

Denitrification and anammox activity in Arctic marine sediments

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Abstract

We measured rates of N_2 production through anaerobic NH_4^+ oxidation with NO_2^- (anammox) and denitrification in permanently cold (from -1.7°C to 4°C) sediments off the east and west coasts of Greenland. The investigated sites (36- to 100-m water depth) covered sediments in which carbon contents ranged from 0.3 to 3.2 dry weight %, O_2 uptake rates ranged from 3.4 to 8.3 $\text{mmol m}^{-2} \text{d}^{-1}$, O_2 penetration depths ranged from 0.25 to 1.70 cm, and bottom-water NO_3^- concentrations ranged from 0.3 to 15.3 $\mu\text{mol L}^{-1}$. Total N_2 production was 34–344 $\mu\text{mol N m}^{-2} \text{d}^{-1}$, of which anammox accounted for 1–92 $\mu\text{mol N m}^{-2} \text{d}^{-1}$ (1–35% of total) and denitrification for 33–265 $\mu\text{mol N m}^{-2} \text{d}^{-1}$. At one of the high-Arctic sites, anammox activity had an optimum temperature (T_{opt}) of 12°C , while that of bacterial denitrification was 24°C . According to the classical temperature scheme for metabolic growth, the anammox response was psychrophilic, while denitrification was psychrotrophic. Although T_{opt} was considerably higher than in situ temperatures, rates of denitrification and anammox were still high at -1.3°C , reaching 17% and 40%, respectively, of those found at T_{opt} . The activation energies, E_a , of anammox and denitrification were 51.0 and 60.6 kJ mol^{-1} , respectively, and the corresponding Q_{10} values were 2.2 and 2.4. Rates of anammox were linearly correlated with bottom-water NO_3^- concentrations ($r^2 = 0.96$, $p < 0.0001$, $n = 11$) at the investigated sites. We suggest that the slow-growing anammox bacteria are favored in sediments with high and stable NO_3^- conditions. This may be a general pattern in deeper waters at other latitudes as well.

Anaerobic ammonium oxidation with nitrite (the anammox process) was recently shown to contribute 24% and 67% of total N_2 production in two temperate continental shelf sediment locations, whereas it was detectable but insignificant relative to denitrification in a eutrophic coastal sediment (Thamdrup and Dalsgaard 2002). The process has also been observed in the water column of anoxic basins, where it can account for 19–35% of total N_2 formation (Dalsgaard et al. 2003; Kuypers et al. 2003). Although limited knowledge exists on this new pathway from natural systems, experiments with temperate shelf sediment have shown that the temperature optimum of anaerobic NH_4^+ oxidation is lower than that of denitrification (Dalsgaard and Thamdrup 2002). This observation led to speculations that anammox would be favored in colder settings, and recently, Rysgaard

and Glud (2004) found that anammox accounted for up to 19% of total N_2 production in the bottom layers of an ice floe in the Greenland Sea. Anammox was undetectable in annual sea ice from the same area, so it was speculated that the higher rates of anammox found in multiyear sea ice were due to the more stable environment compared with annual sea ice. Generally, it appears that stable environments favor slow-growing anammox bacteria (Strous et al. 1998; Jetten et al. 2001).

Despite highly fluctuating seasonal air temperatures in the Arctic, the bottom-water temperature in most coastal areas remains close to 0°C throughout the year. Furthermore, bottom-water nutrient and oxygen conditions are stable, and bacterial mineralization, including denitrification, remains constant throughout the year. An exception is the short, productive summer period, when mineralization is stimulated because of the rapid mineralization of the easily degradable fraction of settling material following the spring bloom (Rysgaard et al. 1998; Berg et al. 2003). Thus, the relatively stable temperature, O_2 , NH_4^+ , and NO_3^- conditions found in these Arctic sediments suggest that anammox is an important process in polar regions.

In this study, we present measurements of denitrification and anammox in various sediments from East and West Greenland, along with organic C and N contents, bottom-water NO_3^- concentrations, O_2 , and NO_3^- sediment–water exchange rates and concentration profiles of O_2 , NO_3^- , NO_2^- , and NH_4^+ . Furthermore, we investigate the temperature regulation of N_2 production.

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Table 1. Site characteristics. Temperature, salinity, and NO_3^- concentrations refer to bottom water. Sediment type, mean porosity, organic carbon content, and C:N ratio were determined for the upper 5 cm of the sediment at the stations investigated.

Station	Date of measurement	Position N	Position W	Water depth (m)	Temp. ($^{\circ}\text{C}$)	Salinity	NO_3^- ($\mu\text{mol L}^{-1}$)	Porosity (v/v)	Org. C (% dry weight)	C:N (mol/mol)
Disko_A	19 Apr 2001	69°17.2'	53°54.2'	50	-0.5	32.8	7.8	0.52	0.67	7.7
Disko_B	19 Apr 2001	69°16.6'	53°56.1'	100	0.5	33.2	15.3	0.60	1.3	10.1
Disko_C	30 Apr 2003	69°15.2'	53°34.5'	50	-1.5	33.2	10.4	0.53	0.28	7.1
Sc_A	11 Sep 2002	70°28.6'	21°57.9'	40	4.0	32.3	2.4	0.56	0.44	17.3
Ella_A	12 Sep 2002	72°52'	25°07'	40	1.7	31.8	0.3	0.54	3.22	—
Dnb_Aa	14 Sep 2002	74°18.6'	20°15.0'	36	-1.3	32.9	2.5	0.67	0.75	9.6
Dnb_Ab	15 Aug 2001	74°18.6'	20°15.0'	36	-1.3	32.9	3.2	0.65	1.1	10.3
Dnb_C	15 Aug 2001	74°18.6'	20°16.9'	85	-1.7	33.0	4.8	0.67	1.1	10.4
Hay_A	17 Sep 2002	75°41.8'	19°31.3'	40	-1.3	32.6	1.4	0.58	0.33	7.9
Dmh_A	18 Sep 2002	76°45.7'	18°40.4'	40	-1.7	32.3	2.0	0.59	—	—
Ile_A	18 Sep 2002	77°37.0'	07°38.6'	40	0.7	31.7	3.4	0.70	0.26	12.3

Materials and methods

Study sites—Rates of denitrification and anammox activity and a number of supporting parameters were measured in 11 coastal sediments in East and West Greenland (Table 1). Data were obtained during 2001–2003. Conditions on the East Greenland coast are dominated by the East Greenland Current, which transports cold low-salinity surface polar water and large amounts of sea ice southward from the Arctic Ocean (Aagaard and Carmack 1989). Nitrate concentrations are usually $<4 \mu\text{mol L}^{-1}$ in the surface layers of the East Greenland shelf water during the summer, but they increase steadily with depth to $13 \mu\text{mol L}^{-1}$ at 250 m (Kattner and Budéus 1997). Conditions in West Greenland around Disko Bay are influenced by both East Greenland polar water and North Atlantic water, which turn westward south of the sill between Davis Strait and the Baffin Bay. During the summer, there is a strong vertical gradient in temperature and salinity as well as horizontal gradients within Disko Bay due to melting of winter ice, runoff from land, and melting of glacier ice from the great glacier near Jakobshavn (Buch 2000). Likewise, during the summer, NO_3^- may be depleted in surface waters due to phytoplankton uptake, but it increases with depth to a steady concentration of 12–15 $\mu\text{mol L}^{-1}$ at 100–300 m (Nielsen and Hansen 1999).

Sampling—Sampling in East Greenland was performed from the Danish military vessel *Vædderen*, and samples from West Greenland were collected from the RV *Porsild*. Bottom water was sampled with a 10-liter Niskin water sampler. Sediment was sampled by a “box-corer” or a modified Kajak sampler. Only undisturbed cores with clear overlying water were used. All cores were transferred to a temperature-controlled incubator, where further processing took place. Subcores for solute exchange, denitrification, and anammox measurements were collected in Plexiglas tubes with a diameter of 5.3 cm. A small Teflon-coated magnet (0.5×3 cm) was placed 5 cm above the sediment surface in each core, which was then submerged in a tank with in situ bottom water and kept in darkness at in situ temperature and air saturation. The Teflon-coated magnets were rotated (60

rpm) to ensure thorough mixing of the overlying water column during measurements (Rasmussen and Jørgensen 1992).

Sediment characteristics—Density and water content (measured as weight loss after drying at 105°C for 24 h) were used to calculate porosity at a depth resolution of 1 cm in two sediment cores from each station. Depth profiles of organic carbon and nitrogen were determined on the same cores after treatment with HCl, freeze-drying, homogenizing, and weighing into sample boats. Analyses were performed on a C/N elemental analyzer (RoboPrep-C/N, Europa Scientific).

Oxygen conditions—At each station, 6–10 vertical O_2 concentration profiles were measured at a resolution of 200–300 μm using a Clark-type O_2 micro-electrode (Revsbech 1989). The microsensors had a measuring tip diameter of 10–20 μm and a low stirring sensitivity of <1 –2% (Glud et al. 2000). The sensors were positioned by a motor-driven micromanipulator, and the sensor current was measured with a pico-ammeter connected to an A/D converter, which transferred the signal to a computer (Revsbech and Jørgensen 1986). Measurements were performed in two selected cores, which were kept in darkness at in situ temperature while the overlying water column of 2 cm was aerated by a flow of atmospheric air to ensure sufficient stirring and fully air-saturated water.

Sediment-water fluxes—Exchange rates of O_2 and NO_3^- between water column and sediment were measured on five intact cores from each station. The sediment height was adjusted to provide a sediment and water column of ~ 12 and ~ 20 cm, respectively. As in the preincubation period, the water column was continuously stirred during the flux experiments. Two blank cores containing only bottom water were incubated along with the sediment cores to correct for water-column activity. In all instances, water-column activity was $<1\%$ of benthic activity.

Flux measurements were initiated by sealing the cores with rubber stoppers, and incubations were performed as described by Risgaard-Petersen and Rysgaard (1995). Samples

were collected from the tank immediately after the cores were sealed and from individual cores after an incubation period of 0.5–2.4 d. During incubation, O₂ concentrations in the water column did not decrease by >20%. O₂ concentrations in the water samples were measured by Winkler titration within 12 h of sampling. Concentrations of NO₃⁻ + NO₂⁻ were determined according to the scheme of Braman and Hendrix (1989). Concentrations of NO₂⁻ were measured by a standard colorimetric technique (Grasshoff et al. 1983).

Denitrification and anammox rates from slurry incubations—At each station, the upper 4 cm from each of three sediment cores was transferred to a gastight container together with Whatman GF/C-filtered bottom water (in a 1:1 ratio), homogenized, and made anoxic by flushing with helium. Then, 1 ml of the anoxic sediment solution was added to a series of gastight vials (12.6-ml Exetainers, Labco), together with 11.6 ml of helium-flushed GFC-filtered bottom water. Three treatments were performed: (1) addition of 50 μmol L⁻¹ ¹⁵NO₃⁻ (99.6 atom %), (2) addition of 50 μmol L⁻¹ ¹⁵NH₄⁺ (99.6 atom %), and (3) addition of 50 μmol L⁻¹ ¹⁵NH₄⁺ (99.6 atom %) and 50 μmol L⁻¹ ¹⁴NO₃⁻ (0.367 atom %). Oxygen microsensor measurements confirmed that the solutions in the vials remained anoxic after addition of the helium-flushed isotopes. The vials were then capped and incubated in the dark at in situ temperature. At different time intervals (after 0.1–7 d of incubation), the incubation was stopped by introducing a 4-ml helium headspace through a septum and injecting 200 μl of a ZnCl₂ solution (50% [w/v]) to preserve samples until later analysis of the ¹⁵N abundance of N₂. The 4-ml sample withdrawn while introducing the 4-ml helium headspace was frozen (–18°C) until later isotopic analysis of NO₃⁻. The abundance and concentrations of ¹⁴N¹⁵N and ¹⁵N¹⁵N were determined using a gas chromatograph coupled to a triple-collector isotopic ratio mass spectrometer (RoboPrep-G⁺ in line with TracerMass, Europa Scientific) as described by Risgaard-Petersen and Rysgaard (1995). The ¹⁵N isotopic distribution in the NO₃⁻ pool was likewise analyzed by isotope ratio mass spectrometry after reduction of NO₃⁻ to N₂ using a denitrifying bacterial culture (Risgaard-Petersen et al. 1993). N₂ production through denitrification and N₂ production by anammox were calculated from the measured isotopes in N₂ (¹⁴N¹⁵N and ¹⁵N¹⁵N) and the ¹⁵N labeling of the NO₃⁻ pool according to Thamdrup and Dalsgaard (2002).

$$\text{Denitrification} = p^{30}\text{N}_2 \cdot F_{\text{N}}^{-2} \quad (1)$$

$$\text{Anammox} = F_{\text{N}}^{-1} \cdot [p^{29}\text{N}_2 + 2 \cdot (1 - F_{\text{N}}^{-1}) \cdot p^{30}\text{N}_2] \quad (2)$$

where $p^{29}\text{N}_2$ is the production of ¹⁴N¹⁵N, $p^{30}\text{N}_2$ is the production of ¹⁵N¹⁵N, and F_{N} is the fraction of ¹⁵N in NO₃⁻.

Denitrification and anammox rates from intact core incubations—At each station, the rate of denitrification was also determined using the isotope pairing technique (Nielsen 1992; Risgaard-Petersen and Rysgaard 1995) and the calculation procedure of Risgaard-Petersen et al. (2003). In short, ¹⁵NO₃⁻ was added to the overlying water (50 μmol L⁻¹ final concentration) to initiate incubation, and cores were closed with rubber stoppers, leaving no headspace. In all,

five sediment cores were incubated, and the sediment cores were processed at different time intervals during the 0.5- to 2.4-d incubation period. After incubation, subsamples of the water column and sediment were collected for analysis of ¹⁵N labeling of N₂ and NO₃⁻. The samples for ¹⁵N abundance in NO₃⁻ were frozen (–18°C) until further analysis, and samples for ¹⁵N-N₂ analysis were preserved in glass vials (Exetainer, Labco) containing 2% (volume) of a ZnCl₂ solution (50% [w/v]).

¹⁵N abundance in and concentrations of N₂ and NO₃⁻ were analyzed as described above. According to Risgaard-Petersen et al. (2003), the N₂ production (p_{14}) in intact sediment cores can be estimated from

$$p_{14} = 2r_{14}[p^{29}\text{N}_2 + p^{30}\text{N}_2(1 - r_{14})] \quad (3)$$

where r_{14} is the ratio between ¹⁴NO₃⁻ and ¹⁵NO₃⁻ in the NO₃⁻ reduction zone, and $p^{29}\text{N}_2$ and $p^{30}\text{N}_2$ are the productions of the isotopes ¹⁴N¹⁵N and ¹⁵N¹⁵N, respectively. Furthermore, the ratio between ¹⁴NO₃⁻ and ¹⁵NO₃⁻ in the NO₃⁻ reduction zone (r_{14}) can be estimated from

$$r_{14} = \frac{(1 - ra) \cdot R^{29} - ra}{(2 - ra)} \quad (4)$$

where R^{29} is the ratio between ¹⁴N¹⁵N and ¹⁵N¹⁵N production, and ra is the contribution of anammox to N₂ production. The contribution of anammox to N₂ production (ra) was found from the slurry incubations described in the previous paragraph. Nitrification rates were calculated by adding denitrification rates based on NO₃⁻ from nitrification to the flux of NO₃⁻ out of the sediment, as no assimilation of NO₃⁻ by benthic microalgae occurred in these sediments (Rysgaard et al. 1993). This is a minimum estimate of nitrification, as it does not include bacterial assimilation.

Temperature dependence—Homogenized sediment from the upper 4 cm of the seafloor of Young Sound (40-m water depth) was used to investigate the temperature dependence of the denitrification and anammox processes. A series of gastight vials (12.6 ml, Exetainers) containing 1 ml of sediment slurry and 11.6 ml of helium-flushed artificial seawater were placed in a 185-cm aluminum temperature-gradient block with three parallel sets of holes drilled along its length, allowing several incubations to be conducted simultaneously. The block was cooled at one end and heated at the other to maintain a stable temperature range from –1.8°C to 33.5°C. The temperature gradient in the block was checked at regular intervals and remained constant within ±0.5°C. Two treatments were performed on the sediment slurries: (1) addition of 50 μmol L⁻¹ ¹⁵NO₃⁻ (99.6 atom %), and (2) addition of 50 μmol L⁻¹ ¹⁵NH₄⁺ (99.6 atom %) and 50 μmol L⁻¹ ¹⁴NO₃⁻ (0.367 atom %). Anoxic conditions were maintained in all vials as measured with O₂ micro-electrodes. Samples were placed in the temperature-gradient block, and incubation was stopped after 0, 11, 24.5, and 51.5 h by introducing a 4-ml helium headspace through a septum and injecting 200 μl of a ZnCl₂ solution (50% [w/v]) to preserve samples until later analysis. The 4-ml sample withdrawn while introducing the 4-ml helium headspace was frozen (–18°C) until later concentration and isotope analysis. Samples were analyzed as described above.

Effects of temperature on bacterial denitrification and anammox activity were modeled using the integrated form of the Arrhenius equation. The activation energy (E_a ; in joules per mole) was estimated from the slope of an Arrhenius plot of $\ln(k)$ as a function of T^{-1} .

$$\ln(k) = \ln(A) + \left(\frac{-E_a}{R} \cdot \frac{1}{T} \right) \quad (5)$$

where k is the reaction rate, A is the Arrhenius constant, R is the gas constant ($8.31 \text{ J K}^{-1} \text{ mol}^{-1}$), and T is the absolute temperature (K).

Q_{10} is a factor by which the reaction rate increases at a temperature increase of 10°C and was calculated as

$$Q_{10} = \exp \left[\frac{E_a \cdot 10}{RT(T + 10)} \right] \quad (6)$$

Vertical concentration profiles of NO_3^- and NO_2^- —Pore-water extractions for measurement of vertical concentration profiles of NO_3^- and NO_2^- were performed on three sediment cores from Young Sound (Dnb_Ab) with a pneumatic squeezer (modified from Reeburgh 1967). The cores were sectioned into 3- to 10-mm slices, and pore-water was extracted through GF/F glass-fiber filters using a pressure of 3–5 bar. Pore-water samples were immediately frozen (-18°C) for later analysis. Concentrations of $\text{NO}_3^- + \text{NO}_2^-$ were analyzed as described above.

Results

Site characteristics for the individual stations are shown in Table 1. During sampling, bottom-water temperatures ranged from -1.7°C to 4°C , and salinities ranged from 31.7 to 33.2, while bottom-water NO_3^- concentrations ranged from 0.3 to $15.3 \mu\text{mol L}^{-1}$. Sediment porosity at the investigated sites ranged from 0.5 to 0.7, and organic C content ranged from 0.3% to 3.2% of dry weight taken as the mean value of the upper 5 cm. Molar C:N ratios ranged from 7 to 17 at the different sites.

Oxygen concentration profiles and exchange rates— O_2 penetration depths ranged from 0.25 to 1.70 cm at the investigated sites, and little variation was observed between the 6–10 profiles measured at each site (Fig. 1). Oxygen consumption as a function of sediment depth was calculated using the numerical procedure for interpretation of O_2 concentration profiles described by Berg et al. (1998). The diffusive O_2 uptake calculated from the mean profiles ranged from $2.8 \text{ mmol m}^{-2} \text{ d}^{-1}$ at Dnb_C to $6.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ at Disko_A (Table 2). At stations Dnb_Aa, Dnb_Ab, Dnb_C, Hey_A, and Ile_A, the highest O_2 consumption was observed near the sediment–water interface due to recent inputs of fresh organic material. At stations Disko_A, Disko_B, Sc_A, and Ella_A, the highest O_2 consumption was observed deeper in the oxic zone, presumably due to the reoxidation of reduced products being transported upward to the oxic zone from deeper sediment layers.

Sediment O_2 uptake determined from intact-core incuba-

tions ranged from 3.4 to $8.3 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Table 2). The ratio between total O_2 uptake and diffusive O_2 uptake ranged from 1 to 2.6 at the investigated sites. Nitrate efflux from all stations, ranging from 0.008 to $0.241 \text{ mmol m}^{-2} \text{ d}^{-1}$, indicated active nitrification in all sediments (Table 3).

Denitrification and anammox rates—The volume-specific denitrification activity, as determined from the NO_3^- -amended anoxic slurry incubations of the upper 4 cm of the sediment, ranged from 23 to $1,211 \text{ nmol N cm}^{-3} \text{ d}^{-1}$, while anammox activity ranged from 4 to $363 \text{ nmol N cm}^{-3} \text{ d}^{-1}$ (Table 3). Thus, anammox contributed 1–35% of total N_2 production. Anammox activity was also evident in the anoxic slurry incubations, because (1) addition of $^{15}\text{NH}_4^+$ caused production of $^{29}\text{N}_2$ in the sediments with high anammox activity and high NO_3^- concentrations, and (2) incubations with $^{15}\text{NH}_4^+$ and $^{14}\text{NO}_3^-$ resulted in an elevated $^{29}\text{N}_2$ production in sediments with anammox activity (data not shown). Subjecting the $^{29}\text{N}_2$ and $^{30}\text{N}_2$ production rates measured in the intact-core experiment to the calculation procedure of Risgaard-Petersen et al. (2003) and applying the relative contribution of anammox to total N_2 production (ra) observed in the slurry experiment resulted in a total production of N_2 of 34 – $344 \mu\text{mol N m}^{-2} \text{ d}^{-1}$, of which denitrification accounted for 33 – $265 \mu\text{mol N m}^{-2} \text{ d}^{-1}$ and anammox for 1 – $92 \mu\text{mol N m}^{-2} \text{ d}^{-1}$ (Fig. 2). Nitrification rates ranged from 30 to $460 \mu\text{mol N m}^{-2} \text{ d}^{-1}$ (Fig. 2).

Temperature dependence—In the permanently cold sediment of Young Sound (annual temperature, $<1^\circ\text{C}$) (Rysgaard et al. 1998), the temperature responses of anammox and denitrification clearly differed (Fig. 3). An optimum temperature of 12°C was observed for anammox activity, while denitrification exhibited maximum activity at 24°C (Fig. 3A). The contribution of anammox to total N_2 production was highest at temperatures of ca. 5°C , where it accounted for 25% of N_2 production. However, the relative importance decreased above and below this optimum temperature, declining to a few percent at 30°C (Fig. 3B). A linear increase in NO_2^- concentration was observed in all incubations and at all temperatures. At -0.7°C , the concentration increased from 0.6 to $0.9 \mu\text{mol L}^{-1}$; at 14.7°C , the concentration increased from 1.0 to $2.4 \mu\text{mol L}^{-1}$; and at 31.2°C , the concentration increased from 1.1 to $2.6 \mu\text{mol L}^{-1}$ during the 51.5-h incubation period (data not shown). This indicates that the NO_2^- concentration did not limit anammox activity during the temperature-block experiment.

The activation energies, E_a , of anammox and denitrification were obtained by plotting the logarithmic rate of the respective processes against the reciprocal of the absolute temperature. The Arrhenius plot of anammox showed a linear relationship ($r^2 = 0.85$, $p = 0.004$) from -1.8°C to 13°C and resulted in an E_a value of 51.0 kJ mol^{-1} , while Q_{10} was 2.2 in the range of 0 – 10°C (Table 4). The Arrhenius plot of denitrification activity showed a linear relationship ($r^2 = 0.90$, $p << 0.001$) from -1.8°C to 25°C and resulted in an E_a value of 60.6 kJ mol^{-1} , while Q_{10} was 2.4 in the range of 0 – 10°C (Table 4).

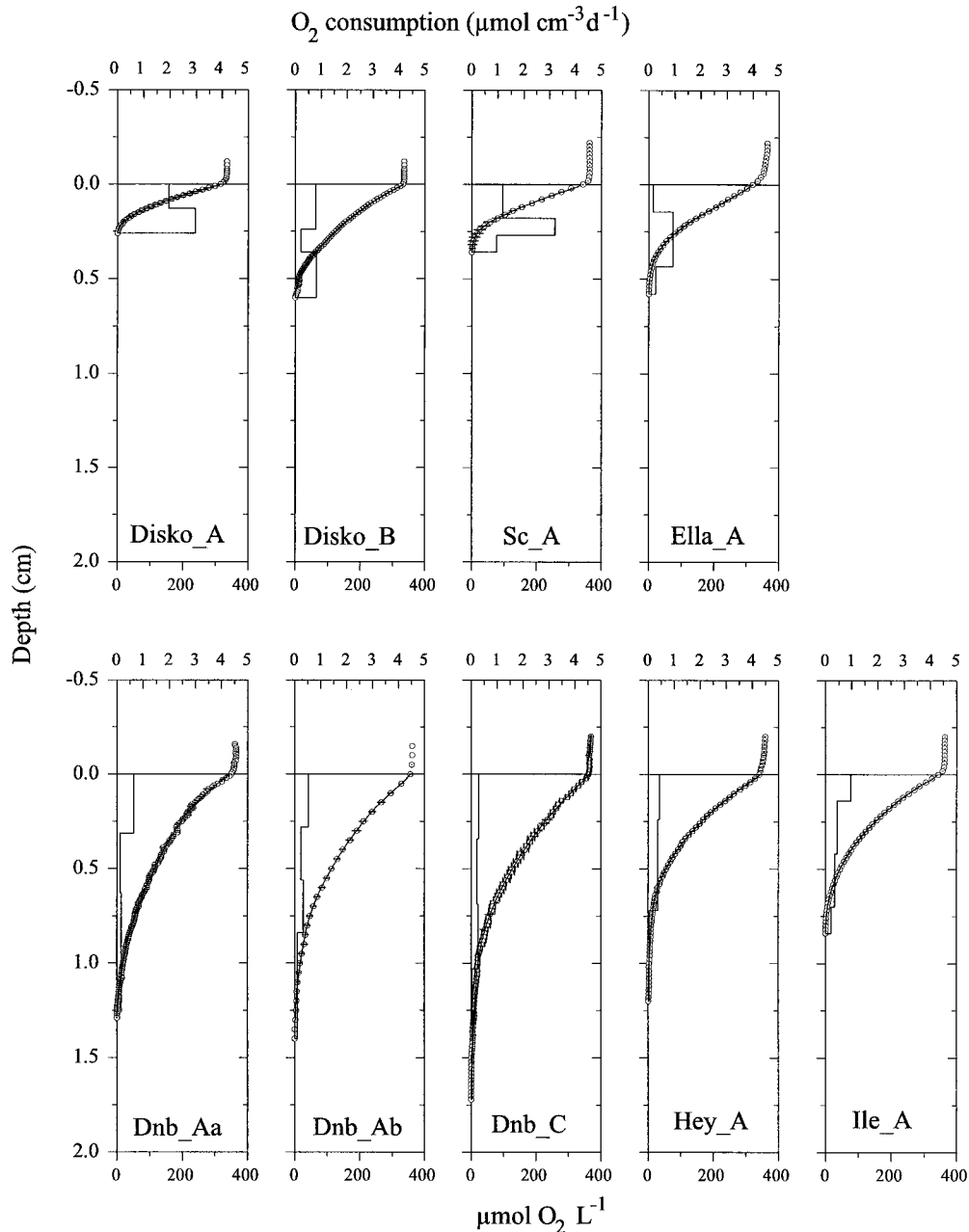


Fig. 1. Vertical O_2 concentration profiles (symbols) and modeled consumption rates (bars) in sediment from the investigated stations. Error bars (horizontal lines) represent $2 \times$ SE of the mean ($n = 6$).

Pore-water concentration profiles of NO_3^- and NO_2^- —Active nitrification activity in the Young Sound sediment was responsible for the concentration peak of NO_3^- in the upper ~ 1 cm where both O_2 and NH_4^+ were present. Below this zone, the NO_3^- concentration decreased due to anaerobic consumption (Fig. 4). A NO_2^- concentration peak of $\sim 2 \mu\text{mol L}^{-1}$ was detected at a depth of 3–4 cm in the sediment.

Correlation analysis—Pearson correlation analysis of area-based anammox rates and the variables measured in the present study showed a highly significant correlation ($r^2 =$

0.96 , $p < 0.0001$) between area-based anammox activity and bottom-water NO_3^- concentration (Table 5; Fig. 5). Using anammox rates from slurry incubations in the correlation analysis proved less significant than area-based anammox rates, because volume-specific rates are dependent on the sediment depth interval used in the slurry incubation in relation to the thickness of the active anammox zone (data not shown). Furthermore, area-based anammox rates correlated with water depth and salinity. However, multiple regression with backward elimination, using water depth, salinity, and NO_3^- concentration as independent variables, showed that

Table 2. Total O₂ uptake measured in flux incubations, diffusive O₂ uptake derived from microelectrode measurements, and nutrient fluxes across the sediment–water interface. Diffusive O₂ uptake is based on the mean concentration profile of 6–10 individual profiles.

Station	Total O ₂ uptake (mmol m ⁻² d ⁻¹)	Diffusive O ₂ uptake (mmol m ⁻² d ⁻¹)	Total O ₂ /diffusive O ₂ uptake	NO ₃ ⁻ efflux (mmol N m ⁻² d ⁻¹)
Disko_A	7.39 ± 0.91	6.38	1.2	0.008 ± 0.0054
Disko_B	4.91 ± 0.79	4.03	1.2	0.023 ± 0.016
Disko_C	6.07 ± 1.51	—	—	0.161 ± 0.096
Sc_A	7.86 ± 1.04	5.86	1.3	0.060 ± 0.014
Ella_A	7.93 ± 1.38	3.06	2.6	0.009 ± 0.022
Dnb_Aa	8.27 ± 2.98	3.50	2.4	0.241 ± 0.080
Dnb_Ab	6.50 ± 1.03	3.61	1.8	0.211 ± 0.072
Dnb_C	3.43 ± 0.58	2.84	1.2	0.140 ± 0.056
Hay_A	6.59 ± 0.62	3.04	2.2	0.073 ± 0.006
Dmh_A	—	—	—	—
Ile_A	3.51 ± 0.75	3.53	1.0	0.052 ± 0.022

only the correlation with bottom-water NO₃⁻ concentration was significant ($p < 0.0001$), as the bottom-water NO₃⁻ concentration is correlated with both salinity ($p = 0.02$) and water depth ($p = 0.008$). Previous data on anammox from a temperate continental shelf sediment (5.5°C, 10.4 μmol L⁻¹ NO₃⁻) fit the general trend of the Arctic locations studied here (Fig. 5). The latter anammox rate was obtained from raw data on $p^{29}\text{N}_2$ and $p^{30}\text{N}_2$ from intact sediment core incubations (Rysgaard et al. 2001) using a contribution of anammox to N₂ production (ra) measured in anoxic slurry incubations (Thamdrup and Dalsgaard 2002) and calculating N₂ production (p_{14}) according to the procedures given by Risgaard-Petersen et al. (2003).

Discussion

Denitrification and anammox—Total N₂ production rates of 34–344 μmol N m⁻² d⁻¹ were observed in the Greenland continental shelf sediments, of which anammox accounted for 1–35% (Fig. 2; Table 3). The present study supports other recent findings that anammox may be responsible for a significant fraction of N₂ production in marine sediments (Thamdrup and Dalsgaard 2002). Furthermore, our data add to the small database on the anammox process, confirming

that it is widespread in nature—occurring in wastewater treatment plants (Mulder et al. 1995), in anoxic water columns (Dalsgaard et al. 2003; Kuypers et al. 2003), in temperate shelf sediments (Thamdrup and Dalsgaard 2002), in multiyear Arctic sea ice (Rysgaard and Glud 2004), and in Arctic shelf sediments (present study).

Correlation analysis of anammox rates and other variables measured in the present study showed high correlation ($r^2 = 0.96$, $p < 0.0001$) between area-based anammox activity and bottom-water NO₃⁻ concentrations (Fig. 5; Table 5). Furthermore, data on anammox from a temperate continental shelf sediment (5.5°C, 10.4 μmol L⁻¹ NO₃⁻) follow the same trend as data from the Arctic locations studied here (Rysgaard et al. 2001; Thamdrup and Dalsgaard 2002). Little is known about the regulation of the anammox process (NH₄⁺ + NO₂⁻ → N₂ + 2H₂O) in marine sediments. However, the K_s value of NH₄⁺ oxidation by anammox bacteria has been reported to be <5 μmol L⁻¹ (Strous et al. 1999). Therefore, pore-water concentrations of NH₄⁺ from the Young Sound site do not indicate that anammox was limited by NH₄⁺ supply, as NH₄⁺ was present in the NO₃⁻-reducing zone in concentrations of 10–20 μmol L⁻¹ (Fig. 4). However, the pore-

Table 3. Specific rates of denitrification and anammox in the upper 4 cm of the sediment as determined from slurry incubations.

Station	Denitrification (nmol N cm ⁻³ d ⁻¹)	Anammox (nmol N cm ⁻³ d ⁻¹)	Anammox in % of total N ₂ production
Disko_A	1,211.0	362.6	23.0
Disko_B	520.8	189.7	26.7
Disko_C	23.2 ± 1.8	5.3 ± 0.3	18.5
Sc_A	516.2 ± 56.1	6.7 ± 0.9	1.3
Ella_A	209.5 ± 84.8	3.6 ± 2.3	1.7
Dnb_Aa	384.1 ± 34.8	6.9 ± 0.8	1.8
Dnb_Ab	58.4 ± 0.6	12.3 ± 0.6	17.4
Dnb_C	24.8 ± 1.2	13.3 ± 0.7	34.9
Hay_A	213.8 ± 54.7	57.0 ± 5.0	21.1
Dmh_A	337.4 ± 45.0	4.9 ± 2.6	1.4
Ile_A	133.3 ± 8.6	15.2 ± 0.5	10.2

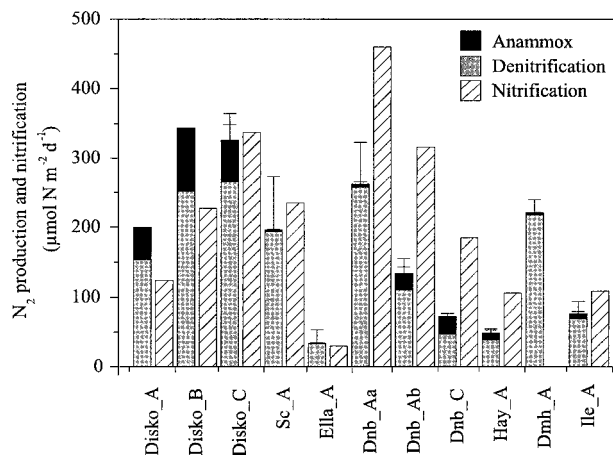


Fig. 2. Rates of denitrification, nitrification, and anammox in West and East Greenland sediments determined from intact-core incubations. Error bars represent SE of the mean ($n = 5$).

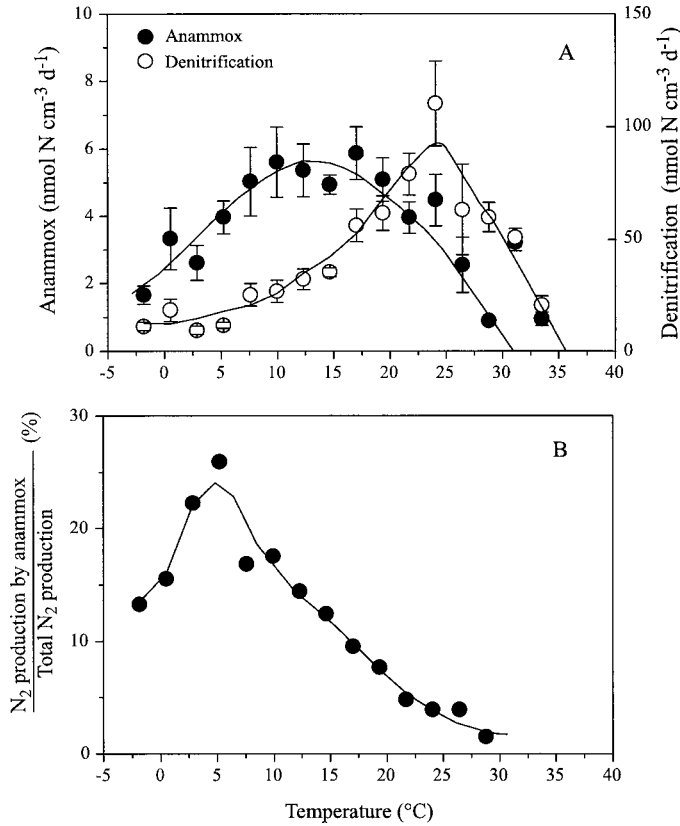


Fig. 3. (A) Rates of N_2 production by anammox and by denitrification as a function of temperature. (B) N_2 production by anammox relative to total N_2 production in the upper 4 cm of sediment from Young Sound. Error bars represent $2 \times \text{SE}$ of the mean ($n = 4$).

water concentration of NO_2^- in the anoxic zone of the Young Sound sediment was only $1\text{--}2 \mu\text{mol L}^{-1}$ and, as the K_s value of the anammox process is $<3 \mu\text{mol L}^{-1} \text{NO}_2^-$ (Dalsgaard and Thamdrup 2002), NO_2^- may have been the limiting factor at this site. Although the pore-water NO_3^- and NO_2^- concentrations observed in our study may be overestimated (Fig. 4), the observation that NO_2^- builds up just below the NO_3^- concentration peak supports recent microsensor measurements in biofilms (de Beer 2000) and freshwater sediments (Stief et al. 2002), indicating that net NO_2^- production takes place mainly in the anoxic zone of the sediment as a result of NO_3^- reduction. Furthermore, Dalsgaard and Thamdrup (2002) observed that increased addition of NO_2^- to anoxic sediment slurries resulted in NO_2^- production from NO_3^- that was four times the NO_2^- consumption rate.

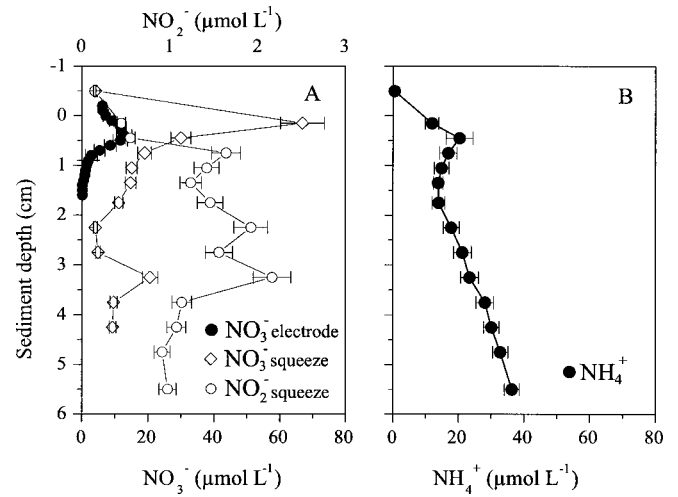


Fig. 4. (A) Pore-water concentrations of NO_3^- and NO_2^- , measured by pore-water squeezing, and $\text{NO}_3^- + \text{NO}_2^-$, measured by microsensor (Rysgaard et al. 1998), in the sediment of Young Sound. (B) Pore-water concentration of NH_4^+ in sediments of Young Sound. Error bars represent $2 \times \text{SE}$ of the mean ($n = 3$). The concentrations of NO_3^- and NO_2^- are most likely overestimated due to the pneumatic squeezing procedure. It has been shown that, during squeezing, internal bacterial and/or phytoplankton NO_3^- pools can be liberated, leading to artificially elevated pore-water concentrations (Lomstein et al. 1990; Fossing et al. 1995). Earlier measurements with nitrate microsensors performed at the same site showed an NO_3^- concentration peak of only $12.7 \pm 2.3 \mu\text{mol L}^{-1}$ (Rysgaard et al. 1998).

Thus, bottom-water NO_3^- concentrations may indirectly control anammox activity through NO_3^- reduction. If the NO_2^- used in the anammox reaction was produced by denitrifying bacteria, the two processes should be correlated. Apart from three sites (Sc.A, Dnb.Aa, and Dmh.A) (Table 3) where anammox rates were very low, a significant correlation was found ($r^2 = 0.85$, $p = 0.001$) between area-based anammox and denitrification activities. The lack of a significant correlation between denitrification and anammox at these three sites may have resulted from low populations of anammox bacteria or different generation times for anammox versus denitrifying bacteria. Denitrifying bacteria may adapt to varying NO_3^- concentrations very quickly due to their rapid doubling time (hours), whereas the slow-growing anammox bacteria, which display doubling times of 11 days under laboratory conditions (Strous et al. 1998), require more stable NO_3^- conditions to maintain an active population. Thus, the reason that a positive correlation was observed between anammox and bottom-water NO_3^- concentrations may be the

Table 4. Optimum temperatures, apparent activation energies, E_a and Q_{10} for the anammox and denitrification processes in sediments of Young Sound, NE Greenland.

Process	T_{opt} ($^{\circ}\text{C}$)	Relative metabolic rate at -1.3°C versus T_{opt} (%)	E_a (kJ mol^{-1})	Q_{10} (0– 10°C)	T range used for calculation of E_a ($^{\circ}\text{C}$)
Anammox	12	40	51.0	2.2	–2 to 13
Denitrification	24	17	60.6	2.4	–2 to 25

Table 5. Pearson correlation coefficients for area-based rates of anammox in Arctic sediments. Temperature, salinity, and NO_3^- concentrations refer to bottom-water conditions. Organic C is given as % dry weight in the upper 5 cm of sediment. TOU is the total sediment O_2 uptake, and DOU is the diffusive sediment O_2 uptake. NO_3^- flux refers to sediment–water exchange rates, and nitrification and denitrification are area-based rates. p -values marked with (*) are statistically significant ($p < 0.05$).

Variable	r	p	n
Water depth	0.73	0.0095*	11
Temperature	0.02	0.95	11
Salinity	0.69	0.017*	11
NO_3^- concentration	0.98	<0.0001*	11
Organic C	-0.08	0.829	10
TOU	0.31	0.38	10
DOU	0.22	0.57	9
NO_3^- flux	-0.16	0.67	10
Denitrification	0.45	0.16	11
Nitrification	0.16	0.66	10

relatively stable NO_3^- conditions in the bottom water of these Arctic sites (Kattner and Budéus 1997; Nielsen and Hansen 1999). Oxygen conditions in these shelf sediments are likewise relatively stable throughout the year, as the mineralization of fresh organic matter is restricted to the short productive summer thaw, while the mineralization of slowly degrading organic matter ensures a stable supply of NH_4^+ for anammox bacteria during the rest of the year (Rysgaard et al. 1998; Berg et al. 2003). The stable O_2 conditions and supply of NH_4^+ would also favor a stable nitrifying population and a steady additional supply of NO_3^- . At present, we need more data in the form of high-resolution NO_2^- and NO_3^- concentration profiles in sediments where anammox occurs to further evaluate the regulation of the process. However, we expect that anammox is favored in relatively deep waters where bottom-water concentrations of NO_3^- are relatively high and stable throughout the year. This may be true not only for Arctic waters, but may also be a general pattern in sediments from other latitudes. Furthermore, denitrification may be limited by the availability of organic matter in sediments underlying deeper waters, which may also increase the relative importance of anammox to total N_2 production (Thamdrup and Dalsgaard 2002).

Anammox and isotope pairing—The isotope pairing technique (Nielsen 1992) has been widely used to estimate N_2 production through denitrification. As discussed by Risgaard-Petersen et al. (2003), the occurrence of anammox is a challenge to this technique, because it leads to violation of the assumption that ^{14}N - N_2 production is independent of added $^{15}\text{NO}_3^-$ and the assumption that the distribution of produced $^{28}\text{N}_2$, $^{29}\text{N}_2$, and $^{30}\text{N}_2$ is binomial. Bacteria with anammox capacity will produce only two of the three possible isotopic N_2 species, $^{28}\text{N}_2$ and $^{29}\text{N}_2$, following addition of $^{15}\text{NO}_3^-$. In systems in which denitrification and anammox coexist, the resultant pool of N_2 produced after $^{15}\text{NO}_3^-$ addition will thus be a mixture of pools with different isotopic compositions and distributions of produced $^{28}\text{N}_2$, $^{29}\text{N}_2$, and $^{30}\text{N}_2$ that cannot be calculated by the classical isotope pairing

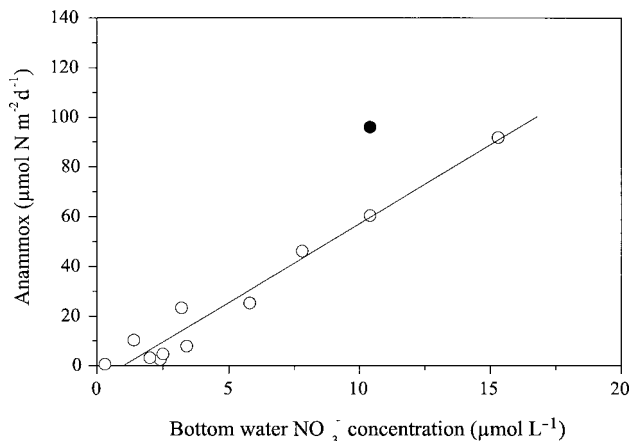


Fig. 5. Rates of N_2 production by anammox as a function of bottom-water NO_3^- concentration. The black dot represents earlier measurements from a temperate continental shelf sediment (Skagerrak S9, see Risgaard-Petersen et al. 2003).

technique. Risgaard-Petersen et al. (2003) showed that the presence of anammox causes an overestimation of N_2 production calculated by the isotope pairing technique. We estimated the error imposed by anammox on the calculation of ^{14}N - N_2 production according to the classical isotope pairing technique for the Arctic sediments studied here where anammox accounted for 1–35% of N_2 production. Compared with the revised estimate used in our study, the overestimation of ^{14}N - N_2 production estimated by the isotope pairing technique amounted to 1–160% (Fig. 6). According to a paired t -test based on individual cores, the apparent overestimation of N_2 production was, however, significant for Disko_A and Disko_C sites only, due to variability in activity. Nevertheless, we still recommend that measurements of anammox activity be included in future studies of nitrogen removal by denitrification activity, as in the present study, or at least that test incubations be made to verify the validity of the assumptions of the isotope pairing technique. Accord-

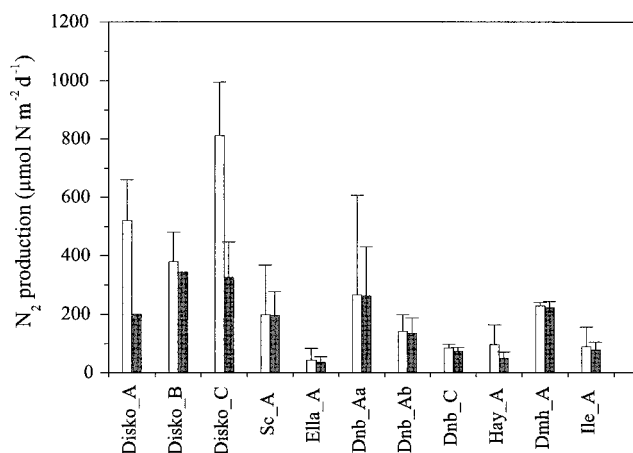


Fig. 6. Estimates of N_2 production calculated from isotope pairing (white columns) and from the procedure of Risgaard-Petersen et al. (2003) (gray columns). Error bars represent SE of the mean ($n = 5$).

ing to the equations developed by Risgaard-Petersen et al. (2003), the error introduced by anammox can be detected by amending cores with different concentrations of $^{15}\text{NO}_3^-$. A positive correlation between the estimated production of $^{14}\text{N-N}_2$ and the added concentration of $^{15}\text{NO}_3^-$ indicates problems with the method. In the same experiment, the true $^{14}\text{N-N}_2$ production calculated by the new equations should be independent of the $^{14}\text{N-N}_2$ production and the concentration of $^{15}\text{NO}_3^-$. Finally, note that quantifying the anammox rate in slurry incubations and using the modified isotope pairing equations show a detailed picture of the N cycle with only minor additional experimental effort.

Temperature dependence—The observed temperature optimum of anammox activity (12°C) (Fig. 3; Table 4) is much lower than that found in wastewater treatment systems (37°C) (Strous et al. 1999; Kuenen and Jetten 2001) but compares with the 15°C optimum found in temperate shelf sediments by Dalsgaard and Thamdrup (2002). The temperature optimum of 12°C found in this study is also comparable with the optimum of psychrophilic sulfate-reducing bacteria from other Arctic (Knoblauch and Jørgensen 1999) and Antarctic (Isaksen and Jørgensen 1996) sediments, while the optimum temperature observed for denitrification (24°C) corresponds to the optimum temperature of oxygen respiration measured in Svalbard sediments (Thamdrup and Fleisher 1998). However, the optimum temperature for denitrification found in the present study is significantly lower than values for bacterial mineralization in temperate sediments, where optimum temperatures of $30\text{--}40^\circ\text{C}$ and rates close to zero at 0°C are typical (Thamdrup et al. 1998).

The temperature optima of activities measured in this study are still much higher than in situ temperatures. This difference between in situ and optimum temperature has also been reported for pure cultures of sulfate-reducing bacteria isolated from other polar environments (Knoblauch and Jørgensen 1999). The observation that both anammox and denitrification activity have optimum temperatures higher than in situ temperatures does not imply that the processes are not adapted to in situ temperature conditions. The important issue is the activity actually achieved at in situ temperatures in relation to the activity found at the optimum temperature of the process. The activities of denitrification and anammox were still high at -1.3°C , reaching 17% and 40%, respectively, of those observed at the optimum temperature (Table 4), which indicates the existence of an efficient and well-adapted community of nitrate/nitrite reducers at these constantly low temperatures.

The activation energy, E_a , of the anammox process (51.0 kJ mol^{-1}) is similar to those measured in wastewater reactors (Strous et al. 1999) and in temperate sediments (Dalsgaard and Thamdrup 2002). The E_a of denitrification (60.6 kJ mol^{-1}) found in Arctic sediments is within the range reported for nitrate-reducing communities in salt marsh sediment (King and Nedwell 1984). Furthermore, the Q_{10} values of sediment anammox and denitrification (2.2 and 2.4, respectively) are close to the value of 2 used in early diagenetic models (Soetaert et al. 1996). At present, we still lack knowledge as to why optimum temperatures and activation

energies of anammox are very similar in the few studies available from temperate and Arctic sediments.

Comparison with other Arctic regions—The anammox rates observed in the present study are the first reported from polar regions, which prevents us from comparing our rates with those from other Arctic regions. Likewise, only few measurements of nitrification and denitrification activity have been made in the Arctic region. The rates of nitrification and denitrification found in the present study are comparable with those reported from Alaskan coastal waters (Henriksen et al. 1993). Furthermore, our denitrification rates are comparable to rates from the Bering Sea (Koike and Hattori 1979) and Barents Sea (Glud et al. 1998) but are in the lower range of those reported from coastal areas of the Chukchi Sea (Devol et al. 1997). Earlier compilations of sediment mineralization data by Jørgensen (1983) show that the major part of the total sediment mineralization of organic matter takes place on the continental shelf. As mineralization of organic matter yields the products NH_4^+ and NO_3^- , these shelf regions are expected to be important sites for denitrification and anammox. The continental shelves make up as much as 52.7% of the total area of the Arctic Ocean and account for $\sim 20\%$ of the total shelf area (0–200 m) of the ocean (Menard and Smith 1966; Jakobsson et al. 2004). Thus, in future compilations of the global nitrogen cycle, it is important to extend measurements of denitrification and anammox activities to encompass the Arctic shelf regions, which are still poorly investigated.

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