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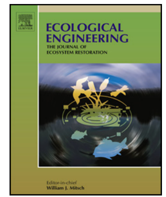

Review

Effects of emergent aquatic plants on abundance and community structure of ammonia-oxidising microorganisms

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ABSTRACT

Clone library construction and quantitative polymerase chain reaction were used to investigate the effects of three emergent aquatic plant species (*Iris pseudacorus*,*Thalia dealbata* and *Typha orientalis*) on the abundances and community structures of ammonia-oxidising archaea (AOA) and bacteria (AOB). The abundances of archaeal and bacterial *amoA* genes ranged from 2.91×10^7 to 3.20×10^9 and 1.03×10^7 to 5.10×10^8, respectively. At the study area, *T. orientalis* showed the highest abundances of AOA and AOB. *I. pseudacorus* harbored higher AOA richness and diversity than *T. orientalis*, but lowest AOB abundance. *Nitrososphaera,* Nitrosopumilus and *Nitrosotalea* clusters were observed in AOA, in which the *Nitrososphaera* cluster was predominant. Moreover, *Nitrosomonas* and *Nitrosospira* clusters were detected in AOB, in which the *Nitrosospira* cluster was dominant. Assays of potential nitrification rates (PNRs) in the samples cultivated with or without ampicillin suggested that AOA may be important in nitrification in unvegetated and *I. pseudacorus*-associated sediments, whereas AOB may dominate nitrification in the other species.

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1. Introduction

The rhizosphere harbors higher amount and metabolism activity, as well as more species and complicated community structures, than those of the non-rhizosphere environment; the thriving roots of plants provide suitable niches for microbial growth and rhizodeposition, resulting in high contents of easily degradable carbon sources for adherent microbes (Avis et al., 2008; Prashar et al., 2013). The roots of emergent macrophytes release oxygen through their special structures (hypertrophic stems and big tissues centered in the stems and roots); this process could stimulate coupled nitrification–denitrification in the rhizosphere compared with that in unvegetated sediments. The microbial communities involved in coupled nitrification–denitrification have been investigated in rice paddy soils; however, those in freshwater sediments, particularly from the rhizospheres of emergent macrophytes, have been rarely reported.

As the first and rate limiting step of nitrification, ammonia oxidation is important in global biogeochemical nitrogen cycle. This process could be catalysed by two groups of prokaryotes, namely, ammonia-oxidising archaea (AOA) and bacteria (AOB). AOA have been recognised as *Crenarchaeota* and then renamed as a new archaeal phylum *Thaumarchaeota* based on the comparative genomics and phylogeny of their concatenated genes (Brochier-Armanet et al., 2008). Studies have demonstrated the ubiquity of AOA and their predominance compared with AOB in terrestrial and aquatic ecosystems (de Gannes et al., 2014; Liu et al., 2014b; Strauss et al., 2014; Wang et al., 2014b; Zeng et al., 2011). However, the relative abundances of these two microbial groups differ in different macrophytes (Herrmann et al., 2009; Trias et al., 2012; Wei et al., 2011), presumably indicating that plant species may affect the abundance of ammonia-oxidising microorganisms. Moreover, AOA and AOB community structures differ in rhizosphere and unvegetated sediments (Herrmann et al., 2009; Zhao et al., 2014), implying the species-specific effects of plants on the microbial community structures.

The present study primarily aimed to investigate the abundances and community structures of AOA and AOB in the rhizosphere and non-rhizosphere sediments of emergent aquatic plants. This study also aimed to determine the effects of the emergent aquatic plants on the abundance and community structure of ammonia-oxidising microorganisms in rhizosphere sediments. Three plant species, namely, *Iris pseudacorus*, *Thalia dealbata* and *Typha orientalis* were obtained from Jinshan Park.

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These species are commonly grown in constructed wetlands (Vymazal, 2013) and characterised by nutrient uptake and extent of radial oxygen loss (ROL). *I. pseudacorus* prefers nitrate as the nitrogen source, whereas *T. dealbata* and *T. orientalis* prefer ammonium (Wang et al., 2014a; Ying et al., 2011). ROL is higher in the roots of *I. pseudacorus* than in the roots of *T. orientalis* (Liu et al., 2010; Li, 2011).

2. Materials and methods

2.1. Site description and sample collection

The rhizosphere and non-rhizosphere sediments of *I. pseudacorus*, *T. dealbata* and *T. orientalis* were collected on 31st July 2013 on the shore of Jinshan Lake, Zhenjiang City, Jiangsu Province, China (32°21′N and 119°41′E). This region is characterised by subtropical monsoon climate with annual mean temperature and rainfall of 15.6 °C and 1088.2 mm, respectively. The Jinshan Lake is a dammed lake, and water level is under artificial control around 4.2 m. At sampling sites, the hydrological condition is stable and similar, and no waterlogging occurs.

Each sampling point contained five plots (about 30 $cm^3 \times 30$ $cm^3 \times 30 cm^3$). Rhizosphere samples were collected by shaking off the sediments that loosely attached to the roots (marked as IP, TD and TO, respectively), whereas the detached sediments were collected as non-rhizosphere samples (marked as N-IP, N-TD and N-TO, respectively). Sediments without vegetation coverage were also collected as control (marked as CT). The collected sediments were immediately transferred into aseptic hermetic bags on an ice bath and transported to the laboratory. The samples from the same plant species were mixed thoroughly and then sieved through a 2-mm mesh to remove plant debris and other impurities. Each sediment sample was divided into two parts for physicochemical and molecular analyses; the samples for the latter were stored at −20 °C.

2.2. Physicochemical analyses

Sediment pH was measured using a PHS-3C numerical pH meter (INESA Scientific Instrument Co., Ltd., China) with a water to sediment ratio of 2.5:1 (Lu, 1999). Organic matter (OM) was measured via dichromate oxidation. Ammonium (NH_4^+-N) and nitrate (NO_3^--N) were extracted with 2 M KCl and then measured using indophenol blue and phenol disulfonic acid methods, respectively. Total nitrogen (TN) was determined using semi-micro Kjeldahl method, and total phosphorus (TP) was colorimetrically measured through ascorbic acid–molybdate blue method. Triplicates were performed for each parameter.

2.3. DNA extraction, PCR amplification and clone library construction

Total DNA was extracted with FastDNA® SPIN kit for soil (MP Biomedicals, Solon, USA) from 0.5 g of sediment samples. Three replicates were performed for each sample, and DNA solutions were homogeneously mixed. Archaeal and bacterial functional gene markers for encoding ammonia monooxygenase alpha subunit were targeted (A*amoA* and B*amoA*, respectively) to detect AOA and AOB in the samples. The primers used were Arch-amoAF/Arch-amoAR for AOA and amoA-1F/amoA-2R for AOB (Table 1) (Francis et al., 2005; Rotthauwe et al., 1997). PCR reactions were performed as described previously (Liu et al., 2014a). PCR products were obtained with PCR gel extraction kit (Sangon, China). The purified gene fragments were then ligated into the pMD™ 19-T vector (TaKaRa, Japan) and transformed into *Escherichia coli* DH5α competent cells. For each sample, a minimum of 50 transformants were randomly selected and analysed using restriction fragment length polymorphism (RFLP) with *Msp*1 and *Hha*1 restriction enzymes (Dang et al., 2009). After RFLP analysis, approximately 20 clones were selected for each library and then sequenced by Sangon Biotech. Incorporated Company (Shanghai, China).

2.4. Phylogenetic analysis

The obtained sequences were analysed online with GenBank database sequences using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) to ensure that these sequences belonged to ammonia-oxidising microorganisms. For AOA and AOB, the clones with more than 95% sequence similarity were classified into the same operational taxonomic unit (OTU). According to the OTUs classification, representative sequences were selected and the most similar sequences to them were searched in GenBank database using BLAST. Moreover, their representative sequences were used for phylogenetic analysis. The representative sequences from each library and various well-known sequences were used for phylogenetic tree construction through neighbor-joining method (based on Jukes–Cantor distance) with MEGA 5.2 program. A bootstrap analysis with 1000 replicates was performed to estimate the confidence values for tree nodes.

2.5. Quantification of archaeal and bacterial amoA gene

Quantitative PCR (qPCR) reaction was performed with CFX96™ real-time PCR detection system (BIO-RAD, USA) using a DyNAmo™ ColorFlash SYBR® green qPCR kit (Thermo, USA). The primer sets described in Section 2.3 were used to quantify the abundance of archaeal and bacterial *amoA* genes in the collected soils. A total volume of 20 μL contained 10 ng of the template, 0.5 mM of each primer and 10 μL of the master mix. The reaction program was 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 53 °C (AOA)/55 °C (AOB) for 30 s and 72 °C for 30 s. Each cycle was recorded. Product specificity was confirmed through melting curve analysis (60–98 °C, 0.5 °C per read, 5 s hold). Plasmids were extracted from the samples by using a SanPrep mini-plasmid kit (Sangon, China) for standards. For each gene and primer set, the reaction was performed with three replicates for the samples and standards.

2.6. Potential nitrification rates (PNRs)

PNRs were measured according to shaken slurry method (Lu, 1999). Briefly, 10 g of fresh soil was added into a 250-mL Erlenmeyer flask containing 100 mL of culture medium and pH was adjusted to 7.4. The culture medium was composed of 0.2 M KH_2PO_4, 0.2 M K_2HPO_4 and 0.05 M $(NH_4)_2SO_4$ with a volume ratio of 3:7:30. The controls of each sample were stored at −20 °C. The experimental samples were shaken at 180 rpm for 1 h and then cultivated in a ZGX-300C constant-temperature incubator (Hangzhou Qianjiang Instruments and Equipment Co., Ltd., China) at 28 °C for 24 h. After cultivation, NO_2^--N and NO_3^--N were extracted with 5 mL of 2 M KCl and then measured. PNRs were calculated based on the changes of NO_2^--N + NO_3^--N concentrations before and after cultivation (Bernhard et al., 2007; Zheng et al., 2014). In a parallel experiment, an additional 1 g L^{-1}

Table 1
PCR primers.

Target	Primer sets	Primer sequences (5′–3′)	Fragment size
AOA amoA gene	Arch- amoA F	STAATGGTCTGGCTTAGACG	635 bp
	Arch- amoA R	GCGGCCATCCATCTGTATGT	
AOB *amoA* gene	amoA-1F	GGGGTTTCTACTGGTGGT	491 bp
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC	

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