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Temperature response of denitrification and anammox reveals the adaptation of microbial communities to in situ temperatures in permeable marine sediments that span 50° in latitude

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Received: 22 August 2013 – Accepted: 23 August 2013 – Published: 3 September 2013

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Published by Copernicus Publications on behalf of the European Geosciences Union.

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Abstract

Despite decades of research on the physiology and biochemistry of nitrate/nitrite-respiring microorganisms, little is known regarding their metabolic response to temperature, especially under *in situ* conditions. The temperature regulation of microbial communities that mediate anammox and denitrification was investigated in near shore permeable sediments at polar, temperate, and subtropical sites with annual mean temperatures ranging from –5 to 23 °C. Total N₂ production rates were determined using the isotope pairing technique in intact core incubations under diffusive and simulated advection conditions and ranged from 2 to 359 μmol N m⁻² d⁻¹. For the majority of sites studied, N₂ removal was 2 to 7 times more rapid under advective flow conditions. Anammox comprised 6 to 14 % of total N₂ production at temperate and polar sites and was not detected at the subtropical site. Potential rates of denitrification and anammox were determined in anaerobic slurries in a temperature gradient block incubator across a temperature range of –1 to 42 °C. The highest optimum temperature (T_{opt}) for denitrification was 36 °C and was observed in subtropical sediments, while the lowest T_{opt} of 21 °C was observed at the polar site. Seasonal variation in the T_{opt} was observed at the temperate site with values of 26 and 34 °C in winter and summer, respectively. The T_{opt} values for anammox were 9 and 26 °C at the polar and temperate sites, respectively. The results demonstrate adaptation of denitrifying communities to *in situ* temperatures in permeable marine sediments across a wide range of temperatures, whereas marine anammox bacteria may be predominately psychrophilic to psychrotolerant. To our knowledge, we provide the first rates of denitrification and anammox from permeable sediments of a polar permanently cold ecosystem. The adaptation of microbial communities to *in situ* temperatures suggests that the relationship between temperature and rates of N removal is highly dependent on community structure.

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1 Introduction

Denitrification and anammox are the dominant processes for nitrogen loss in the ocean. These microbially-mediated reactions convert dissolved inorganic nitrogen to N₂ and are limited to suboxic and anoxic regions of the ocean, including oxygen minimum zones and surficial sediments (Thamdrup and Dalsgaard, 2008). A large portion of nitrogen removal occurs in coastal sediments, where high primary productivity and export production provide abundant organic matter and dissolved inorganic nitrogen that fuels denitrification and anammox. Current nitrogen budgets attribute up to 50 % of the global ocean nitrogen loss to near shore coastal and continental shelf sediments (Codispoti, 2007). Continental shelves are dominated (up to 70 %) by relatively coarse-grained, relict sediments (Emery, 1968); however, the contribution of these permeable continental shelf sediments to nitrogen removal remains poorly constrained (Galloway et al., 2004).

Recent evidence from laboratory and field experiments support the emerging paradigm that sandy permeable sediments serve as a substantial sink for land-derived nitrogen (Deek et al., 2012; Eyre et al., 2013; Deutsch et al., 2010). The transport of solutes and particles by pressure-gradient-driven pore water advection enhances mineralization processes in permeable sediments by delivering dissolved and particulate carbon and removing metabolic end products (Huettel and Rusch, 2000). When pore water advection has been simulated in intact core incubations, an enhancement of denitrification of 1 to 2 orders of magnitude has been observed (Gao et al., 2012; Gihring et al., 2010). Models that account for pore water advection predict higher rates of denitrification due to advective transport of nitrate into the sedimentary denitrification zone (Cardenas et al., 2008), but at the same time, rapid flushing of ammonium from the sediment may decrease coupled nitrification-denitrification (Cook et al., 2006).

To parameterize large-scale models of nitrogen removal from continental shelves, the response of denitrification and anammox to environmental controls must be quantified. Major environmental controls of denitrification and anammox activity have been

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extensively studied in fine-grained sediments and include, but are not limited to, the availability of organic matter, nitrate concentration in the overlying water, O₂ penetration depth, and the extent of bioturbation (Cornwell et al., 1999). Temperature is often considered a master variable because it exerts a modulating effect on multiple physical and biogeochemical processes. The effect of temperature on microbial communities mediating denitrification is limited to a few studies. In previous studies from saltmarsh sediments, seasonal variation in the temperature response of denitrification rates showed that communities with lower optimal temperatures for denitrification became active during winter months (Kaplan et al., 1977; King and Nedwell, 1984). Ogilvie et al. (1997) demonstrated that lower temperatures led to the enrichment of denitrifying isolates, whereas higher temperatures enriched for bacteria that performed dissimilatory nitrate reduction to ammonium, demonstrating that temperature may have an effect on the dominant nitrate reduction pathway. Denitrification and anammox in arctic fjord muds were shown to have optimal temperatures of 25 and 12 °C, respectively, indicating that the anammox bacteria were better adapted to cold conditions (Rysgaard et al., 2004). Together, these studies illustrate that the response of nitrogen removal pathways to the in situ temperature may be strongly dependent on the community structure.

Despite decades of research on the physiology and biochemistry of nitrate-respiring microorganisms, little is known regarding their metabolic response to temperature, especially under in situ conditions. In the present study, we examined the temperature adaptation of denitrifying and anammox communities in near shore permeable sediments spanning a 50° latitudinal range. Permeable sediments were chosen because they dominate continental shelf sediments and are important sites for nitrogen removal. We hypothesized that the temperature response of denitrification and anammox will vary according to the in situ temperature of the sediment. To this end, rate measurements employing the isotope pairing technique (¹⁵N tracer) and temperature gradient block incubations were performed with permeable sediments from subtropical (northeastern Gulf of Mexico), temperate (German Wadden Sea), and arctic (Svalbard, Norway) sites with annual mean temperatures of 23, 11 and –5 °C, respectively. The results

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demonstrate adaptation of denitrifying communities to in situ temperatures and suggest that marine anammox bacteria are psychrophilic to psychrotolerant.

2 Methods

2.1 Study Sites

- 5 Near shore, permeable sediments were sampled at a total of 4 sites from subtropical, temperate and polar temperature regimes (Fig. 1, Table 1). The two subtropical sites were located at St. George Island (SGI), Florida (29.7° N), which is a barrier island in the northeastern Gulf of Mexico. The annual mean water temperature at SGI is 23° C (N.D.B.C., Station SGOF1), and samples were taken in June 2010 from the subtidal
10 zone located approximately 50 m from shore at 1.5 m depth. The temperate site was located at Hausstrand at the island of Sylt (55.0° N) in the German Wadden Sea. The annual mean water temperature at Sylt in 2007 was 11.0° C and comparable to the long term mean (1984–2007) of 10.5° C (Hedtkamp, 2005). Samples were taken on intertidal flats in March (mean temperature 5.6° C) and June 2007 (mean temperature
15 17.9° C). Polar permeable sediments were sampled in August 2010 on an intertidal flat at Ymerbukta (78.3° N) on the island of Spitsbergen (Svalbard). This region has an annual mean temperature of -5° C (Forland and Hanssen-Bauer, 2003), and sediments remain frozen from fall to spring (Sawicka et al., 2010).

2.2 Denitrification and anammox rates determined using intact cores

- 20 At each site, 15 cm long sediment cores with 15 cm of overlying water were collected by hand using 3.5 cm diameter core liners. Cores were immediately transported to temperature-controlled rooms and stored at the in situ temperature until incubation experiments were initiated (less than 24 h). All incubations were maintained at the in situ temperature measured at the time of core collection. Denitrification and anammox rate determinations were performed using the ^{15}N isotope pairing technique (IPT, Nielsen,
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1992) with modification to account for anammox according to Risgaard-Petersen et al. (2003). For each site and sample date, IPT experiments were performed in the dark with and without pore-water perfusion as described in de Beer et al. (2005). Overlying water was removed from the cores and replaced with air-equilibrated site water

5 to which 50 µM Na¹⁵NO₃⁻ (99 atom %; Cambridge Isotope Laboratories, Andover, MA) was added.

One set of cores was immediately capped without headspace and submerged in water collected from the field site. A second set of cores was perfused with 20 mL of Na¹⁵NO₃⁻-amended site water by slowly pumping water with a syringe through a valve in the top stopper closing the core liners, while excess water was re-

10 leased through a valve in the bottom stopper of the core liners. The core diameter, sediment porosity, and volume of water perfused were used to calculate the depth of perfusion, which was approximately 5.0 cm for the subtropical and temperate sites and 7 cm for the polar site. Mixing of the water column in the cores was maintained by magnetic stir bars that were continuously rotated using an external magnet. Triplicate

15 cores were sacrificed at each time point by the addition of 1 mL of ZnCl₂ (50 % w/v) to the water in the core liners, resealing the core without headspace, and inverting to homogeneously mix sediment, ZnCl₂, and water. Water samples were then collected by a syringe while avoiding the introduction of air bubbles and transferred to 12 mL Exetainer® vials containing 250 µL of 50 % ZnCl₂ added as a preservative.

20 Linear regressions of excess ²⁹N₂ or ³⁰N₂ concentration (see analytical procedures) against time were used to calculate the rates of ²⁹N₂ and ³⁰N₂ production. All regression slopes were determined to be significantly greater than 0 (*t* test, *p* < 0.05), and the standard error of the regression slopes was used to calculate the error of denitrification and anammox rates. Volumetric calculations of denitrification and anammox

25 were adjusted to areal rates using the sediment height, core diameter, and sediment porosity. At sites where anammox was negligible, the denitrification rate, *D*₁₄, was calculated according to Nielsen (1992). Where anammox was detected, anaerobic slurry incubations were performed to determine the relative contribution of anammox to N₂ production (ra), and rates were calculated according to Risgaard-Petersen et al. (2003).

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The slurries were performed by adding 1 mL of homogenized wet sediment from the surface (0–5 cm) to a 12 mL Exetainer®, which was then filled without headspace with site water containing 100 µM Na¹⁵NO₃⁻. Vials were incubated for 24–48 h at the in situ temperature and sampled every 6 h in triplicate by addition of 250 µL of 50 % ZnCl₂.

5 The calculations of Thamdrup and Dalsgaard (2002) were used to determine the contribution of anammox.

2.3 Vertical profiles of denitrification and anammox

Vertical profiles of the potential rates of denitrification and anammox were determined in the Sylt sediments in June 2007. Cores were sectioned at 2 cm intervals, and sediment was slurried with bottom water in sealed gas-impermeable bags. The bottom

10 water was amended with 100 µM Na¹⁵NO₃⁻ (99 atom %), and the bags were flushed with helium to ensure anaerobic conditions. The slurries were incubated at the in situ temperature. Samples for the determination of ²⁹N₂ and ³⁰N₂ production were taken at 3, 6, 12, and 20 h and preserved in 6 mL Exetainers with ZnCl₂ as described above.

15 Potential denitrification and anammox were calculated according to the equations of Thamdrup and Dalsgaard (2002). Vertical profiles of pore-water nitrate, nitrite, and ammonium concentrations were measured at the time of core collection using Rhizon samplers (Rhizosphere Research Products) that were inserted into ports drilled at 1 cm intervals in polycarbonate core liners (9.5 cm diameter) (Seeberg-Elverfeldt et

20 al., 2005). Rhizons were rinsed with the first 0.5 mL of extracted pore-water, and 2 mL were collected for each 1 cm depth interval for a total depth of 10 cm.

2.4 Temperature gradient block experiments

The temperature response of potential denitrification and anammox rates was determined using temperature gradient block incubators (cf., Sagemann et al., 1998) located at either the Max Planck Institute for Marine Microbiology (Bremen, Germany) or Florida State University (Tallahassee, Florida). The two temperature blocks were identi-

cal in size: each consisted of a 2 m-long (cross section 15 × 15 cm) insulated aluminum block that was electrically heated at one end and cooled at the other by a circulating water bath. Each block had 4 parallel rows of 30 vertical wells (1.8 cm diameter, 15 cm length), with 3 rows for samples and one row for temperature monitoring.

- At the time of intact core collection, samples for temperature gradient experiments were collected from the upper 5 cm of the sediments into pre-sterilized plastic containers and stored at the in situ temperature. Potential rates of denitrification and anammox were determined in time series incubations of anaerobic slurries across a temperature range of –1.0 to 42 °C. Slurries were prepared by adding 1 mL of wet sediment to a 12 mL Exetainer® vial followed by filling of the vials with helium-degassed site water that was amended with Na¹⁵NO₃ to a final concentration of 100 µM. One set of samples was sacrificed at each time point by the addition of 250 µL of 50 % ZnCl₂ to each vial, followed by vigorous mixing. Four time points were sampled for each experiment over a period of 15–24 h. Parallel anaerobic slurries were incubated at the in situ temperature and sampled in triplicate at each time point to verify homogeneity between vials. The production of labeled N₂ was calculated as described for the core incubations by the regression of ²⁹N₂ and ³⁰N₂ against time, and the calculations of Thamdrup and Dalsgaard (2002) were used to partition ²⁹N₂ and ³⁰N₂ production into potential denitrification and anammox rates.
- An additional temperature block experiment was performed determine anammox activity at each site, whereby production of ²⁹N₂ and the absence of ³⁰N₂ after addition of 100 µM ¹⁵NH₄Cl and 100 µM Na¹⁴NO₂[–] tracer in anaerobic slurries indicated anammox activity. Nitrification can lead to artifacts when using ¹⁵NH₄⁺ tracer, but the absence of ³⁰N₂ production indicated that the slurries were sufficiently anaerobic to preclude nitrification. The rate of N₂ production by anammox was determined from the regression of ²⁹N₂ production against time, which was then corrected for the fraction of ¹⁵N-labeled NH₄⁺ according to Holtappels (2011).

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The optimum temperature (T_{opt}) was visually determined from plots of rates against temperature. Additionally, a range for T_{opt} was defined where rates did not fall below 90 % of the optimum rate. The slope of temperature response was modeled using the Arrhenius function. A regression through the linear range of the plot of inverse temperature against the natural log of denitrification (or anammox) rate provided an estimate of activation energy according to the equation:

$$\ln(k) = \ln(A) + \left(\frac{-E_a}{RT} \right) \quad (1)$$

where k is the reaction rate, and E_a is the activation energy (kJ mol^{-1}). In addition to the activation energy, the ratio of the potential rate at 5 °C to the rate at in situ temperature was calculated as an indicator of psychrophilic adaptation.

2.5 Analytical procedures

Dissolved nitrate + nitrite (NO_x^-) and nitrite (NO_2^-) were determined by chemiluminescence detection after reduction to NO gas on a Thermo model 42i NO_x analyzer (Thermo Scientific). Nitrate + nitrite were reduced with an acidic VnCl_2 solution (Braman and Hendrix, 1989), and nitrite was reduced using an acidic iodide solution (Garside, 1982). Dissolved ammonium (NH_4^+) was determined by the colorimetric method of Bower and Holm-Hansen (Bower and Holm-Hansen, 1980). Concentrations of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ in excess of natural abundance were measured by either isotope ratio mass spectrometry (IRMS) (Sylt) or membrane-inlet mass spectrometry (St. George and Ymerbukta). For IRMS, a gas sample from the helium-equilibrated (2 mL) headspace of each Exetainer was analyzed. The membrane inlet mass spectrometer was configured and calibrated according to An et al. (2001). An air-equilibrated standard of 0.2 μm filtered site water or seawater was maintained at in situ temperature in a thermostatic water bath and was analyzed every 20 min to correct for instrument drift. Oxygen was removed from the vacuum system inlet before entering the mass spectrometer using a heated (600 °C) quartz column with copper turnings.

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3 Results

Sediment characteristics for each site are shown in Table 1. Sediments were comprised of fine-to-medium grained sands, except at the polar site, which contained a mix of sand and very fine gravel (Table 1). The median grain size was 211–223 µm at the subtropical sites and 338 and 824 µm at the temperate and polar sites, respectively. Permeabilities ranged from 1.0×10^{-10} to $4.8 \times 10^{-11} \text{ m}^2$ and were sufficiently high to permit advective pore-water exchange (Huettel and Rusch, 2000). The organic carbon contents were < 0.1 % at the subtropical and temperate sites, and C : N ratios for these sites were between 7.1–8.4. The polar site had an organic carbon content of 0.49 % and a C : N ratio of 22.6.

3.1 Rates and pathways of N₂ production

The isotope pairing technique, modified according to Risgaard-Petersen et al. (2003), was used to estimate the rate of unlabeled N₂ production, p14, in intact 3.5 cm diameter cores. Areal rates of p14 varied between $1.9\text{--}359 \mu\text{mol m}^{-2} \text{ d}^{-1}$ (Table 2). Simulated pore-water advection by perfusion led to substantial increases in N₂ production rates at all sites. An approximately 2-fold increase in p14 was observed upon perfusion of the subtropical sediments. Perfusion led to a larger increase of p14 in the winter temperate and polar (5-fold) and summer temperate (7-fold) sediments. The highest denitrification rate ($359 \mu\text{mol m}^{-2} \text{ d}^{-1}$) was observed with sediment perfusion at the temperate site. For all sites, denitrification was the dominant pathway for N₂ production. At Sylt, the contribution of anammox to N₂ production was 14 % in winter and 6 % in summer, and anammox was responsible for 6.5 % of N₂ production at Ymerbukta. The contribution of anammox was below the detection limit in sediments from St. George Island.

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3.2 Vertical profiles of denitrification and anammox

Pore-water DIN profiles and potential rates of denitrification and anammox at Sylt (June) revealed a vertical structure of N-removal mechanisms within the sediment (Fig. 2). Pore-water DIN profiles showed elevated ammonium and nitrate concentrations below 4 cm. Concentrations were constant from 4–10 cm, and were approximately 12 µM for both nitrate and ammonium. Potential denitrification rates exhibited a maximum of $120 \mu\text{mol L}^{-1} \text{ d}^{-1}$ between 2–6 cm, whereas, anammox gradually increased with depth to a maximum rate of $20 \mu\text{mol L}^{-1} \text{ d}^{-1}$ at 8–10 cm. The anammox trend with depth was confirmed in parallel incubations with $^{15}\text{NH}_4^+$ tracer additions (data not shown).

3.3 Temperature response of denitrification and anammox

Potential rates of denitrification and anammox were determined between –1 and 42 °C in a temperature gradient block incubator. The optimum temperature for denitrification (T_{opt}) varied with the in situ temperature, with a T_{opt} of 21 °C observed at the polar site and 35–36 °C observed at the subtropical sites, respectively (Fig. 3). The temperate site exhibited a seasonal pattern in the T_{opt} of denitrification, with a T_{opt} of 26 °C observed in the winter and 34 °C observed in the summer. Both the temperate and polar site had broad T_{opt} peaks with widths of 7 to 9 °C (Table 3), whereas the subtropical site had well-defined, narrow peaks (2 °C or less).

At the polar site, a relatively high rate of denitrification was observed at 5 °C, corresponding to 34 % of T_{opt} ; in contrast, rates at 5 °C comprised only 1 % of the T_{opt} rate at the subtropical site (Table 3). The ratio of rates at 5 °C relative to the T_{opt} at the temperate site was similar during both summer and winter field samplings (11–17 %). Activation energies (E_a) calculated from Arrhenius plots also indicated latitudinal variation in the temperature response of denitrification (Table 3). The E_a values at the temperate and polar sites were similar at $52\text{--}65 \text{ kJ mol}^{-1}$. In contrast, the activation energies for denitrification at the subtropical site ranged from 102.7 to $123.5 \text{ kJ mol}^{-1}$,

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corresponding to a 1.5- to 2-fold higher activation energy than at the temperate and polar sites. Normalization of the temperature response curves to the denitrification rate at T_{opt} revealed two trends: T_{opt} increased with increasing environmental temperature, and temperate and polar sediments had higher relative rates at low temperatures as compared to subtropical sediments (Fig. 5).

The temperature response of anammox was only determined for Sylt and Ymerbukta, as anammox could not be detected at the subtropical SGI sites. Rates from the temperature gradient block experiments using $^{15}\text{NH}_4^+$ tracer additions are reported here rather than rates determined using $^{15}\text{NO}_3^-$ tracer. Because denitrification rates were at least an order of magnitude higher than anammox rates in temperature block experiments, underestimation of the denitrification rate (estimated by production of $^{30}\text{N}_2$) could produce a false anammox signal in experiments with $^{15}\text{NO}_3^-$ tracer. The use of $^{15}\text{NH}_4^+$ tracer is generally accepted as providing more conclusive evidence of anammox activity (Holtappels et al., 2011), and thus, these experiments were chosen as the best approach for estimating the anammox temperature response. Temperature optima of 9 and 26 °C were observed for anammox at the polar and temperate sites, respectively (Fig. 4). For both sites, anammox rates at 5 °C comprised 35 % of the T_{opt} rate. The activation energy for anammox was 35 kJ mol⁻¹ at the temperate site (Table 3), but activation energy could not be accurately calculated from the temperature response curve at the polar site. Normalization of the temperature response curves to the anammox rate at T_{opt} clearly shows a higher T_{opt} at the temperate site versus the polar site, but similar relative rates at lower temperatures are observed (Fig. 5).

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4 Discussion

4.1 Nitrate removal rates in sands under high nitrate loading

Intact core incubations from the temperate site, Sylt, showed the highest rates of N_2 production of all sites studied. The Sylt-Romo basin receives nitrate from the Elbe and Weser Rivers, with nitrate concentrations in the List tidal basin ranging from up to 60 μM in winter to less than 1 μM in summer (van Beusekom et al., 2008). A recent study of sediment nitrogen dynamics in the same area measured N_2 effluxes under diffusive conditions ranging from 144 to 1128 $\mu mol\ N\ m^{-2}\ d^{-1}$ in February and April but did not detect N_2 production in summer when water column nitrate concentrations were low (Deek et al., 2012). In the present study, intact core incubations showed higher rates of N_2 production in March relative to June under both diffusive and simulated pore-water advective conditions. These results indicate that seasonal variation in nitrogen loading is likely the dominant driver of the seasonal variability in N removal. The production of N_2 in winter months at Sylt is likely sustained by the advective transport of nitrate-rich water into the denitrification zone, whereas in summer, nitrate must be supplied from within the sediment. In June, pore-water profiles showed that 10–12 μM nitrate was present in the upper 10 cm of sediment, despite being nearly absent in the overlying water. This nitrate is likely supplied via nitrification, enhanced by bioturbation and bioirrigation of macrofauna (Huettel, 1990), as well as groundwater sources that have been shown to locally contribute inorganic nitrogen to the pore-water at Sylt (Hedtkamp, 2005).

The rates from the temperate site at Sylt (74 – $359\ \mu mol\ N\ m^{-2}\ d^{-1}$) add to a limited number of observations of N_2 production in advection-dominated permeable sediments under high nitrogen loading. Rates from sands in the nitrogen-impacted Baltic Sea were found to be lower (12 – $108\ \mu mol\ N\ m^{-2}\ d^{-1}$) but were measured under diffusive conditions (Deutsch et al., 2010). At least two studies have observed higher rates of N loss in nutrient-impacted permeable sediments under advective conditions. The average N_2 production rate under simulated pore-water advection in southern German Wadden

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Sea tidal flats was $8.3 \text{ mmol N m}^{-2} \text{ d}^{-1}$ (Gao et al., 2012), nearly 20-fold higher than rates observed at Sylt in the northern Wadden Sea. These higher rates in the southern Wadden Sea may be due to higher organic matter loading as compared to the less eutrophic Wadden Sea around Sylt (Van Beusekom et al., 2012). Likewise, rates of N loss were found to be high ($0.8\text{--}2.2 \text{ mmol N m}^{-2} \text{ d}^{-1}$) in continuous advection chambers in tropical carbonate sediment and increased to $11.5 \text{ mmol N m}^{-2} \text{ d}^{-1}$ in response to the deposition of coral spawning material (Eyre et al., 2008). We observed that N_2 removal rates that were 4 to 7 times more rapid under simulated advective flow in comparison to diffusive transport conditions for Wadden Sea sediments. In general, these studies show high but variable N_2 removal rates in sands with high nitrogen loading, reflecting the dynamic nature of biogeochemical cycling in permeable sediments.

4.2 Temperature adaptation of denitrification

The adaptation of denitrifying microbial communities to in situ temperatures was inferred from multiple parameters of the temperature response curves, including the temperature optima (T_{opt}), activation energies (E_a), and the ratio of denitrification at 5°C to T_{opt} , all of which varied directly with in situ temperatures. It is important to note that the T_{opt} of growth rates are used to define psychrophiles and mesophiles according to the classification scheme of Morita (1975), but T_{opt} for respiration rates were measured in the present study. Temperature gradient block incubations were carried out over short (<24 h) timescales, and T_{opt} values most likely reflect the optimal respiratory enzyme activity of the in situ microbial community, which may deviate from the T_{opt} for growth.

The T_{opt} for denitrification fell into two ranges: a T_{opt} of approximately $20\text{--}25^\circ\text{C}$ for polar and cold temperate sediments (Sylt winter) and a T_{opt} of approximately 35°C for subtropical and warm temperate sediments (Sylt summer). All T_{opt} values were greater than the in situ sediment temperature, although the subtropical sites had the smallest difference between T_{opt} and the in situ temperature, suggesting that these communities may be closer to their maximum efficiency at in situ temperatures than are the polar

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and cold temperate communities. However, many purified psychrophilic enzymes have optimal catalytic rates between 15–30 °C, and the primary adaptation in psychrophilic enzymes is the maintenance of relatively high specific catalytic rates at low temperature (Feller and Gerdau, 2003). The T_{opt} values for permeable sediments examined in this study are consistent with previous results from cold Arctic muds, where T_{opt} values of 20–28 °C have been observed for aerobic respiration, denitrification, and sulfate reduction (Arnold et al., 1998; Rysgaard et al., 2004). The community at the polar site (where T_{opt} was lowest at 21 °C) may still be psychrophilic based on T_{opt} values for growth, as denitrifying isolates obtained from this site had optimal growth rates between 5–15 °C (Canion et al., 2013). Similar results from pure cultures of psychrophilic sulfate-reducing bacteria corroborate our observations: values of T_{opt} for growth were found to be up to 10 °C lower than those for sulfate respiration (Isaksen and Jorgensen, 1996). Results indicate that the properties of respiratory enzymes may be important in determining the T_{opt} for growth of mesophiles, whereas metabolic pathways other than respiration limit the T_{opt} for growth in psychrophiles.

Activation energy (E_a) can also be used as an indicator of adaptation to low temperatures in conjunction with the T_{opt} for denitrification. The E_a values for the denitrification temperature response integrate multiple enzymatic reactions of denitrifiers with a range of thermal adaptation, although lower values of E_a generally indicate high relative rates at low temperatures as a result of psychrophilic adaptation. Subtropical sediments had E_a values of 102–123 kJ mol⁻¹, approximately twice that of the polar and temperate sites, which is interpreted as the presence of an exclusively mesophilic community. In contrast, the lower E_a values at the polar and temperate sites were indicative of a significant contribution of psychrophilic to psychrotolerant populations. The range of E_a we observed for polar and temperate sites (50–65 kJ mol⁻¹) is comparable to previous observations of E_a of 60.6 kJ mol⁻¹ for denitrification in Arctic marine muds (Rysgaard et al., 2004). The ratio of denitrification at 5 °C to T_{opt} also confirms an increasing contribution of psychrophilic communities to denitrification across the gradient from subtropical to polar sediments. Specifically, polar sites exhibited rates of denitrification at 5 °C that

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were 34 % of the rate at T_{opt} , whereas the temperate and subtropical sites had rates at 5 °C that were 14 % and 1 % of T_{opt} , respectively.

Seasonal variation in T_{opt} was observed at the temperate site, Sylt, and the broadening of the T_{opt} in summer, rather than a clear shift towards a higher T_{opt} , suggests that psychrophilic to psychrotolerant denitrifying communities remain active in the summer when mesophilic activity is also apparent. Similarly, the E_a for denitrification at Sylt was similar between summer and winter months, indicating that psychrophilic/psychrotolerant populations still contributed to denitrification in the summer. Seasonal variation in T_{opt} for nitrate reduction has been observed in temperate salt-marsh sediments, where two distinct temperature optima for nitrate reduction at 12.8 and 28 °C were observed in winter and spring, respectively (King and Nedwell, 1984). These two temperature optima were interpreted as evidence of two nitrate-reducing communities with distinct temperature adaptation. In the present study, such a low temperature optima in the winter was not observed; however, the T_{opt} of 12.8 °C in the study of King and Nedwell (1984) may have been the result of a seasonal development of an anammox community (see below), but the study was conducted prior to the discovery of anammox.

4.3 Temperature adaptation of anammox

Multiple lines of evidence in this study indicate that the anammox process is well-adapted to cold conditions. At both the polar and temperate sites, low T_{opt} and E_a values of anammox relative to denitrification and the ratio of the anammox rate at 5 °C to T_{opt} (35 %) all support cold adaptation. Anammox activity was also absent from the subtropical sites. A T_{opt} of 9 °C at the polar site is consistent with the optima of 12–15 °C previously observed in Arctic fjord and deep North Sea muds, with in situ temperatures of –1.3 and 6 °C, respectively (Dalsgaard and Thamdrup, 2002; Rysgaard et al., 2004). Nitrification, which supplies nitrite for the anammox reaction, was found to have T_{opt} values of 8–16 °C in Svalbard fjord muds, closely corresponding to the T_{opt} for anammox (Thamdrup and Fleischer, 1998). The T_{opt} of 26 °C at the temperate

site is similar to previously reported T_{opt} values of approximately 25 °C for enrichments of marine anammox bacteria performed at 15–30 °C (Kawagoshi et al., 2012; van de Vossenberg et al., 2008). The temperate T_{opt} of 26 °C for anammox indicates a psychrotolerant rather than a mesophilic response (see the previous section for rationale).

5 Based on previous observations of the T_{opt} for respiration and growth on sulfate in pure cultures of mesophiles (Isaksen and Jorgensen, 1996), a T_{opt} for anammox higher than 30 °C would be expected if the anammox community at the temperate site were dominated by mesophiles.

Anammox bacteria detected in the marine environment belong almost exclusively 10 to the genus *Scalindua* within the Planctomycetes, and evidence for temperature-selected ecotypes has been found in enrichments from temperate fjord sediments (van de Vossenberg et al., 2008). However, reports of anammox activity in marine samples are generally limited to environmental temperatures of 25 °C or less. The compilation 15 of the marine anammox activity measurements in Schmid et al. (2007) includes oxygen minimum zone water columns, temperate sediments, and polar sediments with a range of in situ temperatures between –1.5 and 17 °C, with one observation at 20 °C. Anammox activity has been detected in temperate estuarine sediments with in situ temperatures ranging from 15–19 °C (Dale et al., 2009; Teixeira et al., 2012), and rates were depressed or undetectable in summer months when temperatures were above 20 °C. 20 The presence of anammox has been confirmed in subtropical and tropical mangrove sediments (Fernandes et al., 2012; Meyer et al., 2005); however, anammox communities in brackish mangrove sediments may be more metabolically diverse than those of marine sediments exposed to higher salinities (Amano et al., 2011). Along with the evidence from temperature block incubations in the present study, these studies suggest 25 that marine anammox bacteria (*Scalindua*) are predominately psychrophilic to psychrotolerant. However, more surveys of the activity and diversity of anammox bacteria are required to thoroughly test this observation.

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4.4 Implications for N removal on continental shelves

Rates of nitrogen removal from sands under simulated pore water advection in the present study support the emerging paradigm that permeable shelf sediments are important sites of N removal. To our knowledge, we provide the first report of denitrification and anammox activity from permeable sediments of a polar or permanently cold ecosystem. The contribution of arctic permeable shelf sediments to global N removal is likely significant, given the vast continental shelf of the Arctic Ocean.

Microbial community adaptation to in situ temperatures has been previously demonstrated in fine-grained marine sediments for aerobic respiration, hydrolysis, nitrate respiration, and sulfate reduction (Rysgaard et al., 2004; Arnosti et al., 1998; Thamdrup et al., 1998). In permeable continental shelf sediments, we observed a strong temperature adaptation of microbial communities that mediate denitrification to in situ temperature, and marine anammox bacteria appear to thrive in sediments exposed to colder conditions. Thus, other environmental parameters are likely to outweigh temperature as the dominant control on sediment N loss rates from permeable sediments.

Acknowledgements. This study was supported by grants from the National Science Foundation (OCE-0424967 and OCE-0726754), Florida State University (PEG 513680014), and the Max Planck Society. Partial support was provided to J. E. Kostka by the Hanse Wissenschaftskolleg. T. M. Gehrke was supported in part by a fellowship from the Estuarine Reserves Division, National Oceanic and Atmospheric Administration. We thank Patrick Chanton, John Kaba, and Yingfeng Xu for their assistance with sample collection and analysis. We also acknowledge cruise participants and Captain Stig Hennigsen for assistance with sample collection in Svalbard in 2010, and we thank the Florida Department of Environmental Protection for permission to sample at St. George Island State Park.

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Table 1. Sample site locations and sediment characteristics. Sites at Ymerbukta and Sylt were intertidal, and the sites at SGI were subtidal.

Site	Coordinates	Mean water (range) temperature	Sediment Type	Median (µm) Grain Size	Permeability (m ⁻²)	C _{org} (wt %)	C:N
SGI Gulf (Gulf of Mexico) ¹	29°41.73' N 84°46.25' W	23 °C (14 to 33)	Slightly gravelly fine sand	223	2.4×10^{-11}	0.04	8.1
SGI Bay (Gulf of Mexico) ¹	29°39.86' N 84°52.38' W	23 °C (14 to 33)	Slightly gravelly fine sand	211	1.3×10^{-11}	0.08	8.4
Sylt (Wadden Sea) ²	55°00.90' N 08°26.20' E	11 °C (2 to 19)	Moderately well sorted medium sand	338	4.8×10^{-11}	0.13	7.1
Ymerbukta (Svalbard) ³	78°16.42' N 14°01.96' E	-5 °C (-20 to 9)	Sandy very fine gravel	824	1.0×10^{-10}	0.49	22.6

¹ Grain size and permeability from Chipman et al. (2012).

² Sediment characteristics from Hedtkamp (2005).

³ Permeability estimated for Ymerbukta site according to the Carman-Kozeny equation.

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Table 2. Isotope pairing technique (IPT) rate measurements of denitrification and anammox. The production of unlabeled N₂ (p14) was calculated according to Risgaard-Peterson et al. (2004), and the contribution of anammox (% Anammox) was determined in anaerobic slurries according to Thamdrup and Dalsgaard (2002). Error estimates for p14 were determined from the standard error of the regression slope.

Site	Date	Sediment <i>T</i> (°C)	Bottom Water NO ₃ ⁻ (μM)	p14 (μmol N m ⁻² d ⁻¹)			% Anammox
				Diffusive Conditions	Simulated Advection		
SGI Gulf (Gulf of Mexico)	June 2011	29.9	1.7	8.8 ± 1.1	15 ± 3.7	0 %	
SGI Bay (Gulf of Mexico)	June 2011	31.8	2.2	1.9 ± 0.6	3.4 ± 0.7	0 %	
Sylt (Wadden Sea)	March 2007	5.3	53.9	79.7 ± 2.7	359 ± 75	14.0 %	
Sylt (Wadden Sea)	June 2007	19	0.8	6.5 ± 1.7	47.6 ± 29.6	6.0 %	
Ymerbukta (Svalbard)	August 2010	6.8	0.5	9.9 ± 2.8	49 ± 6.8	6.5 %	

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Table 3. Optimum temperatures (T_{opt}), apparent activation energy (E_a), and ratio of rates at 5 °C relative to T_{opt} for anammox and denitrification in permeable sediments. The range of the T_{opt} peak where rates did not fall below 90 % of the optimum rate is noted in parentheses.

		SGI Gulf	SGI Bay	Sylt Summer	Sylt Winter	Ymerbukta
Denitrification	$T_{\text{opt}}(^{\circ}\text{C})$	36 (35–37)	35	34 (26–35)	26 (24–32)	21 (17–24)
	Rate at 5 °C relative to T_{opt}	1.4 %	0.4 %	17.1 %	11.2 %	34.4 %
	$E_a(\text{kJ mol}^{-1})$	102.7 ± 2.1	123.5 ± 4.0	51.7 ± 3.8	65.0 ± 4.0	57.8 ± 4.4
Anammox	$T_{\text{opt}}(^{\circ}\text{C})$	N.A.	N.A.	26 (22–29)	N.D.	9
	Rate at 5 °C relative to T_{opt}	N.A.	N.A.	34.8 %	N.D.	35 %
	$E_a(\text{kJ mol}^{-1})$	N.A.	N.A.	35.0 ± 4.9	N.D.	N.D.

N.A. = no detectable activity; N.D. = not determined.

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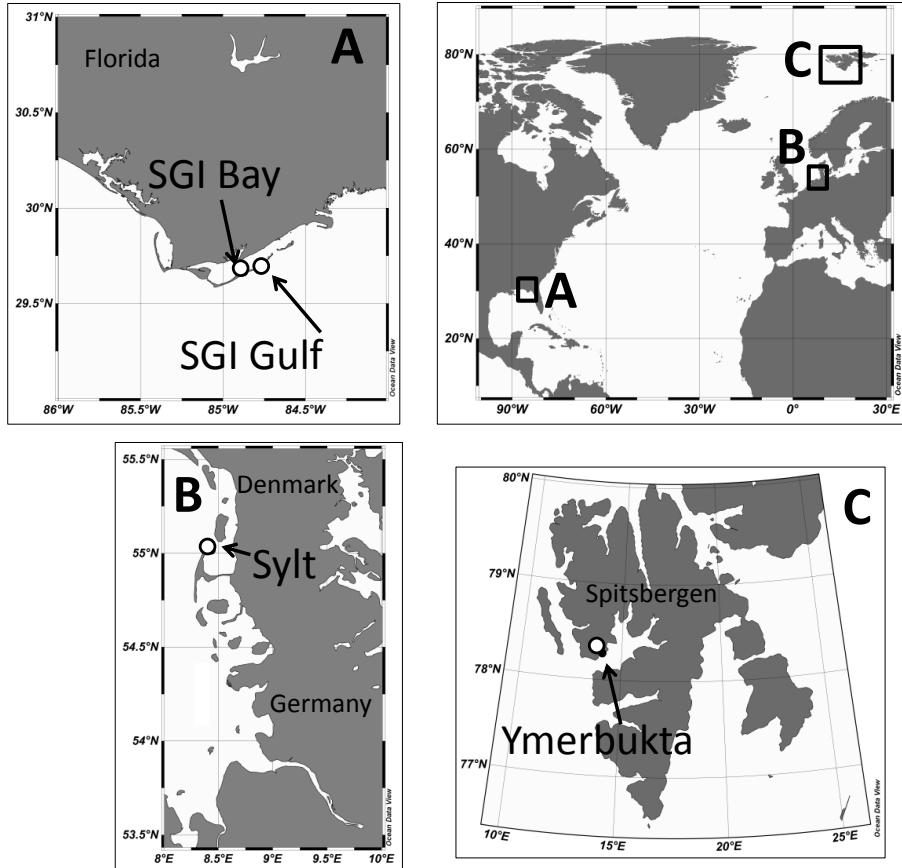


Fig. 1. Location of the sample sites.

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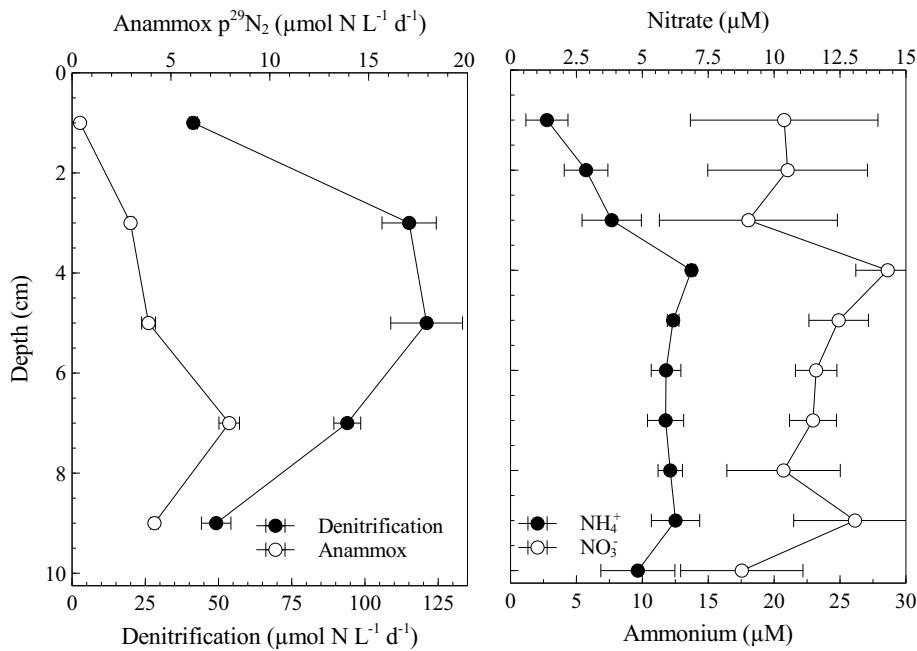


Fig. 2. Left: Depth resolved potential rates of denitrification and anammox from Sylt in June 2007. Experiments using $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ tracer were used to estimate denitrification and anammox, respectively. Right: Pore water profiles of nitrate and ammonium from Sylt in June 2007. Error bars represent the standard error of 3 replicate profiles.

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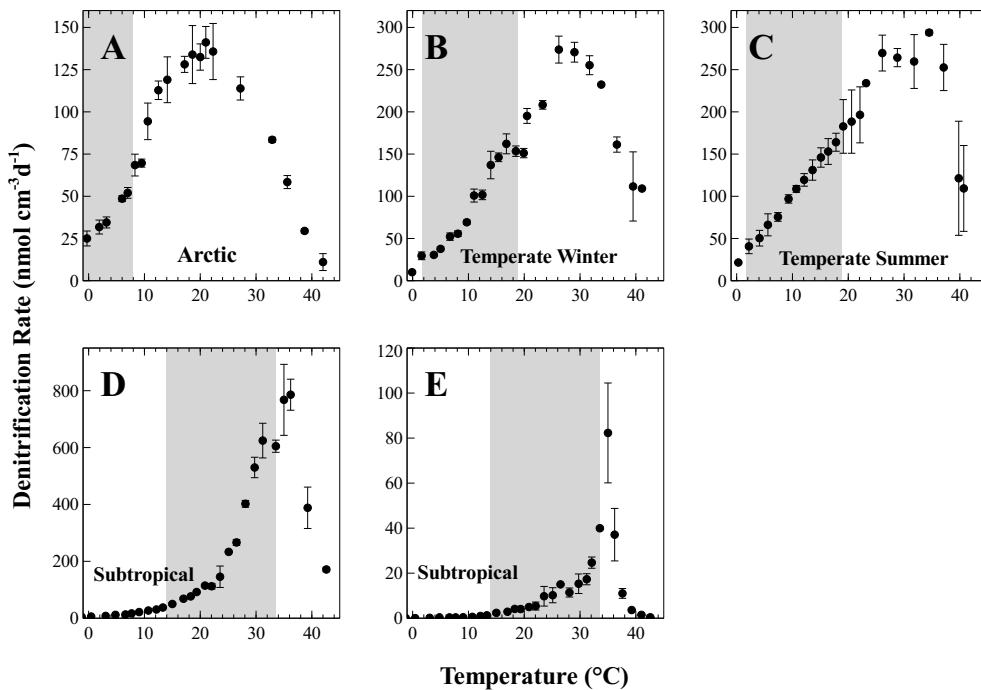


Fig. 3. Potential denitrification rates measured in temperature gradient block experiments for (A) Ymerbukta, (B) Sylt (March 2007), (C) Sylt (June 2007), (D) SGI Gulf, and (E) SGI Bay. The seasonal temperature range is indicated by gray rectangles (Note that the seasonal minimum for A is -20 °C). Error bars represent the standard error of the regression slope calculated at each temperature. Note different y axis scaling.

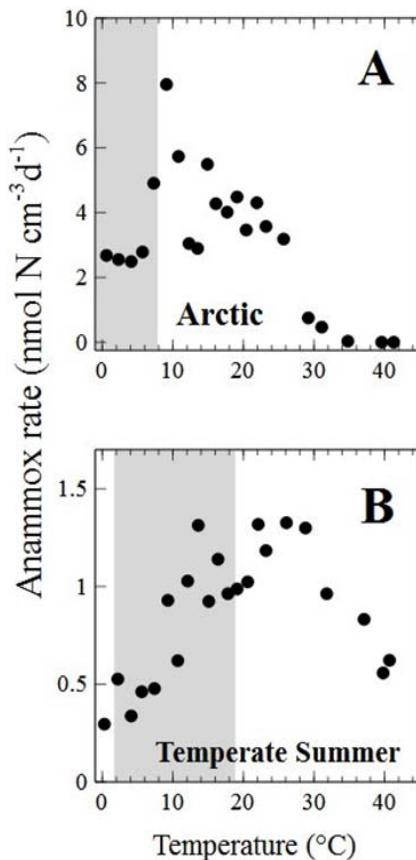


Fig. 4. Potential anammox rates measured in temperature gradient block experiments for **(A)** Ymerbukta and **(B)** Sylt (June 2007). Rates were measured with $^{15}\text{NH}_4^+$ label. Estimates of standard error were not made because rates were calculated from two time points. Note different y-axis scaling.

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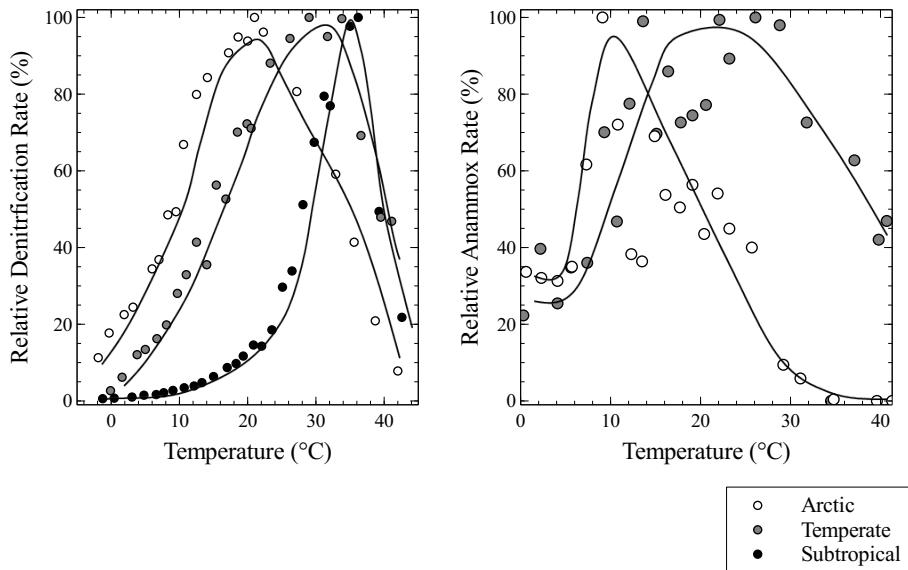


Fig. 5. Left: Normalized temperature response of denitrification for subtropical, temperate, and arctic sites. Right: Normalized temperature response of anammox for temperate and arctic sites. Error bars are omitted for clarity