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Rates of N_2 production and diversity and abundance of functional genes associated with denitrification and anaerobic ammonium oxidation in the sediment of the Amundsen Sea Polynya, Antarctica

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ABSTRACT

A combination of molecular microbiological analyses and metabolic rate measurements was conducted to elucidate the diversity and abundance of denitrifying and anaerobic ammonium oxidation (anammox) bacteria and the nitrogen gas (N_2) production rates in sediment underlying the highly productive polynya (Stns. 10 and 17) and the sea-ice zone on the outer shelf (Stn. 83) of the Amundsen Sea, Antarctica. Despite the high water column productivity, the N_2 production rates by denitrification (0.04–0.31 nmol N cm^{-3} sed. h^{-1}) and anammox (0.13–0.26 nmol N cm^{-3} sed. h^{-1}) were lower than those measured in other polar regions. In contrast, gene copy number (10^6–10^7 copies cm^{-3} of *nirS* and *nosZ* genes targeting denitirifiers and 10^5–10^7 copies cm^{-3} of 16S rRNA genes related to anammox bacteria) of the two bacterial groups at Stn. 17 was similar compared to those of other organic-rich environments. The majority of the *nirS* sequences were affiliated with *Gammaproteobacteria* (54% and 61% of the total *nirS* gene at Stns. 17 and 83, respectively), which were closely related to *Pseudomonas aeruginosa*. Most *nosZ* sequences (92% and 72% of the total *nosZ* genes at Stns. 17 and 83, respectively) were related to the *Alphaproteobacteria*, which were closely related to *Ruegeria pomeroyi* and *Roseobacter denitrificans*. Most (98%) of the sequences related to anammox bacteria were affiliated with *Candidatus* Scalindua at Stn. 17. Consequently, despite the low metabolic activity, the abundance and composition of most denitrifying and anammox bacteria detected from the ASP were similar to those reported from a variety of marine environments. Our results further imply that increased labile organic matter production resulting from a shift of the phytoplankton community from *Phaeocystis* to diatoms in response to rapid melting of sea ice stimulates metabolic activities of the denitrifying and anammox bacteria, thereby enhancing the N removal process in the ASP.

1. Introduction

Denitrification and anaerobic ammonium oxidation (anammox) are the two major nitrogen (N) removal processes, accounting for ~70% of fixed N loss in the marine N cycle (Codispoti, 2007; Thamdrup and Dalsgaard, 2008). Denitrification is a respiratory process by which organic carbon (OC), as well as reduced iron and sulfur species, are oxidized by facultative anaerobic bacteria using nitrate or nitrite as an electron acceptor (Thamdrup and Dalsgaard, 2008; Zumft, 1997). Generally, denitrification has been considered a key N removal mechanism in coastal marine sediments, including estuaries (Risgaard-Petersen et al., 2004; Trimmer et al., 2003), coastal bays (Rich et al., 2008), wetlands (Koop-Jakobsen and Gibilin, 2009), and mangroves (Fernandes et al., 2012; Meyer et al., 2005). Recently, anammox that oxidizes NH_4^+ to N gas (N_2) has been reported as an alternative N removal process in a variety of environments (Thamdrup, 2012), such as deep-sea sediment (Engström et al., 2009; Thamdrup and Dalsgaard, 2002), manganese rich sediment (Trimmer et al., 2013), oxygen minimum zones in the water column (Kuypers et al., 2003), Arctic sediments (Gihring et al., 2010; Rysgaard et al., 2004), and even in sea ice (Rysgaard and

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Glud, 2004). The relative contribution of denitrification and anammox to N_2 production in sediments is highly variable. Organic loading appears to be a major controlling factor, and the contribution of anammox thereby increases with increasing water depth and the associated attenuation of the detrital organic flux, ranging from $<10\%$ of total N_2 production in shallow coastal sediments to $\sim 50\%$ in the deep sea (Thamdrup, 2012; Trimmer and Engström, 2011). The highest anammox contribution observed in sediments is $\sim 80\%$, which was reported in a manganese-rich continental basin (Thamdrup, 2012; Trimmer et al., 2013).

Although the final product of denitrification and anammox is the same (i.e., N_2), the two processes are different in terms of their biochemical pathways and the microorganisms involved (Kartal et al., 2012; Strous et al., 2006; Zumft, 1997). Molecular microbiological studies revealed that bacterial groups related to facultative *Alpha*-, *Beta*-, and *Gammaproteobacteria* were associated with denitrification (Canfield et al., 2005; Thamdrup and Dalsgaard, 2008). To elucidate their diversities, various functional genes such as *nirS*, *nirK*, and *nosZ*, which encode nitrite and nitrous oxide reductase, have been studied in marine environments (Braker et al., 2000; Dong et al., 2009; Li et al., 2013a,b; Liu et al., 2003; Oakley et al., 2007; Smith et al., 2007; Wang et al., 2014a,b). Additionally, the diversity of anammox bacteria has been explored using 16S rRNA, *hzo* (hydrazine oxidoreductase), *hzs* (hydrazine synthase), and *Scalindua-nirS* genes as molecular markers (Li et al., 2010, 2013a,b; Wang et al., 2012). The known anammox bacteria belong to the phylum *Planctomycetes* and include five candidate genera: Brocadia, Kuenenia, Anammoxoglobus, Jettenia, and Scalindua (Jetten et al., 2009; Kartal et al., 2007, 2008; Kuenen, 2008; Schmid et al., 2003), with Scalindua typically dominating in marine settings (Borin et al., 2013; Kong et al., 2013; van de Vossenberg et al., 2008; Woebken et al., 2008). To better understand the biogeochemical N cycles and controls in marine environments, quantifying N_2 production rates and characterizing the microbial communities involved in the N removal processes are essential (Canfield et al., 2005; Thamdrup and Dalsgaard, 2008; Thamdrup, 2012).

The Amundsen Sea Polynya (ASP) is reported to be the most productive coastal polynya among the 37 coastal polynyas around Antarctica (Arrigo and van Dijken, 2003; Arrigo et al., 2012). The intrusion of relatively warm, salty, and nutrient-rich Circumpolar Deep Water (CDW) causes the rapid melting of the ice shelf that contains iron, thereby supporting phytoplankton blooms within coastal polynyas during the spring and summer (Alderkamp et al., 2012; Fragoso and Smith, 2012; Gerringa et al., 2012). Moreover, the high primary production in the water column of the ASP has the potential to profoundly affect the rates and pathways of organic matter mineralization in sediments (Hartnett et al., 2008; Thamdrup, 2012). Detrital organic matter in the sediment is processed by a variety of microbial groups using an array of different electron acceptors such as O_2, NO_3^-, MnO_2, FeOOH, and SO_4^{2-} (Canfield et al., 2005; Rysgaard et al., 1998), and the relative significance of each carbon oxidation pathway is largely determined by the availability of OC and electron acceptors (Canfield et al., 2005; Rysgaard et al., 1998).

Benthic denitrification is stimulated when both organic matter and NO_3^- are consistently supplied from the overlying water column (Rysgaard et al., 1998, 2004). Since the N cycle is tightly coupled to the carbon cycle (Thamdrup, 2012), characterizing the microbes related to denitrification and anammox, and elucidating their interactions with N removal processes, are particularly important to better understand benthic–pelagic coupling in the highly productive ASP. However, both denitrification and anammox rates together with the microbes responsible for the N_2 production are poorly understood in Antarctic sediments (Hartnett et al., 2008), and no information is available, especially in the ASP. The major objectives of this study were to estimate the rates and relative importance of denitrification and anammox, and to elucidate the diversities and compostions of both denitrifying and anammox bacterial communities in two contrasting sediments in the highly productive polynya and in the sea-ice zone in the outer shelf of the Amundsen Sea.

2. Materials and methods

2.1. Study area and sediment sampling

Sampling was conducted from February 18 to March 7, 2012, onboard the Korean icebreaker research vessel R/V *Araon* at the center of the polynya (Stns. 10 and 17) and in the sea-ice zone on the outer shelf (Stn. 83) of the Amundsen Sea (Table 1 and Fig. 1). Ice coverage on the surface water column was presented by others (e.g., Hahm et al., 2014; Kim et al., 2014). The average chlorophyll *a* concentration was 2 times higher in the polynya than in the sea-ice zone (Table 1). Sediments were collected using a box-corer (Marine Tech Korea, Changwon, Korea). Duplicate or triplicate subsamples for geochemical analyses, N_2 production rate measurements, and microbiological analyses were taken using acrylic cores (9 cm diameter × 35 cm length). The cores were immediately sealed with butyl rubber stoppers and stored in a cold room (<4 °C) until processing within 6 h.

2.2. Geochemical analysis

For determination of dissolved inorganic N (DIN), pore water was extracted in the cold room using soil moisture samplers (Rhizon SMS, Rhizosphere Research Products, Wageningen, The Netherlands) inserted through predrilled holes at 1-cm intervals from the top of the core to a depth of 12 cm that had been sealed with rubber tape before the sampling. Pore water was filtered through 0.2-μm cellulose ester syringe filters (Advantec Toyo Rashi Kaisha, Ltd., Tokyo, Japan) and stored at -20 °C. Concentrations of NO_X ($NO_2^- + NO_3^-$) were analyzed using a nutrient autoanalyzer (Proxima, Alliance Instruments, Frepillon, France). NH_4^+ concentrations were measured by flow injection analysis with conductivity detection (Hall and Aller, 1992).

To analyze particulate OC (POC) in the sediment, the sediment core was sectioned into 1-cm intervals from the top to a depth of 10 cm, and a 2-cm interval between 10 and 12 cm depth. The

Table 1
Characteristics of the sampling stations.

Location	Station	Latitude	Longitude	Water depth (m)	Temp. (°C)	Salinity (psu)	Chl-a^a (μg L^{-1})
Polynya	10	73.250°S	114.998°W	825	−1.1	34.0	3.60
	17	73.496°S	114.008°W	730	−1.2	33.5	5.16
Outer shelf	83	71.699°S	114.037°W	530	−1.8	33.6	2.55

[a] The concentrations within the surface mixed layer from Hyun et al. (2015).

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