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Diversity and abundance of ammonia-oxidizing archaea and bacteria in polluted mangrove sediment

Huiluo Cao[a], Meng Li[a], Yiguo Hong[b], Ji-Dong Gu[a,*]

[a] School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China
[b] South China Sea Institute of Oceanography, Chinese Academy of Sciences, 164 Xingang Road West, Guangzhou 510301, People's Republic of China

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ABSTRACT

Ammonia oxidation by microorganisms is a critical process in the nitrogen cycle. Recent research results show that ammonia-oxidizing archaea (AOA) are both abundant and diverse in a range of ecosystems. In this study, we examined the abundance and diversity of AOA and ammonia-oxidizing beta-proteobacteria (AOB) in estuarine sediments in Hong Kong for two seasons using the ammonia monooxygenase A subunit gene (*amoA*) as molecular biomarker. Relationships between diversity and abundance of AOA and AOB and physicochemical parameters were also explored. AOB were more diverse but less abundant than AOA. A few phylogenetically distinct *amoA* gene clusters were evident for both AOA and AOB from the mangrove sediment. Pearson moment correlation analysis and canonical correspondence analysis (CCA) were used to explore physicochemical parameters potentially important to AOA and AOB. Metal concentrations were proposed to contribute potentially to the distributions of AOA while total phosphorus (TP) was correlated to the distributions of AOB. Quantitative PCR estimates indicated that AOA were more abundant than AOB in all samples, but the ratio of AOA/AOB (from 1.8 to 6.3) was smaller than most other studies by one to two orders. The abundance of AOA or AOB was correlated with pH and temperature while the AOA/AOB ratio was with the concentrations of ammonium. Several physicochemical factors, rather than any single one, affect the distribution patterns suggesting that a combination of factors is involved in shaping the dynamics of AOA and AOB in the mangrove ecosystem.

Introduction

Nitrification, the microbial oxidation of ammonia to nitrate via nitrite, plays a critical role in the global nitrogen cycle. Two distinct monophyletic groups of chemolithoautotrophic ammonia-oxidizing bacteria (AOB), β-proteobacterial genera *Nitrosomonas* and *Nitrosospira* [24,54,55] and the γ-proteobacterial genus *Nitrosococcus* [69], have long been thought to be responsible for ammonia oxidation which is the first and also the rate-limiting step in nitrification. However, recent metagenomic analysis of archaea [60] and the isolation of ammonia-oxidizing crenarchaeote *Nitrosopumilus maritimus* [28] have proved the existence of ammonia oxidizing archaea (AOA), as well as the critical role of them based on a series of surveys of marine and terrestrial ecosystems for contributions to the nitrogen cycle [4,6,11,20,22,34,35,43,70,72]. Although numerous studies show the global distribution of AOA, only a few studies have been focused on the estuarine ecosystem and relationships of AOA with other nitrifying microbes [13,20]. Mangrove ecosystems in some estuaries are the major component of the tropical and subtropical coastal wetlands featured by high turnover rates of organic matter and nutrient cycling between the ocean and terrestrial habitats [48]. It has been shown that microbes are the main drivers in the nitrogen and phosphorus cycles in mangroves [56,66,68]. Denitrifying bacteria [18], N_2 fixing [18,56,66] and the newly discovered anammox bacteria [42] have been detected in mangrove ecosystems. These studies indicate nitrogen cycling is complex in such systems. The diversity and abundance of AOA in mangrove ecosystems are currently less known.

The contributions of archaea comparing with bacteria to aerobic ammonia oxidation in environments have been summarized recently [53]. AOA are one to two orders of magnitude more abundant than AOB in many marine and terrestrial ecosystems [43,47,70]. However, contrary results also appeared in a few studies, indicating that AOB are more dominant [27,45]. To date, a few environmental factors have been suggested to affect the community structure of AOA or AOB and relative contributions, for example, temperature [15,67], pH [23,49,62], salinity [4,5,9,20,43,45,58,59], and phosphate [25]. More recently, it is proposed that AOA might play an important role in the nitrogen cycle in low-nutrient, low-pH, and sulfide-containing environments [17]. However, the dynamics between these two distinct ammonia-

* Corresponding author. Tel.: +852 2299 0605; fax: +852 2559 9114.
E-mail address: jdgu@hkucc.hku.hk (J.-D. Gu).

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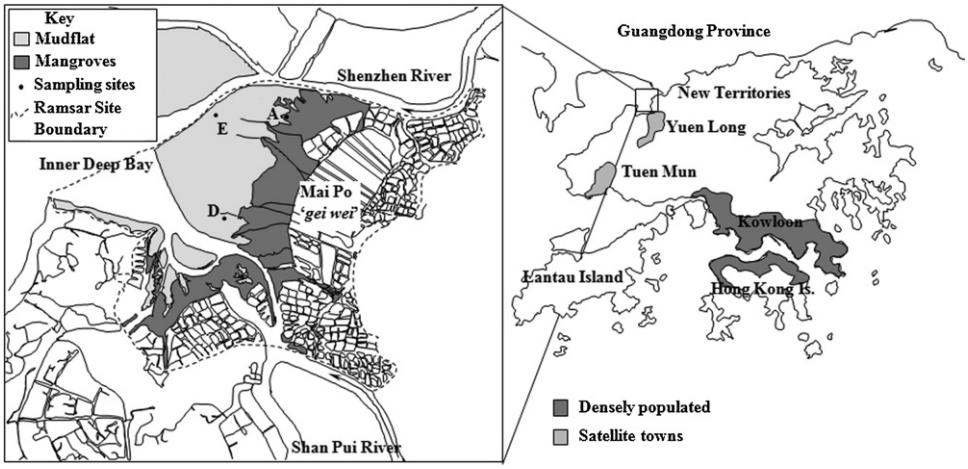

Fig. 1. Location of sampling sites in Mai Po and Inner Deep Bay of Hong Kong.

oxidizing groups are likely to be more complex because of their common substrate and ecological niche. Further studies are still necessary to determine the physicochemical parameters affecting their abundance and diversity, especially pollution related ones including metals, because some critical enzymes involved in the microbial nitrogen cycle are metalloenzymes, and trace metal availability should be a key factor to regulate nitrogen transformations [44]. For example, the AMO (ammonia monooxygenase) which oxidizes ammonia to hydroxylamine [26] was considered as a Cu and possibly Fe containing enzyme [16,71]. Recently, Li et al. [36] showed that in Cu polluted soil AOA seemed more tolerant to Cu contamination than AOB. Additionally, Mosier and Francis [45] also presented that the correlation between AOB community composition and nickel concentrations and the abundance of AOA *amoA* gene was strongly correlated with lead (Pb) concentrations in San Francisco Bay. However, the effect of heavy metals in sediments of estuary on the community structure is still not well known.

The Pearl River (Zhujiang) Estuary is one of the most complex estuarine systems in the world because of branches, sub-estuaries and the large quantity of wastewater discharged, which is located in one of the most rapidly developing areas of the world economy in the past two decades [12]. Large quantities of domestic and industrial wastewater discharged into this area and eventually the South China Sea through three sub-estuaries, resulting in ammonium contamination and hypoxia. Lingdingyang is the largest sub-estuary surrounded by a number of metropolises such as Guangzhou, Shenzhen, Macau and Hong Kong. Mai Po Nature Reserve Ramsar Site (22°30′N, 114°02′E) of Hong Kong, is located at this sub-estuary and the northwestern part of the New Territories, which comprises sub-tropical mangroves, inter-tidal mudflats, fishponds and drainage channels [38]. It is a shallow estuary with an average water depth of about 2.9 m and the mean tidal range of 1.4 m and also the largest wetland in Hong Kong [31]. Because this area is between Shenzhen Special Economic Zone and Hong Kong, a large quantity of wastewater including domestic sewage and industrial wastewater is discharged by Shenzhen River and inland rivers of Hong Kong, resulting in the contamination of the sediments. The sediments are mainly polluted by heavy metals, e.g. Cu, Pb, Hg, organic pollutants and anthropogenic nitrogen [30,37].

The *amoA* gene encoding the AMO subunit A has been proved as a suitable molecular marker to investigate AOA and AOB [54,55] and now is popular in the molecular ecological studies of AOA and AOB. Thus, the *amoA* gene was amplified to determine the diversity and distributions of nitrifying microbes, and then was quantified using quantitative PCR (q-PCR) for the different sites in different seasons. Statistical analyses were also employed to correlate these results with physicochemical parameters to identify potential contributors to the diversity and abundance of AOA and AOB.

Materials and methods

Sample collection and analyses of physico-chemical characteristics

Sampling sites of this study at Mai Po Nature Reserve of Hong Kong were chosen for two distinctive regions: one (site A) covered with mangrove trees *Kandelia obovata* (formerly *Kandelia candel*) while the other two sites (sites D and E) without any vegetation (Fig. 1). In 2008, samples were taken in May of the monsoon season and in November the dry season. Sediment core samples were collected from the surface layer (1–2 cm) using the acrylic tube with 10 cm diameter at each of the three sites (Table 1) and were put into plastic bag and then stored in an ice-cold cooler for transport from the field back to the laboratory within 4 h. For the same site and same season, triplicate subcores were selected and then mixed together for homogenization. Each sediment sample was further split into two equal parts, one for DNA isolation and another for chemical analyses.

Table 1
Physical and chemical characteristics of the sediment samples used in this study.

Sampling site	Season	pH	T (°C)	Redox (mV)	Ammonium (μM)	NO_x (μM)	TKN (mg/kg)	TN (mg/kg)	TP (mg/kg)	S^{2-} (mg/kg)	TOC (mg/kg)	As (mg/kg)	Cd (mg/kg)	Cu (mg/kg)
A	May	6.2	28.0	−59.2	266.4	2.4	1160.3	1170	1128	53	13.7	27.0	0.45	103.7
	November	7.4	19.6	−96.3	246.6	1.1	997.0	1006	956	552	17.9	17.5	0.60	98.3
D	May	7.2	28.0	−116.0	55.2	2.2	872.9	881	1608	365	11.7	31.0	0.45	124.7
	November	7.5	19.6	−59.0	575.7	2. 7	605.0	614	836	771	13.6	18.2	0.55	104.7
E	May	7.2	28.0	−154.0	79.0	9.3	847.2	864	1657	101	9.2	16.0	0.25	103.7
	November	7.5	19.6	−189.0	344.8	0.5	711.0	718	399	132	10.0	17.0	0.20	44.7