Communities of sediment ammonia-oxidizing bacteria along a coastal pollution gradient in the East China Sea

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ABSTRACT

Anthropogenic nitrogen (N) discharges has caused eutrophication in coastal zones. Ammonia-oxidizing bacteria (AOB) convert ammonia to nitrite and play important roles in N transformation. Here, we used pyrosequencing based on the *amoA* gene to investigate the response of the sediment AOB community to an N pollution gradient in the East China Sea. The results showed that AOB assemblages were primarily affiliated with *Nitrosospira*-like lineages, and only 0.4% of those belonged to *Nitrosomonas*-like lineage. The *Nitrosospira*-like lineage was separated into four clusters that were most similar to the sediment AOB communities detected in adjacent marine regions. Additionally, one clade was out grouped from the AOB lineages, which shared the high similarities with *pmoA* gene. The AOB community structures substantially changed along the pollution gradient, which were primarily shaped by NH_4^+–N, NO_3^-–N, SO_4^{2-}–S, TP and *Eh*. These results demonstrated that coastal pollution could dramatically influence AOB communities, which, in turn, may change ecosystem function.

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1. Introduction

Ongoing anthropogenic pollution has accelerated the eutrophication of coastal waters and thereby caused the deterioration of marine ecosystems (Bonsdorff et al., 1997). Multiple pollutants have been discharged into seawater and deposited into benthic sediments. The increasing nitrogen (N) input, primarily ammonia nitrogen (NH_4^+–N), has accelerated eutrophication in coastal areas. Increasing NH_4^+–N input can destroy the balance of the N cycle system (Goulding et al., 1998), and excessive NH_4^+–N can be toxic to aquatic animals (Dong et al., 2011). Therefore, it is particularly important to remediate ammonia nitrogen pollution.

Microbial nitrification, which oxidizes ammonia to nitrate via nitrite, plays an important role in the global nitrogen cycle and the biological oxidation of ammonia nitrogen in natural environments (Arrigo, 2005; Guo and Peng, 2008). Ammonia oxidizing bacteria (AOB) convert ammonia to nitrite, the first and rate-limiting step in chemoautotrophic nitrification (Beman and Francis, 2006). It has been reported that these functional organisms could potentially transform and eliminate nitrogen from water (Lam et al., 2007) and sediments (McCaig et al., 1999). Therefore, it is critical to understand the spatial distribution of AOB communities and the factors shaping this pattern.

Traditional studies based on the isolation of AOB have shown the diversity and functional importance of AOB communities (Smorczewski and Schmidt, 1991; Hirota et al., 2002). However, the low growth rates and yields of AOB have hindered the analysis of their diversity because these analyses were time consuming and had a low level of coverage (Ma et al., 2008). The rapid development of molecular biology techniques such as fluorescence *in situ* hybridization (FISH) (Hallin et al., 2005), denaturing gradient gel electrophoresis (DGGE) (Ziembińska et al., 2009), single strand conformation polymorphism (SSCP) (Henne et al., 2013), terminal restriction fragment length polymorphism (T-RFLP) (Osborn et al., 2000) and construction of *amoA* gene clone libraries (Zheng et al., 2012) have provided new insights into AOB diversity in natural environments. The currently verified AOB are primarily restricted to two distinct monophyletic classes of chemolithoautotrophic bacteria, namely, the Betaproteobacterial genera *Nitrosomonas* and *Nitrosospira* (Head et al., 1993; Purkhold, 2003) and the Gammaproteobacterial genus *Nitrosococcus* (Teske et al., 1994). AOB exploit ammonia monooxygenases (AMOs) to catalyze the oxidization of ammonia. The *amoA*-based assay, first developed by Rotthauwe et al. (1997), has been widely used as a molecular

biomarker to investigate the structure and diversity of AOB communities in diverse environments such as nitrifying activated sludge (Ziembińska et al., 2009), wastewater treatment plants (Baek et al., 2010), soil (Li et al., 2012) and marine sediments (Francis et al., 2003; Zheng et al., 2012). These previous studies reported that *Nitrosomonas* and/or *Nitrosospira* were the dominant AOB in different environments and that their distribution was influenced by specific environmental variables, such as the availability of ammonia, oxygen, temperature and pH. However, these methods also have several drawbacks; for example, they are time consuming and involve low-flux processes (Muyzer and Smalla, 1998; Sun et al., 2009). Recently, the development of high-throughput sequencing techniques, such as pyrosequencing, has overcome these deficiencies in a number of cases and has enabled us to trace rare microorganisms and detect subtle variations in microbial communities in response to external environmental changes (Zhang et al., 2011; Pester et al., 2012; Xiong et al., 2012; Ma et al., 2013). For example, Zhang et al. (2011) detected AOB with relative abundances under 1% (ranging from 0.29% to 0.64%) in various activated sludge samples. In these samples, AOB were considered to represent the primary contributors to ammonia oxidation and elimination.

Recent studies have extensively reported that the microbial community could mirror environmental quality (Dang et al., 2008; Yeo et al., 2013; Zhang et al., 2014). For example, sediment bacterial assemblages could be used as biological indicators of pollution levels (Xiong et al., 2014). Sediment AOB communities have responded to specific environmental disturbances, such as eutrophication, in Jiaozhou Bay (Dang et al., 2010) and thus hold the potential to serve as a biological indicator of perturbations in coastal environments (Dang et al., 2010; Cao et al., 2011).

Xiangshan Bay, in the Ningbo region and connected to the East China Sea, is famous for its aquaculture and seafood production. However, the excessive input of nutrients due to industrial and agricultural discharge has accelerated eutrophication in this region, resulting in frequent algal blooms (Wang and Wu, 2009) and threatening marine aquaculture. It is necessary to reveal the impacts of coastal eutrophication on marine microbial assemblages, which, in turn, may be applied as a biological index to evaluate the ecological risk of eutrophication. To test this hypothesis, we collected sediment samples along a transect that showed a clear N pollution gradient (Xiong et al., 2014). In this study, we applied 454 pyrosequencing based on the *amoA* gene to (1) investigate the diversity and structure of AOB communities along a coastal pollution gradient in the East China Sea and (2) assess the environmental factors that shape the pattern of the AOB community.

2. Materials and methods

2.1. Sampling and environmental factor analysis

Sediment samples were collected on October 25, 2012 from five sites along a transect (ranging from 29°22′43″ to 29°22′58″ N, 122°2′30″ to 122°34′48″ E) from nearshore to offshore in Xiangshan Bay, the East China Sea. Five biological replicates were sampled within a 50 m by 50 m area at each site. The sampling transect displayed a clear N pollution gradient; sediment biogeochemical data were recorded in our previous study (Xiong et al., 2014).

2.2. DNA extraction, bacterial amoA gene amplification and 454 pyrosequencing

DNA was extracted from 0.5 g sediment using a Power Soil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA)

according to the manufacturer's protocol. An aliquot (50 ng) of purified DNA from each sample was used as a template for PCR amplification, with the following primer set: *amoA*-1F (5′-GGGGTTTCTACTGGTGGT-3′), which contained the Roche 454 'A' pyrosequencing adapter and a unique 11-bp barcode sequence, and *amoA*-2R (5′-CCCCTCTGCAAAGCCTTCTTC-3′) (Rotthauwe et al., 1997), which contained the Roche 454 'B' sequencing adapter at the 5′-end. Each sample was amplified in triplicate in a 50 μL reaction under the following conditions: initial denaturation for 2 min at 95 °C; then 35 cycles consisting of 30 s at 95 °C (denaturation), 30 s at 60 °C (annealing) and 30 s at 72 °C (elongation); and a final elongation for 10 min at 72 °C (Rotthauwe et al., 1997). The PCR products were purified using the AxyPrepDNA QIAquick Gel Extraction Kit (AXYGEN, Silicon Valley, California, USA), and all PCR products were fluorometrically quantified using QuantiFluor™-ST (Promega, Madison, Wisconsin, USA) and mixed in a sequencing volume ratio. Sequencing was performed using the GS FLX Titanium platform (454 Life Sciences, Branford, CT, USA).

2.3. Processing of pyrosequencing data

Sequencing reads were quality filtered, and chimeras were eliminated using the Mothur software package (http://www.mothur.org, Schloss et al., 2009) with minor modifications, as described previously (Xiong et al., 2012). Briefly, the bacterial reads whose lengths were outside the bounds of 200 and 491 bp (the length of PCR products) and the cases in which the homopolymer run exceeded 6 were removed using PyroNoise (Quince et al., 2009). Sequences with the same barcode were then assigned to the same sample (Caporaso et al., 2010). The bacterial phylotypes were identified using uclust (Edgar, 2004) and assigned to operational taxonomic units (OTUs, 97% similarity). Representative sequences from each OTU were aligned using PyNAST (Caporaso et al., 2010), and the most abundant sequence in the OTU was selected as the representative sequence. The taxonomic identity of each phylotype was determined using the nucleotide database from NCBI (http://ftp.ncbi.nlm.nih.gov/blast/db/). To avoid sample size-based artifacts, we used a randomly selected subset of 1730 sequences (corresponding to the smallest sequencing effort for any of the 25 samples) per sample for further analysis (Xiong et al., 2012).

2.4. Statistical analysis

Metrics of the *amoA* community alpha diversity (Shannon-Wiener *H* and Simpson *D*) and richness estimators (Ace and Chao 1) were calculated using the OTU occurrence (weighted) data. The phylogenetic tree was constructed using the representative sequences of the OTUs and reference sequences using the neighbor-joining method. Cluster analysis and principal coordinate analysis (PCoA) (Lozupone and Knight, 2005; Lozupone et al., 2007) were applied to evaluate the differences in AOB communities based on Euclidean distances using PAST software (Clarke, 1993). The raw environmental data were standardized for comparative purposes using the equation SNDE = (x − mean of the raw data)/ standard deviation of the raw data, where SNDE represents the standard normal deviate equivalents and x represents the raw data for one sample. A Mantel test was used to screen the sediment properties that were significantly correlated with an AOB community, and a redundancy analysis (RDA) (Lepš and Šmilauer, 2003) was then used to correlate the AOB communities with selected environmental factors in the R environment using the "vegan" package (Team, 2012).