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# Seasonal population changes in the ammonia-oxidizing bacteria community structure of Songhua Lake, China

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## ABSTRACT

Ammonia-oxidizing bacteria (AOB) and the effects of environmental characteristics on the AOB community distribution were investigated in the Songhua Lake in May, August, October and December of 2011. Profiles of the AOB communities were generated using denaturing gradient gel electrophoresis (DGGE) to assess the expression of 16S rRNA genes followed by DNA sequence analysis. The dominant AOB groups were affiliated with *Nitrosomonas* and *Nitrospira* spp. The effects of environmental characteristics on the AOB community distribution were analyzed by the ordination technique of redundancy analysis. The environmental characteristics significantly influencing the AOB community structure were different in the four seasons. The suspended solids (SS), ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ) and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) differed in May, the pH,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and total nitrogen (TN) differed in August, and the  $\text{NH}_4^+\text{-N}$ , DO and TN differed in December. None of the assessed environmental variables displayed heterogeneity in October. This study suggested that seasonal variation and nutrient differences might be responsible for the differences in the key environmental characteristics used to determine the distribution of the AOB communities in all seasons.

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## 1. Introduction

Nitrification is a critical pathway in the nitrogen cycle and plays a key role in the biogeochemical cycling of nitrogen (Casciotti et al., 2003). It is an important process required to remove excess ammonia to nitrate via nitrite (Camargo and Alonso, 2006). Excess ammonia can stimulate the growth of ammonia-oxidizing bacteria (AOB). This process catalyzes the oxidization of ammonia ( $\text{NH}_4^+\text{-N}$ ) to nitrite ( $\text{NO}_2^-\text{-N}$ ), which is the first and rate-limiting step of nitrification

(Arp and Stein, 2003). AOB have traditionally been taxonomically classified into two monophyletic groups: beta- and gamma-proteobacteria. Gamma-proteobacteria are the minor member of the ammonia-oxidizing community (Ward, 2005). They have been observed in tight clusters within the beta-proteobacteria based on analyses of 16S rRNA sequences with *Nitrosomonas* and *Nitrospira* members (Dang et al., 2010; Phillips et al., 2000).

A number of studies have suggested that the variation in the AOB community composition is a response to

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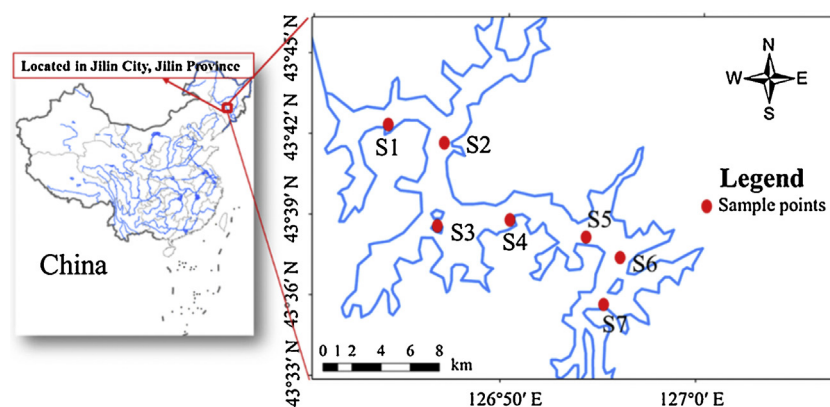


Fig. 1 – Map of Songhua Lake with sampling locations.

physiological and ecological differences, such as pH, salinity and ammonia concentration in estuarial coastal and deep-sea ecosystems (Cilia et al., 1996; de Bie et al., 2001). However, the partial information from these different ecological niches could not explain the distribution in the AOB community structure that responded to environmental changes in complex environments, such as anthropogenic activity-impacted lakes. Furthermore, information on the relationships between the environmental characteristics and the AOB community structure is not available.

Songhua Lake is the third largest artificial lake in China. Eutrophication has become one of the most severe problems in Songhua Lake since 2008 (Shi et al., 2008). Due to the noticeable temperate monsoon climate changes that occur over the four seasons, seasonally alternations in the climatic conditions control the distribution and biodiversity of microbes. The diversity and composition of the AOB and environmental characteristics must also be affected by the seasonal changes (Sims et al., 2012). Therefore, it is necessary to determine the seasonal population changes in the AOB community composition and their relationship with the water quality.

Recent studies on the seasonal variation of ammonifying and denitrifying bacteria in Songhua Lake have provided a partial understanding of N biogeochemical cycling along a gradient of the surface layers in Songhua Lake (Zhao et al., 2015). However, although partial knowledge is emerging, the correlation between environmental characteristics and AOB community structure, abundance and distribution across the four seasons is not well understood in a comprehensive way. Thus, research was carried out to determine to what extent the environmental characteristics affect the AOB community composition with seasonal population changes.

In order to achieve a deep understanding of the AOB process related to water quality and climatic variations, this study aimed to determine whether changes in the AOB community composition could be related to the water quality and to differences in key environmental characteristics caused by seasonal changes in the hydrodynamic conditions.

## 2. Materials and methods

### 2.1. Sample collection and environmental characteristic measurements

Seven sample sites were established in Songhua Lake, which is a component of a large drainage system with a catchment area that comprises 22.7% of Jilin. This body of water possesses an average water-carrying capacity of  $133.2 \times 10^6 \text{ m}^3$

and a depth of 35 m. The sample sites were designated from upstream to downstream in Songhua Lake based on the distribution of the water system (Fig. 1). In 2011, freshwater samples were collected in triplicate from the main affluent, which was divided into three sections: an upstream (S1, S2), middle stream (S3, S4, S5) and downstream (S6, S7) section in spring (May), summer (August), fall (October), and winter (December), respectively. The deepest point of Songhua Lake is 70 m. All samples were collected from the middle level (at a depth of 16 m) of the lake, as the surface water (0 m) was vulnerable to various natural factors, including the weather. The bottom level of the water contains little oxygen, and the AOB have difficulty surviving in low-oxygen conditions. Approximately 5 L of water was collected for chemical and molecular analyses. The samples were then rapidly transferred to the laboratory on ice. Water samples (200 mL) were filtered through filters with a pore size of  $0.22 \mu\text{m}$  and a diameter of 45 mm (Durapore). The filters were stored at  $20^\circ\text{C}$  until DNA extraction for molecular biological analysis.

The temperature, pH, DO concentration,  $\text{BOD}_5$  and suspended solids (SS) were constantly monitored in situ using a multiparameter water quality probe (6600EDS, YSI, USA). The total nitrogen (TN), ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrite nitrogen ( $\text{NO}_2^-\text{-N}$ ), and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) were measured according to a published procedure (Gilliam et al., 2001).

### 2.2. DNA extraction, PCR amplification and DGGE analysis

DNA extraction was performed using method C with ultrasonic pretreatment according to a published procedure (Zhang et al., 2011). Briefly, the DNA extraction procedure was performed in accordance with a method described by Zhou et al. (1996). High-energy sonication (HES, with a constant frequency of 20 kHz and 40 W power input) was applied to the sample for 2 min (Magic-Knezev and Van Der Kooij, 2004). Nest amplification of the PCR products was achieved using the AOB 16S rRNA gene fragments as templates. The first round of amplification was conducted using primers pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATC-CAGCCGCA-3') (Rowan et al., 2003; Salles et al., 2004) followed by amplification with the AOB specific primers CTO189f (5'-CCGCCGCGCGGGCGGGCGGGGGCAGGGGGGAGRA-AAGYAGGGGATCG-3') and CTO654r (5'-CTAGCYTTGTAGTTT-CAAACGC-3'). These primers were designed to amplify partial 16S rDNA sequences (465 bp) from the beta-proteobacteria AOB (Kowalchuk et al., 1997). PCR was conducted using an Omni-E programmable thermal cycler (Hybaid Ltd., Middlesex,

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