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Original article

Community structure and distribution of planktonic ammonia-oxidizing archaea and bacteria in the Dongjiang River, China

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Abstract

Ammonia-oxidizing archaea (AOA) and bacteria (AOB) are widely distributed in the natural environment and play crucial roles in the nitrification process and the removal of nitrogen (N). Although planktonic microbial community plays an important role in river biogeochemical cycles, few studies have attempted to address the characteristics of AOA and AOB in the water column of river ecosystems. This study examined the community structures, distributions and abundance of planktonic AOA and AOB in the Dongjiang River and their responses to the changes in environmental parameters through quantitative polymerase chain reaction, cloning, and sequencing of ammonia mono-oxygenase (*amoA*). The abundance ratio of AOB to AOA varied from 0.07 to 9.4 along the river and was positively correlated with the concentration of ammonium. Significantly positive correlations were observed between the abundance of AOB and potential nitrification rates, which suggested that the contribution of AOB to nitrification was greater than that of AOA in the river. Phylogenetic analyses showed that AOA communities could be divided into three branches of *Thaumarchaeota*: Group 1.1a, Group 1.1a associated and Group 1.1b, with most sequences belonging to Group 1.1a. All AOB sequences fell within *Nitrosomonas* and *Nitrosospira* species, and the majority of sequences were affiliated with the latter. Multivariate statistical analyses indicated that the community distributions of AOA and AOB were significantly correlated with the concentrations of nitrate and total suspended solids, respectively. These findings fundamentally improved our understanding of the role of planktonic AOA and AOB in nitrogen cycling and their responses to changes in environmental factors in the river ecosystem.

Keywords: River ecosystem; *amoA* gene; Planktonic ammonia oxidizer

1. Introduction

Nitrification is one of the most important processes in N cycling, which starts from the sequential oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and eventually to nitrate (NO_3^-); then the coupling process of denitrification or anaerobic ammonium oxidation (anammox) leads to the N_2 loss to the atmosphere. The limiting process of nitrification (NH_4^+ oxidation to NO_2^-) is mainly mediated by a set of phylogenetically restricted groups of *Beta*- and *Gammaproteobacteria* (ammonia-oxidizing bacteria, AOB) and Groups 1.1a and 1.1b of *Thaumarchaeota* (ammonia-oxidizing archaea, AOA) [1]. Given the important role of ammonia oxidation in limiting nitrification and the fact that the contributions of AOA or AOB to ammonia oxidation likely vary with different environments [2], a more thorough understanding of the interrelationship between ammonia oxidizers and various environmental factors is necessary. The distribution, composition and abundance of

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AOA and AOB have been demonstrated to be influenced by salinity, temperature, pH, or N fertilization in marine and terrestrial environments [3–11]. Indeed, freshwater ecosystems were identified as one of the largest reservoirs of archaea and bacterial diversity [12], but they are by far less thoroughly sampled than marine habitats [13] and the sampling for studying ammonia oxidizers in freshwater ecosystems is biased towards oligotrophic or eutrophic lakes and low-salinity estuaries [13–19]. Rivers represent one typical inland water system with heterogenous properties and play important roles in regenerating and circulating nutrients, in which nitrogen (N) has received particular interest because of the significant concerns of excess anthropogenic N pollution [20,21]. However, planktonic AOA and AOB community composition and abundance in freshwater rivers is still poorly understood, as is the relative influence of deterministic environmental factors on their dynamic changes, although the importance of AOA and AOB has been preliminarily established in river sediments in recent years [22–24].

Although the existence or high abundance of a functional gene does not necessarily demonstrate functional activity [1], the abundance of functional genes integrates recent environmental history and recent process activity, and therefore is a good predictor of potential functional activities [25]. Increasingly convincing evidence for the relative roles of AOA and AOB in ammonia oxidation was provided by correlation of *amoA* gene abundance with nitrification rates in the marine pelagic environment, sediment and soil [10,17,26–29]. However, the contribution of AOA versus AOB to nitrification in the water column of river ecosystems is still poorly understood.

The Dongjiang River is the eastern tributary of the Pearl River in Guangdong province and provides vital drinking water to Hong Kong and several other cities in the Pearl River Delta (PRD). The booming economic growth and urban development along the river basin have led to excessive release of wastes into the river region [30]. Nonetheless, the overall water quality of this river is still within the acceptable surface water quality extent, which could be attributed to the high pollutant transformation efficiency of the microorganisms living in the river [31]. Consistent with this, a high abundance of AOA and AOB was found in the Dongjiang River [24,32], and in addition, the community distribution of AOA in river sediments was shown to be clearly related to pH and TC in our previous reports [24]. Distinct ammonia oxidizers in different niches (such as sediments and the water column of the rivers) may result from differences in the environmental conditions within these niches, and may lead to significant variations in nitrification in the environment [33]. However, the factors that influence community abundance and composition of planktonic AOA and AOB along the river remain unclear and the contributions of AOA and AOB to nitrification have yet to be determined.

To better understand the characteristics of planktonic AOA and AOB and their responses to dynamic environment changes in the Dongjiang River, five sampling sites from upstream to downstream along the river were selected according to the extent of economic development and nutrient gradients. The community compositions and abundance of AOA and AOB and the potential nitrification rate (PNR) were investigated for each site. The aims were to identify coexistence patterns of planktonic AOA and AOB communities in the Dongjiang River, to determine the relationships between these patterns and environmental factors, and to evaluate the relative contributions of planktonic AOA and AOB to nitrification in the river ecosystem.

2. Materials and methods

2.1. Study sites and field sampling

The Dongjiang River basin has a subtropical climate with a mean annual temperature of ~21 °C. Front- and typhoon-type rainfalls are predominant and there are large seasonal variations in rainfall and runoff within the basin. Most annual rainfall and runoff (80%) occur in wet seasons, and only 20% occur in dry seasons [34]. In this study, water sampling was carried out for five days within one week during the dry season (March) in 2011 (Fig. 1). Among the five sampling sites, Heyuan (site 2), Guzhu (site 3), Huizhou (site 4) and Qiaotou (site 5) were located in the mainstream and Xinfeng (site 1) was located in a tributary in the upper reaches of the Dongjiang River. Detailed information on the sampling sites was previously reported [24].

For chemical and biological analyses, triplicate water samples from each sampling site were collected using a plexiglass water sampler (WB-PM, Beijing Purity Instrument Co., Ltd, China). In order to reflect the true conditions of the river water, each sample contained water of 1–5 m depth below the water surface. Microbial cells from 1 to 2 L river water were collected through a membrane nucleopore filter of 0.22 μm pore size (diameter: 47 mm) within 12 h between water collection and filtration in the laboratory. After filtration, each filter was kept at −80 °C until DNA extraction.

2.2. Water physicochemical analysis

The water samples were determined for pH, temperature, dissolved oxygen (DO), ammonium (NH_4^+−N), nitrite (NO_2^-−N), and nitrate (NO_3^-−N) according to the methods described by Liu et al. [32]. The contents of total suspended solids (TSS) and the permanganate index (PI) were estimated using the methods of ISO 11923-1997 and ISO 8467-1993. The concentrations of total carbon (TC), total organic carbon (TOC), total inorganic carbon (TIC) and total nitrogen (TN) were determined on a Total Organic Carbon Analyzer (Elementar, Germany).

2.3. Potential nitrification rate

Potential nitrification rates (PNRs), which provide a rough estimate of the potential activity of ammonia oxidizers, were determined using the chlorate inhibition method described by Kurola et al. [6] with minor modifications. Briefly, the