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Seasonal variation of anaerobic ammonium oxidizing bacterial community and abundance in tropical mangrove wetland sediments with depth

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ABSTRACT

Anaerobic ammonium oxidation (anammox) process was found to make a nonnegligible contribution to nitrogen loss in estuarine and coastal ecosystems in recent years. In order to better understand ecological characteristics of anammox bacteria in coastal ecosystems, 16S rRNA gene clone library and real-time quantitative PCR (qPCR) were used to investigate the diversity and abundance of anammox bacteria in sediments of the Dongzhai Harbor mangrove wetland in Hainan, China. Sediments at different depths along a transect from mangrove trees to mudflats were studied in March (spring) and August (summer). The dominant genera of anammox bacteria observed in both seasons were *Kuenenia* spp. and *Scalindua* spp. The diversity and abundance of anammox bacteria showed not only seasonal variations, but also differences with depths and habitats. Anammox bacteria appeared to be more abundant and had higher diversity in August than in March. In addition, lower layer (40–45 cm) sediments and mangrove sediments had higher abundance and diversity of anammox bacteria than surface layers (0–2 cm) and non-mangrove areas. It was suggested that water pH, salinity and sediment nitrite, nitrate and ammonium might be key factors shaping the community structure and distribution of anammox bacteria. This study further indicated that the mangrove ecosystem might provide a favorable niche for anammox bacteria and thus plays an important role in nitrogen cycling. Overall, these results extend our current knowledge about the structure and dynamics of anammox bacterial community in the mangrove ecosystem.

1. Introduction

Coastal ecosystems are threatened by habitat destruction and high anthropogenic pollutants inputs, such as excess nitrogen, due to anthropogenic activities like aquaculture, agriculture, human inhabitation, tourism, overharvesting, and wastewater discharges, etc. (Canfield et al., 2010; Reis et al., 2016; Vikas and Dwarakish, 2015). Excessive nitrogen from anthropogenic emission in estuarine and coastal ecosystems has already resulted in critical environmental problems such as eutrophication and water quality degradation in many parts of the world, which has become a global concern (Jickells et al., 2017; Seitzinger and Phillips, 2017; Selman et al., 2008). Mangrove forests, intertidal coastal wetlands occurring in the confluence of terrestrial and marine system (Alongi et al., 2002), are considered important in stabilizing the shoreline and filtering nutrients and pollutants, and in modifying the physical and biogeochemical properties of estuarine waters and sediments (Alongi, 2001). Among the nitrogen transformation process in mangrove ecosystems, anaerobic ammonium oxidation (anammox) is one of the important nitrogen removal pathways (Cao et al., 2017; Fernandes et al., 2012).

Anammox converts ammonia with nitrite as electron acceptor to dinitrogen gas under anoxic conditions without emission of nitrous oxide (N_2O) (Kuypers et al., 2003; Van de Graaf et al., 1995). This process is efficient and environmentally sustainable as it has no need for organic carbon inputs and aeration, and no greenhouse gases release (Kartal et al., 2010). The anammox process is driven by anammox bacteria affiliated with the phylum *Planctomycetes* (Strous et al., 1999), which has been widely observed in various anoxic habitats, such as marine (Bale et al., 2014; Li et al., 2013b), estuarine (Lisa et al., 2015; Teixeira et al., 2014), freshwater (Smith et al., 2015; Yang et al., 2017), and terrestrial environments (Shen et al., 2015; Wang and Gu, 2013), even from some extreme environments such as high temperature petroleum reservoirs (Shartau et al., 2010), hydrothermal vents (Russ et al., 2013) and hypersaline environments (Borin et al., 2013; Speth et al., 2017).

Anammox activity was found to play a potentially important role in

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Table 1
Information of sampling sites and physicochemical properties of overlying water samples.

Sample location	Coordinate	Distance (m)	Season	T (°C)	pH	NH_4^+-N (μM)	NO_2^--N (μM)	NO_3^--N (μM)	TP-P (μM)	PO_4^{3-}-P (μM)	Salinity (‰)	Redox potential (mV)
S1 mangrove area	19°59′54.88″N, 110°36′32.71″E	0	Mar.	24.4	7.88	–	–	–	–	–	29.5	−265.3
			Aug.	27.5	6.45	8.2 ± 0.4c	3.8 ± 0.1b	4.3 ± 0.0a	16.2 ± 2.4	5.0 ± 0.5ab	29.0	−253.3
S2 the edge of mangroves	19°59′54.13″N, 110°36′31.55″E	10	Mar.	24.6	8.03	–	–	–	–	–	31.0	−232.8
			Aug.	27.6	6.68	13.5 ± 0.3a	2.1 ± 0.1c	0.4 ± 0.0c	BD	6.0 ± 1.1a	29.4	−200.2
S3 mudflat	19°59′52.63″N, 110°36′26.97″E	1000	Mar.	24.5	8.11	–	–	–	–	–	31.0	−244.7
			Aug.	27.5	7.29	11.7 ± 0.4b	4.6 ± 0.2a	0.5 ± 0.1b	6.2 ± 0.3	4.6 ± 0.2b	30.1	−218.1

BD: below detection limit.
Different letters indicate significant differences ($P < 0.05$).

nitrogen losses in some mangrove systems, and to fluctuate with seasons, habitats and depths. The report by Fernandes et al. showed that anammox activity at deeper depths (8–10 cm) in the anthropogenically influenced mangrove ecosystem was five times higher than that in the relatively pristine mangrove ecosystem, accounting for 67–96% of the total N_2 production (Fernandes et al., 2012). In the Jiulong mangrove system, rates of anammox in mangrove surface sediments were mainly higher than that in the bare flats, contributing 33% of the total N_2 production in winter and 57.5% in summer (Cao et al., 2017). The genera of *Scalindua*, *Kuenenia*, *Brocadia* and *Jettenia* anammox bacteria were detected in the Maipo mangrove wetland where has been strongly influenced by anthropogenic input. Moreover, the abundance of the anammox bacterial *hzo* gene was higher in the surface mangrove sediments than in mudflats (Li et al., 2011). So far, reports on anammox process and anammox bacteria in subtropical and tropical mangrove ecosystems are still limited. Most of the related researches were mainly focused on the activity of anammox bacteria and contribution of anammox to N_2 production in mangrove sediments. The characteristics of anammox bacterial ecological distribution and factors in mangrove ecosystems within different seasons, habitats and depths are still poorly understood. This study was thus aimed to gain better insight into the ecological niches and physiological characteristics of anammox bacteria in mangrove wetland sediments.

The Dongzhai Harbor of Hainan Island is the first national mangrove wetlands reserve established in China. In the course of economic development, patterns of land use in this area significantly changed during the past decades. Aquaculture pond areas and construction areas increased drastically, while the mangrove area steadily decreased (Xin et al., 2014). Tourism, discharge of aquaculture effluents and intensive livestock resulted in excessive nitrogen and phosphorus in the seawater of this area (Wen et al., 2015). Information about anammox bacteria in this tropical mangrove wetland is still in absence. Therefore, the Dongzhai Harbor mangrove wetland was chosen as an example to study the important biogeochemical transformation processes. The investigation of these processes contributes to an improved understanding of the global nitrogen cycle.

Previous studies indicated that anammox activity in mangrove system differed greatly in vegetated and non-vegetated areas, sediment depths and seasons (Cao et al., 2017; Fernandes et al., 2012). Therefore, it could be assumed that anammox bacterial community and abundance could differ in different habitats of mangrove system and in seasons. Thus, the present study was to investigate the community structures and abundance of anammox bacteria in mangrove and non-mangrove sediments at different depths in the Dongzhai Harbor mangrove wetland in two seasons. Moreover, the correlations between the anammox bacterial community structures and environmental factors were evaluated, which will provide additional information of anammox bacteria in mangrove ecosystems.

2. Materials and methods

2.1. Study area and sampling

The Dongzhai Harbor mangrove wetland is situated in the northeastern part of Hainan Island, China (N 19°51′–20°01′, E 110°30′–110°37′), covering 1578.2 ha of mangroves and 1759.4 ha of intertidal zone. It is the most abundant mangrove wetland in China with 84 km coastline (Zhao et al., 2014). This area features a tropical oceanic monsoon climate. The annual sunshine duration is up to 2200 h and the average temperature is 23.8 °C (Liao et al., 2009). The Dongzhai Harbor has the average monthly low tide level of 0.99–1.32 m. In the mangrove wetlands, the dominant mangrove trees species are *Ceriops tagal*, *Bruguiera sexangula*, *Bruguiera gymnorrhiza*, *Kandelia obovata*, and *Aegiceras corniculatum* (Xin et al., 2014). The soil type is classified as Subaqueous Histosols (Sulfiwassists) (Soil Survey Staff, 2014).

Three sites (S1, S2 and S3) along a transect from the mangrove forest to the coastal water in the Dongzhai Harbor mangrove wetland were sampled in March (spring) and August (summer) 2015, respectively (Table 1). Site S1 was located in the mangrove forest, while site S2 was near the edge of the mangrove forest, about 10 m away from S1. Site S3 is located on the tidal mudflats (no covering vegetation), with a distance of 1000 m to site S1. Three distinct sampling locations (about 100 m apart) in each of the three sampling area (S1, S2 and S3) were selected. At least four replica points at each location were selected. Sediment samples in the surface layer (0–2 cm), mediate layer (20–25 cm) and lower layer (40–45 cm) were collected by the core sampler from each point. The four replica sediment samples at the same depth from the same location were mixed and homogenized. Sediment samples were stored at 4 °C for physicochemical analysis and −20 °C for subsequent molecular analysis.

2.2. Environmental parameter analysis

Concentrations of tested substances in water samples including nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), ammonia nitrogen (NH_4^+-N), total phosphorus (TP) and reactive phosphate (PO_4^{3-}-P) were measured according to the Chinese national standard methods (GB/T 12763.4-2007, China). NO_2^--N, NO_3^--N and NH_4^+-N concentrations of sediment samples were determined using KCl extraction following the National Environmental Protection Standard methods (HJ634-2012, China). UV–Vis spectral analyses were conducted by using a Perkin-Elmer Lambda-25 spectrophotometer. All analyses were performed in triplicates, and standard curves were generated during each batch experiment. The overlying water temperature, pH and redox potential were determined *in situ* by a Hanna HI8424 (Hanna Instruments, Milan, Italy). The overlying water salinities were measured using a Hanna RHS (Hanna Instruments, Milan, Italy).