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Depth-specific distribution and importance of nitrite-dependent anaerobic ammonium and methane-oxidising bacteria in an urban wetland

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ABSTRACT

Anaerobic ammonium oxidation (anammox) and nitrite-dependent anaerobic methane oxidation (n-damo) are two recently discovered processes in the nitrogen cycle that are catalysed by anammox bacteria and n-damo bacteria, respectively. Here, the depth-specific distribution and importance of anammox bacteria and n-damo bacteria were studied in an urban wetland, Xixi Wetland, Zhejiang Province (China). Anammox bacteria related to *Candidatus* Brocadia, *Candidatus* Kuenenia and *Candidatus* Anammoxoglobus, and n-damo bacteria related to "*Candidatus* Methylomirabilis oxyfera" were present in the collected soil samples. The abundance of anammox bacteria ($2.6–8.6 \times 10^6$ copies g^{-1} dry soil) in the shallow soils (0–10 cm and 20–30 cm) was higher than that ($2.5–9.8 \times 10^5$ copies g^{-1} dry soil) in the deep soils, whereas the abundance of n-damo bacteria ($0.6–1.3 \times 10^7$ copies g^{-1} dry soil) in the deep soils (50–60 cm and 90–100 cm) was higher than that ($3.4–4.5 \times 10^6$ copies g^{-1} dry soil) in the shallow soils. Anammox activity was detected at all depths, and higher potential rates (12.1–21.4 nmol N_2 g^{-1} dry soil d^{-1}) were observed at depths of 0–10 cm and 20–30 cm compared with the rates (3.5–8.7 nmol N_2 g^{-1} dry soil d^{-1}) measured at depths of 50–60 and 90–100 cm. In contrast, n-damo was mainly occurred at depths of 50–60 cm and 90–100 cm with potential rates of 0.7–5.0 nmol CO_2 g^{-1} dry soil d^{-1}. This study suggested the niche segregation of the anammox bacteria and n-damo bacteria in wetland soils, with anammox bacteria being active primarily in deep soils and n-damo bacteria being active primarily in shallow soils.

1. Introduction

Anaerobic ammonium oxidation (anammox) and nitrite-dependent anaerobic methane oxidation (n-damo) are two of the most recent discoveries in the microbial nitrogen cycle. Anammox and n-damo are microbially mediated processes, which anammox allows ammonium to be oxidised via nitrite (van de Graaf et al., 1995), whereas n-damo allows methane to be oxidised via nitrite (Raghoebarsing et al., 2006). The anammox process has been recognised as an important pathway for the production of dinitrogen gas (N_2) in the marine nitrogen cycle, which is estimated to be responsible for approximately 50% of the nitrogen loss in marine ecosystems (Arrigo, 2005; Brandes et al., 2007; Hu et al., 2011a). So far, there are five genera of anammox bacteria have been described, including *Candidatus* Brocadia (Strous et al., 1999; Kartal et al., 2008; Hu et al., 2010), *Candidatus* Kuenenia (Schmid et al., 2000), *Candidatus* Scalindua (Kuypers et al., 2003; Schmid et al., 2003; Woebken et al., 2008; van de Vossenberg et al., 2013), *Candidatus* Anammoxoglobus (Kartal et al., 2007) and *Candidatus* Jettenia (Quan et al., 2008; Hu et al., 2012). Until now, anammox bacteria have been found in various marine ecosystems (Schmid et al., 2007; Hu et al., 2012a; Shao et al., 2014), freshwater ecosystems (Hu et al., 2012b; Wenk et al., 2013; Ding et al., 2014) and terrestrial ecosystems (Humbert et al., 2010; Shen et al., 2013).

The n-damo process is reported to be catalysed by "*Candidatus* Methylomirabilis oxyfera" (*M. oxyfera*) (Ettwig et al., 2009). This process links carbon and nitrogen cycles, and may act as an important and overlooked sink of the greenhouse gas methane

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(Shen et al., 2012). Methane is 25-fold more effective at trapping heat than is carbon dioxide (CO_2) on a per-molecule basis and is responsible for 20% of global warming since the industrial age (Knittel and Boetius, 2009). The n-damo process can alleviate the greenhouse effect by converting methane to CO_2. So far, studies have reported the distribution and importance of n-damo bacteria in many environments, including freshwater lakes (Deutzmann and Schink, 2011; Kojima et al., 2012), rivers (Shen et al., 2014a), reservoirs (Kojima et al., 2014), wetlands (Wang et al., 2012a; Hu et al., 2014; Zhu et al., 2014; Shen et al., 2014b, 2014c) and marine ecosystems (Chen et al., 2014; Shen et al., 2014d).

The distribution and importance of anammox bacteria and n-damo bacteria have been recorded in various habitats as mentioned above, subsequently stimulating a need to define the ecological niches in which they are most likely to persist. Both the anammox bacteria and n-damo bacteria use the same electron acceptor (nitrite), thus they can potentially compete for nitrite in nature. So far, the co-existence of anammox bacteria and n-damo bacteria has been reported in several enrichment cultures (Luesken et al., 2011a; Haroon et al., 2013; Shi et al., 2013; Ding et al., 2014). It was observed that if ammonium was supplied in excess, n-damo bacteria were outcompeted from the enrichment culture, suggesting that anammox bacteria have a higher affinity for nitrite (Luesken et al., 2011a). The potential interaction between the anammox bacteria and n-damo bacteria in natural environments in currently poorly known though they are able to coexist in nature (Wang et al., 2012b; Shen et al., 2014c).

Wetlands are one of the most important nitrogen sinks, which can offset roughly 17% of anthropogenic nitrogen inputs to the environment (Jordan et al., 2011). In the meantime, wetlands represent one of the most important sources of greenhouse gas methane, which are responsible for 20–40% global methane emissions (Denman et al., 2007; Bastviken et al., 2011). Furthermore, wetland soils are saturated with water, which could create anoxic conditions in the soil. The anoxic conditions of the wetland soils theoretically provide suitable habitats for both anammox bacteria and n-damo bacteria. In addition, the wetland system exhibits a high dynamic of nitrogen cycling, and significant vertical gradient of the nutrients (like the ammonium and nitrate) exists in wetland soils. Therefore, the wetland system may serve as a model system for studying the ecology of anammox bacteria and n-damo bacteria. It would be interesting to study the vertical distribution patterns of anammox bacteria and n-damo bacteria in wetland soils to see if there any niche segregation for these two groups of bacteria.

To gain a better insight into the ecological niches of anammox bacteria and n-damo bacteria in wetland soils, the vertical distribution and importance of these two groups of bacteria have been investigated. For this purpose, an urban wetland, Xixi wetland was selected. The Xixi wetland is an urban wetland, which has high concentration of inorganic nitrogen and suffers frequent eutrophication due to the increased anthropogenic activities (e.g. agricultural run-off) in recent years. Further, significant vertical gradient of nitrogen concentration exists in this wetland. Activity tests and molecular analyses showed that the anammox bacteria mainly occurred in the shallow soils of the wetland, whereas the n-damo bacteria mainly occurred in the deep soils, suggesting the niche segregation of the two groups of bacteria.

2. Materials and methods

2.1. Site description and sample collection

The Xixi Wetland, located in Zhejiang Province, is a rare urban wetland with a total area of 11.5 km^2. It is the first and the only wetland in China that provides vital service for both urban life and farming (Cheng and Wu, 2006). This wetland receives abundant precipitation (annual rainfall of 1435 mm), has four distinct seasons and an annual average temperature of 16.2 °C. Three sampling sites (XXA, XXB and XXC) with average intervals of 1–2 km were selected in the Xixi Wetland. The three sampling sites are located in different regions of the wetland system with different soil hydrological conditions. The sampling site XXA is permanently submerged in water, while the site XXC is permanently no water covered on its surface soil. The site XXB is temporally submerged by water, which is water-covered when sampling. In each site, at least five soil cores were collected as replicates in September 2012 using a stainless steel ring sampler (5 cm in diameter and 100 cm in length). The soil cores were sliced at 10-cm intervals and mixed for each depth in the field to form one composite sample in each site. A total of ten composite samples were collected from each site because the sampling depth is 100 cm. Among the 10 composite samples, the samples collected from four representative depths (0–10 cm, 20–30 cm, 50–60 cm and 90–100 cm) in each site were selected to activity tests and molecular analyses. So a total of 12 soil samples from the three sampling sites were used for subsequent activity tests and molecular analyses. The soil samples were immediately placed in sterile containers, sealed, and transported to the laboratory on ice within 12 h. The collected soil samples were subsequently divided into three parts. The first part was incubated to determine the potential rates of anammox process and n-damo process immediately after arrival at the laboratory, the second part was stored without oxygen at 4 °C for subsequent physical and chemical property analyses, and the third part was stored at −80 °C for later molecular analyses.

2.2. Chemical analyses

The temperature and pH of the intact soil were measured by using an IQ150 pH meter (IQ Scientific Instruments Inc., Carlsbad, CA, USA). Soil ammonium, nitrite and nitrate were extracted using 2 M KCl, as previously described (Shen et al., 2013). The soil total nitrogen content was determined using the FOSS Kjeltec™2300 analyser (FOSS Group, Höganäs, Sweden), and the soil organic carbon content was determined using the $K_2Cr_2O_7$ oxidation method. The below-ground soil gas samples were gathered through soil gas samplers as previously described (Hu et al., 2014). The methane concentration in soil gas was measured as previously described (Hu et al., 2014).

2.3. Isotope tracer experiments

The potential anammox rate and n-damo rate in the examined wetland soils were determined using ^{15}N and ^{13}C stable isotope labelling experiments, respectively. Soil samples of known weight were transferred into the He-flushed, 75-ml glass vials, together with He-purged deionised water (50 ml). The soil slurries were pre-incubated under anoxic conditions for at least 30 h to remove the residual NO_x^- and to ensure completely anoxic conditions in the soil slurries. The slurries were subsequently split into five treatment groups: (a) $^{15}NH_4^+$ (^{15}N at 99.6%), (b) $^{15}NH_4^+ + NO_2^-$, (c) $^{15}NO_2^-$, (d) $^{13}CH_4$ (^{13}C at 99.9%) and (e) $^{13}CH_4 + NO_2^-$. Subsequently, He-purged stock solution was injected into each vial, resulting in a final concentration of 100 μM N in treatments (a), (b), (c) and (e). Two milliliters of headspace gas in each vial of treatments (d) and (e) was removed and replaced with an equal volume of $^{13}CH_4$, resulting in a final concentration of 10% methane (by volume) in the headspace. The treatment (a) was used to exclude the possibility of the production of ^{15}N labelled dinitrogen gas though nitrification and subsequent denitrification or anammox. The treatments (b)