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Shifts between ammonia-oxidizing bacteria and archaea in relation to nitrification potential across trophic gradients in two large Chinese lakes (Lake Taihu and Lake Chaohu)

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ABSTRACT

Ammonia oxidation plays a pivotal role in the cycling and removal of nitrogen in aquatic ecosystems. Recent findings have expanded the known ammonia-oxidizing prokaryotes from Bacteria to Archaea. However, the relative importance of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) in nitrification is still debated. Here we showed that, in two large eutrophic lakes in China (Lake Taihu and Lake Chaohu), the abundance of AOA and AOB varied in opposite patterns according to the trophic state, although both AOA and AOB were abundant. In detail, from mesotrophic to eutrophic sites, the AOA abundance decreased, while the AOB increased in abundance and outnumbered the AOA at hypertrophic sites. In parallel, the nitrification rate increased along these trophic gradients and was significantly correlated with both the AOB abundance and the numerical ratio of AOB to AOA. Phylogenetic analysis of bacterial *amoA* sequences showed that *Nitrosomonas oligotropha*- and *Nitrosospira*-affiliated AOB dominated in both lakes, while *Nitrosomonas communis*-related AOB were only detected at the eutrophic sites. The diversity of AOB increased from mesotrophic to eutrophic sites and was positively correlated with the nitrification rate. Overall, this study enhances our understanding of the ecology of ammonia-oxidizing prokaryotes by elucidating conditions that AOB may numerically predominated over AOA, and indicated that AOA may play a less important role than AOB in the nitrification process of eutrophic lakes.

1. Introduction

Eutrophication of lakes and reservoirs is a world-wide environmental problem (Vollenweider, 1992), particularly in China, due to rapid economic development (Jin et al., 2005). High levels of nitrogen loading are one of the major causes of eutrophication. Fortunately, much of the external nitrogen can be diminished through tightly coupled processes in the microbial nitrogen cycle. Coupled microbial processes can convert ammonia to nitrite and nitrate through nitrification, which can then be lost to the atmosphere as N_2 gas via denitrification or anammox, thereby reducing the effects of excessive nitrogen inputs and eutrophication (Mosier and Francis, 2008). However, recent discoveries, such as the

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existence anammox bacteria and ammonia-oxidizing archaea (AOA), have suggested that our understanding of these processes is incomplete (Francis et al., 2007).

The first key step of coupled nitrification–denitrification, the oxidation of ammonia to nitrite which is often directly determining the removal efficiency of nitrogen, is catalyzed by two groups of prokaryotes, the ammonia-oxidizing bacteria (AOB) and the only recently recognized ammonia-oxidizing archaea (AOA) (De La Torre et al., 2008; Hatzenpichler et al., 2008; Konneke et al., 2005). Despite the very short research history, the archaeal type of the *amoA* gene has been detected in a wide range of environments (Francis et al., 2005; Leininger et al., 2006; Sims et al., 2012; Wuchter et al., 2006), and the numerical dominance of archaea in ocean (Lam et al., 2007), soil (Jia and Conrad, 2009; Leininger et al., 2006) and lake (Herrmann et al., 2009; Wu et al., 2010) has been established. All of these results indicate that archaea may be important in natural nitrification processes.

Although the ammonia-oxidizing prokaryotes play an important role in removing excessive nitrogen from aquatic systems, to date, little information has been available regarding these prokaryotes in natural freshwater sediments, especially eutrophic lakes. Understanding the major factors influence ammonia-oxidizing prokaryotes, including the abundance, diversity and activity, is very important for a better understanding of the nitrogen cycling processes in aquatic ecosystems. Our previous work indicated that both the abundance and the community of AOB were greatly different between a eutrophic lake and an oligotrophic lake (Chen et al., 2009), and another study at estuary gave similar results (Dang et al., 2010). A relative work of lakes found an increase in diversity of AOA and AOB from oligotrophic lakes to mesotrophic lakes (Herrmann et al., 2009). Additionally, a previous study of Lake Taihu found that sediment organic substances, as an indicator of trophic state, greatly affected the AOA abundance (Wu et al., 2010). Based on these previous works, we hypothesized that the trophic state may be a major factor shaping the abundance and community structure of AOA and AOB in aquatic systems. However, whether the varied trends of AOA and AOB communities are similar along the trophic gradients and the relative importance of these groups in ammonia oxidation in freshwater ecosystems remain unclear (Leininger et al., 2006; Nicol and Schleper, 2006).

To validate our hypothesis and to address the questions mentioned above, two large shallow subtropical freshwater lakes (Lake Taihu and Lake Chaohu), located in the middle and lower reaches of the Yangtze River in China, were chosen as model systems. The large size of both lakes results in spatial heterogeneity of environmental parameters even within the same lake. Furthermore, although the two lakes were separated geographically, they were similar in the trophic state in some regions, thus providing an ideal model to distinguish the role of trophic gradients from other factors such as localized dispersal in shaping the abundance, community composition and function of AOA and AOB.

In this study, sediment samples were collected from sites of different trophic state to analyze the abundance and community composition of AOB and AOA. The objectives of this study were to test whether and how AOB and AOA community abundance and composition vary along a gradient of lake trophic states, to determine the major environmental factors affecting the relative abundance of AOA and AOB, and to assess the relative contributions of AOB and AOA to the nitrification of eutrophic lakes.

2. Materials and methods

2.1. Site description and sample collection

Lake Taihu (30°55′–31°32′N, 119°52′32″–120°36′10″E) is the third largest freshwater lake in China. This lake has a surface area of 2338 km^2 and a mean depth of 1.9 m with high spatial variability and includes regions that are dominated by algae or macrophytes (Zhang et al., 2007) (Fig. 1). The water quality of the lake decreases from south to north and from east to west, due to the discharge of pollutants from the northern and western areas (Qin, 2008).

Lake Chaohu (31°25′–31°42′N, 117°16′–117°51′E) is the fifth largest freshwater lake in China. This lake has a surface area of 760 km^2 and a mean depth of 2.69 m (Fig. 1). The lake can be divided into two parts from Zhongmiao Temple to Qitouzui Cape. The water quality decreases from east to west, due to the discharge of pollutants from the industrial and municipal wastewater of Hefei City located at the western part of Lake Chaohu (Shang and Shang, 2007).

Eutrophication is a serious environmental problem for both Lake Taihu and Lake Chaohu. The trophic state of both lakes has been extensively investigated (Cai et al., 2012; Guang-wei, 2008; Jiao et al., 2006; Shang and Shang, 2007; Yu et al., 2011) using the Trophic State Index (TSI) developed by Carlson (1977) and revised by Aizaki (1981). Based on these studies, Lake Taihu can be divided into the following three parts according to the trophic state: (1) its North Bays (Meiliang Bay and Zhushan Bay) are in eutrophic to hypertrophic states; (2) much of the open areas (Central region and Western region) and Gonghu Bay are in mesotrophic to eutrophic states, and (3) the East Bays are mainly in the mesotrophic state; Similarly, Lake Chaohu can be divided into two parts from the Zhongmiao Temple to Qitouzui Cape, with the western part in a eutrophic to hypertrophic state and the eastern part in a mesotrophic state.

To investigate whether and how the trophic state influences the abundance, community composition and function of AOA and AOB, sampling sites were chosen based on the following principles: (1) these sites should exhibit a trophic gradient within each of the two study lakes; (2) each group of different trophic states should have repeats within each lake; (3) each group of different trophic states should have repeats from different lakes. Complying with the above principles and taking into account the ecological zone, geographical features and sediment distribution, totally 18 sites (8 for Lake Taihu and 10 for Lake Chaohu) were chosen for investigation. According to previous descriptions (Qin, 2008; Shang and Shang, 2007) and our own survey, this sampling design could address the goal of understanding the effect of trophic states on AOA and AOB communities.

Using a beaker-type sampler, sediment cores were collected from Lake Taihu on April 27, 2011 and from Lake Chaohu on May 19, 2011. The surface sediment samples down to a 3-cm depth were transferred to sterile plastic bags stored in an ice cooler and brought back to the laboratory. One part was stored