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Distribution characteristics of ammonia oxidizing microorganisms in rhizosphere sediments of cattail

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ABSTRACT

The abundance, diversity and community structure of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) were investigated, using *amoA* gene as a molecular biomarker, to examine how cattail (*Typha orientalis*) growth affects the abundances and communities of AOB and AOA in the rhizosphere sediment. Clone libraries and qPCR (quantitative polymerase chain reaction) results indicated the *amoA* gene copies numbers, relative abundance of AOA and AOB *amoA* gene clone libraries were highest in rhizosphere sediment, followed by non-rhizosphere sediment and control, indicating that cattail growth has a remarkable effect on the abundance and community structure of ammonia oxidizing microorganisms. Diversity index (Chao1 and Shannon H) have an obvious variation in cattail rhizosphere, non-rhizosphere and control sediments. The average values of Shannon *H* of AOA and AOB in the rhizosphere sediment were higher than the non-rhizosphere sediment and control. The average value of Chao1 was highest in non-rhizosphere sediment for AOA, whereas, was highest in the rhizosphere sediment for AOB. The abundance, diversity and community structure of AOA and AOB were obvious different in the cattail's three developmental stages, and higher abundances and more complex community structure were obtained in the maturity stage, which may be attributable to the higher root exudation owing to fast growth rates and strong metabolisms in the maturity stage. In addition, despite higher abundant of AOA versus AOB was demonstrated in the cattail rhizosphere, non-rhizosphere sediment and control, bacteria, not archaeal, have more ecosystem functions and play a more important role in dominating ammonia oxidation under the conditions of this study according to the two findings from the present study. One is that AOB had a more diverse and more complex community structure than AOA; the other is that the average value of potential nitrification rates (PNRs) significantly decreased (by 49.08%, 53.69% and 10.39%, respectively) in rhizosphere, non-rhizosphere sediment and control, as compared to PNRs without the ampicillin, when ammonia-oxidizing bacteria were inhibited by ampicillin.

1. Introduction

Nitrogen (N) is the principal pollutant in freshwater ecosystems and often considered responsible for eutrophication (Elser et al., 2007; Wei et al., 2011; Hou et al., 2013a; Howarth and Marino, 2006; Vollenweider, 1992). Aquatic macrophytes are crucial components in the freshwater ecosystem (Srivastava et al., 2008; Zhao et al., 2014), which play key roles in sustaining the clear water state (Wang et al., 2009). Previous studies had demonstrated aquatic macrophytes could affect nutrient cycling through influencing rhizosphere microbial communities (Bais et al., 2006; Wu et al., 2007; Zeng et al., 2012; Zhao et al., 2014), as oxygen released from the roots of macrophyte species stimulates nitrification and coupled nitrification-denitrification in the rhizosphere, as compared to the bulk sediment (Bodelier et al., 1996; Herrmann et al., 2009; Ottosen et al., 1999; Risgaard-Petersen and Jensen, 1997). Therefore, rhizosphere-associated biogeochemical transformations of nitrogen (N) are crucial to the understanding of nutrient cycling (Herrmann et al., 2008; Ottosen et al., 1999) in the aquatic ecosystems.

As the first and rate-limiting step of nitrification, ammonia oxidation, the microbial oxidation process of ammonia to nitrate via nitrite, plays a critical role in global N cycle

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(Kowalchuk and Stephen, 2001). This process is carried out by two groups of microorganisms, ammonia-oxidizing archaea (AOA) (Könneke et al., 2005) and ammonia-oxidizing bacteria (AOB) (Koops et al., 2003). Numerous studies have demonstrated aquatic macrophytes had strong influences on the diversity, abundance and community structure of AOA and AOB (Arroyo et al., 2015; Chen et al., 2008; Herrmann et al., 2008, 2009; Trias et al., 2012; Wei et al., 2011). For example, Herrmann et al. (2009) found the diversity and abundance of archaeal and bacterial *amoA* gene have obvious differences in three rooted macrophytes (*Littorella uniflora*, *Juncus bulbosus*, and *Myriophyllum alterniflorum*), and release of oxygen and organic carbon from the macrophyte rhizospheres increased the abundance of archaeal *amoA* gene. Herrmann et al. (2008) also found that AOA, but not AOB, were also strongly enriched in the rhizosphere of the freshwater macrophyte *L. uniflora* in a mesotrophic Danish lake, suggesting that AOA were primarily responsible for increased rates of nitrification in the rhizosphere of this plant. Wei et al. (2011) reported that AOA and AOB can attach to three floating aquatic macrophytes (*Eichhornia crassipes*, *Pistia stratiotes* and *Ipomoea aquatic*) suspending root systems in a eutrophic water environment, and AOB were the predominated ammonia-oxidizers on the rhizoplanes of macrophytes. Zhao et al. (2014) reported that submerged macrophytes (*Ceratophyllum demersum*, *Vallisneria spinulosa*, and *Potamogeton crispus*) exhibited no significant effect on the abundance and diversity of archaeal *amoA* gene, while *C. demersum* and *V. spinulosa* increased the abundance and diversity of bacterial *amoA* gene in the rhizosphere sediment. Our previous studies also demonstrated that abundances and community structures of AOA and AOB differed considerably in the rhizosphere sediments of three species, i.e. *Iris pseudacorus*, *Thalia dealbata* and *Typha orientalis* (Zhang et al., 2015). Apart from species-specific, plant age or developmental stage may also affect the predominant bacterial strains in the rhizosphere due to different composition of root exudates (MacDonald et al., 2004; Prashar et al., 2014; Smalla et al., 2001), as radial oxygen loss from aerenchymatous roots is linked to the respiratory activity of the roots, which varies with root age. Clearly, the distribution characteristics of rhizosphere-associated AOA or AOB varied with plant growth stages, plant species, and different genotypes.

Cattail, as one of the most common emergent aquatic macrophyte species growing in freshwater ecosystems, play an important roles in improving water quality, providing valuable habitats for microorganism, etc., in various natural and artificial ecosystems. Therefore, the information about ammonia-oxidizing communities around the plant cattail roots is crucial to the understanding of nutrient cycling in freshwater ecosystem dominated by this plant species. To date, minimal information is available regarding the influence of cattail on ammonia-oxidizing microorganisms in sediment. The existing research only involved certain growth stage. For example, Zhang et al. (2015) sampled rhizosphere sediments of species *Typha orientalis, Iris pseudacorus* and *Thalia dealbata* on their mature stage and found that *Typha orientalis* rhizosphere sediment showed higher abundance of AOA and AOB than species *Iris pseudacorus* or *Thalia dealbata*. To date, little is known about how the cattail affects the community composition, abundance and diversity of AOA and AOB, particularly in the rhizosphere during their growth process. Furthermore, information is lacking on AOA and AOB contribution to ammonia oxidation in the cattail rhizosphere sediment and subsequent effect on cattail growth is scarcely studied. Therefore, further research is needed to bridge the gap of knowledge.

In the present study, sediment samples were collected from cattail rhizosphere, non-rhizosphere, and areas without vegetation coverage at three growth stages to investigate the difference of abundance, diversity and community composition of ammonia oxidizing microorganisms; to examine the effect of cattail growth on the abundances and communities of AOB and AOA; and to estimate the relative contributions of AOA and AOB to nitrification in the cattail rhizosphere sediment.

2. Materials and methods

2.1. Study region and sample collection

Sampled sites were located at shore of Jinshan lake park, Zhenjiang City, Jiangsu Province, China (32°21′ N and 119°41′ E). The Jinshan lake park is a dammed lake, and water level is under artificial control around 4.2 m. For all the sampling sites, the hydrological conditions were stable and uniform (Zhang et al., 2015).

Five replicate cattail (*Typha orientalis*) roots were excavated from five separate plots with approximately $30\,cm^3 \times 30\,cm^3 \times 30\,cm^3$ using shovel on 20-May (seedling stage, marked as M), 31-July (maturity stage, marked as J) and 16-November (decline stage, marked as N) of 2013, respectively. Then, each of the five cattails roots was drained of water and shaken off the loosely adhered soil. The detached soils were collected as non-rhizosphere sediment samples (marked as NCA), and the adhered soil were collected as rhizosphere sediment samples (marked as CA). Meanwhile, un-vegetated sediment was collected as control (marked as CK). Collected sediment sample (five replicates) was placed into sterile plastic bags on an ice bath before transported to the laboratory. In laboratory, each collected sediment sample (five replicates) was thoroughly mixed and sieved through a 2-mm mesh to remove visible root pieces remaining in the rhizosphere soil and other impurities. Then each sediment sample was divided into two portions. One was stored at −20 °C for molecular and potential nitrification rates (PNRs) analysis, and the other was freeze dried with a freeze dryer (LGJ-12, Songyuan, China), powdered, sieved through a 100-mesh nylon sieve for the analyses of sediment physico-chemical properties.

2.2. Physicochemical analyses

Sediment pH was extracted according to the method of (Lu, 1999) and measured using a PHS-3C digital pH meter (INESA Scientific Instrument Co., Ltd., China). Total N (TN) was determined using the semi-micro Kjeldahl method (Lu, 1999). Ammonium (NH_4^+-N) and nitrate (NO_3^--N) of sediment were extracted with 2 M KCl and measured using indophenol blue and phenol disulfonic acid methods, respectively (Bao, 1981). Total phosphorus (TP) was extracted by the SMT protocol method (González Medeiros et al., 2005) and the extracts were determined through ascorbic acid-molybdate blue method (Lu, 1999). For heavy metals (Cu, Cr, Pb, Cd and Zn), sediment samples were digested with a solution of HNO_3-HF-$HClO_4$ and then measured using inductively coupled plasma optical emission spectrometry (ICP-OES, VISTA-MPX, Varian Co., Ltd., Australia). Organic matter (O. M) of sediment was measured via dichromate oxidation following Lu (1999) method. All parameters were performed in three replicates.

2.3. Molecular analyses

Three replicates total DNA of 0.5 g sediment samples was extracted using FastDNA® SPIN Kit for Soil (MP Biomedicals, LLC, USA) according to the manufacturer's instruction, then those DNA solutions were mixed. The *amoA* gene fragments of bacterial and archaeal were amplified with the primer pair *amoA*-1F and *amoA*-2R (Rotthauwe et al., 1997) and Arch-*amo*AF and Arch-*amo*AR (Francis et al., 2005), respectively. The primer sequences and PCR programs were performed as described previously (Liu et al., 2014a). Then, the purified gene fragments with PCR gel extraction kit (Sangon, China) were subsequently ligated into the pMD™ 19-T