 2.5 μl 10× PCR buffer (without $MgCl_2$; Thermo, USA), 1.5 μl $MgCl_2$ (25 mM; Thermo, USA), 1 μl dNTP (2.5 mM; Thermo, USA), 1 μl each primer (5 μl M; Sangon, China), 0.2 μl *Taq* DNA