Similar temperature responses suggest future climate warming will not alter partitioning between denitrification and anammox in temperate marine sediments

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Key words: denitrification, anammox, marine sediment, climate change, temperature dependence, activation energy

Paper type: Primary research

Abstract

Removal of biologically available nitrogen (N) by the microbially mediated processes denitrification and anaerobic ammonium oxidation (anammox) affects ecosystem N availability. Although few studies have examined temperature responses of denitrification and anammox, previous work suggests that denitrification could become more important than anammox in response to climate warming. To test this hypothesis, we determined whether temperature responses of denitrification and anammox differed in shelf and estuarine sediments from coastal Rhode Island over a seasonal cycle. The influence of temperature and organic C availability was further assessed in a 12-week laboratory microcosm experiment. Temperature responses, as characterized by thermal optima (T_{opt}) and apparent activation energy (E_a), were determined by measuring potential rates of denitrification and anammox at 31 discrete temperatures ranging from 3 to 59°C. With a few exceptions, T_{opt} and E_a of denitrification and anammox did not differ in Rhode Island sediments over the seasonal cycle. In microcosm sediments, E_a was somewhat lower for anammox compared to denitrification across all treatments. However, T_{opt} did not differ between processes, and neither E_a nor T_{opt} changed with warming or carbon addition. Thus, the two processes behaved similarly in terms of temperature response, and this response was not influenced by warming. This led us to reject the hypothesis that anammox is more cold-adapted than denitrification in our study system. Overall, our study suggests that temperature responses of both processes can be accurately modeled for temperate regions in the future using a single set of parameters, which are likely not to change over the next century as a result of predicted climate warming. We further conclude that climate warming will not directly alter the partitioning of N flow through anammox and denitrification.

Introduction

Marine nitrogen (N) availability affects both regional and oceanic primary productivity as well as regional susceptibility to eutrophication (Ryther & Dunstan, 1971; Perry & Eppley, 1981; Diaz & Rosenberg, 2008). An important oceanic N sink is via microbially mediated N removal, particularly in coastal and continental shelf sediments, which receive and remove 50-80 Tg N y^{-1} from terrestrial and marine sources (Howarth *et al.*, 1996; Galloway *et al.*, 2004; Gruber & Galloway, 2008). Benthic N removal occurs through denitrification and anaerobic ammonium oxidation (anammox), both of which are anaerobic processes that reduce NO_3^- or NO_2^- to N_2. While denitrification is primarily a heterotrophic process that uses NO_3^- to oxidize organic carbon, anammox uses NO_2^- to oxidize NH_4^+ and is primarily autotrophic. However, anammox depends on organic carbon mineralization indirectly as a source of NH_4^+. Both denitrification and anammox are microbially mediated enzymatic processes that may respond differently to changes in temperature (Dalsgaard & Thamdrup, 2002; Rysgaard *et al.*, 2004; Brin *et al.*, 2014). As temperatures in coastal waters are predicted to continue to rise over the next century (Nixon *et al.*, 2004; Christensen *et al.*, 2007), differences in temperature responses between processes could alter the flux of N through denitrification versus anammox.

The temperature response of an enzymatic process can be described by its activation energy (E_a), which reflects the increase in rate with increase in temperature (temperature dependence), as well as its thermal optimum (T_{opt}), the temperature at which rates are maximal (Arrhenius, 1915). In nature, the temperature response of a biogeochemical processes is determined by the combined temperature response of the assemblage of organisms performing the reactions in any given environment (Allen *et al.*, 2005; Hall *et al.*, 2008, 2010; Yvon-Durocher *et al.*, 2014). Ecosystem level processes may display distinct temperature dependence,

as has been demonstrated for photosynthesis and respiration (Yvon-Durocher *et al.*, 2010; Demars *et al.*, 2011). For microbially mediated processes, changes in temperature responses could reflect: 1) changes at the cellular level, through physiological acclimation by individual microbial strains; or 2) changes at the microbial population level, through changes in abundance of strains adapted to different temperatures (Angilletta Jr., 2009; Hall *et al.*, 2010; Crowther & Bradford, 2013). However, rates or temperature responses may be more strongly limited by other factors than temperature in the environment, such as substrate supply. Thus, in some cases there may not be a strong selective advantage to adapt to changes in temperature (Hartley *et al.*, 2007, 2008; Crowther & Bradford, 2013).

The hypothesis that temperature may be a key driver of the relative importance of denitrification and anammox as N loss pathways was provided by studies in permanently cold sediments, which found that anammox was relatively more favored over denitrification at colder temperatures (Dalsgaard & Thamdrup, 2002; Rysgaard *et al.*, 2004). More recent studies examining seasonal patterns or temperature responses of anammox and denitrification rates in marine sediments also support anammox being cold-adapted or hindered at higher temperatures (Teixeira *et al.*, 2012; Brin *et al.*, 2014; Canion *et al.*, 2014a, 2014b). Besides temperature, availability of organic C likely exerts a strong influence on the relative importance of anammox and denitrification as N loss pathways, with organic C favoring denitrification over anammox (Thamdrup & Dalsgaard, 2002; Engström *et al.*, 2005). As temperature also influences organic matter decomposition rates and therefore organic C availability, the effects of temperature could be mediated indirectly through changes in organic C availability rather than as a direct result of inherent differences in enzyme kinetics between the anammox or denitrification pathway (Isaksen & Jørgensen, 1996; Canion *et al.*, 2014a; Brin *et al.*, 2015).

Despite indications that anammox and denitrification rates may respond differently to temperature, this control has only been examined in a few studies (Dalsgaard & Thamdrup, 2002; Rysgaard *et al.*, 2004; Canion *et al.*, 2014a, 2014b). Furthermore, it is unknown whether changes due to climate warming may alter not only rates but also the temperature dependence of each process (King & Nedwell, 1984; Acuña *et al.*, 2008; Robador *et al.*, 2009; Perkins *et al.*, 2012). Differences in temperature dependence of each process over the range of temperatures experienced *in situ* could alter the relative rates of each process, and thus its contribution to N_2 production (Holtan-Hartwig *et al.*, 2002). Furthermore, climate warming could have indirect effects on temperature dependence by influencing organic C availability. This could occur if warming alters the deposition of organic C to benthic sediments, e.g. via changes in spring phytoplankton blooms in coastal ecosystems (Sommer & Lengfellner, 2008; Nixon *et al.*, 2009; Lewandowska & Sommer, 2010), or the rate of consumption of sediment organic C (Alsterberg *et al.*, 2012).

We have examined controls on anammox and denitrification in temperate marine sediments previously by measuring potential rates in field collected samples over a seasonal cycle and in a separate microcosm experiment (Brin *et al.*, 2014, 2015). In this paper, we report new measurements on the temperature responses of anammox and denitrification rates in the same sediments, to directly test the hypothesis that anammox and denitrification have different temperature responses. We asked whether T_{opt} or E_a 1) vary between anammox and denitrification, 2) vary by sampling site or season within each process, and 3) can be altered by manipulations of temperature or organic C availability in a microcosm experiment.

Materials and Methods

Seasonal study

To determine how temperature responses varied by site and season, two study sites were sampled in coastal Rhode Island, USA: an inner continental shelf site, Rhode Island Sound (RIS2) and an estuarine site, Providence River Estuary (PRE) (i.e., Heiss *et al.*, 2012; Brin *et al.*, 2014). These sites will be referred to as shelf and estuarine sites, respectively. The shelf site had a water depth of 38 m, and bottom water temperatures were between 7 and 17ºC during sampling dates. The estuarine site had a water depth of 5 m and greater seasonal temperature variation, with measured bottom water temperatures between 3 and 22ºC across sampling dates. Sediments at both sites were fine-grained, with a higher organic carbon content at the estuarine site (2.6%) than the shelf site (0.8%) (NC2100 Elemental Analyzer).

The shelf site was sampled in January, June, July, and September 2011 and March 2012, and the estuarine site was sampled in June and August 2011 and January 2012. At the shelf site, PVC tubes were fastened to the inside of a box core that was deployed from the research vessel to obtain intact sediment cores. At the estuarine site, intact cores were collected into PVC tubes (10 cm inner diameter) using a pull corer. After collection, the cores were immediately transported back to the laboratory at near-*in situ* temperature. Sediment cores were held in the dark at *in situ* temperature under air-bubbled site water in aquaria. This approach was taken because water columns at the sites were generally well mixed, indicating that bottom water was near air saturation. O_2 microprofiles were measured in the cores 1-4 days after sample collection to determine the O_2 penetration depth, as described previously (Brin *et al.*, 2014). Cores were then removed from aquaria and a 1 cm depth layer of sediment just below the O_2 penetration depth (<0.5 cm) was extruded from the core tube, sliced off, and collected for temperature

response measurements. This depth interval was the focus of this study as it contained the NO_3^- reducing layer, based on O_2 penetration depth and concentration of NO_3^- in porewater profiles (Brin *et al.*, 2014). Sediment from 4-5 cores corresponding to any given site and sampling date were pooled to obtain enough sediment to conduct temperature response measurements.

Microcosm experiment

A total of fifteen microcosms were set up and maintained as described previously, using sediment collected at the shelf site in March 2012 (Brin *et al.*, 2015). Briefly, microcosms consisted of sieved (1 mm) surface sediment (0-4 cm depth interval) layered approximately 4 cm deep in glass pans, each placed in an aquarium containing 6 L of 0.2 μm-filtered Narragansett Bay seawater (salinity 32), which was kept air saturated with aquarium pumps. Half of the overlying water was replaced every two weeks to prevent buildup of nutrients or other compounds. All microcosms were initially held at 4°C for 16 days, after which three microcosms were destructively sampled, and potential rate experiments were conducted (t_0 experiments). The microcosms were then exposed to temperature treatments by maintaining half of the microcosms at 4°C and shifting the other half to 17°C. This temperature manipulation represents seasonal minimum and maximum temperatures at the site (Emery & Uchupi, 1972; Brin *et al.*, 2014). Carbon was added biweekly to half of the microcosms at either temperature in the form of *Chlorella* algae, in the form of a suspension that was gently mixed into the top 1 cm of sediment at a rate equivalent to 3.1 μmol C cm^{-2} d^{-1}, which is expected to maintain sediment labile C availability (Brin *et al.*, 2015). This resulted in four treatments in a full factorial design, referred to here as 4°C, 4°C+C, 17°C and 17°C+C, with three replicate aquaria in each treatment. O_2 consumption was increased by both carbon addition and temperature. O_2 penetration into the

154 sediment was at most 0.5 cm, with shallower penetration in sediments with greater O_2
155 consumption, indicating that added organic C reached anoxic layers in all the microcosms.
156 Treatments were maintained for 12 weeks, after which point the overlying water was aspirated
157 off and the contents of each pan were collected into a beaker for temperature response
158 measurements.
159
160 *Temperature responses of denitrification and anammox potential rates*
161 Sediment from a given site or microcosm replicate was homogenized in a beaker, and 1.5
162 mL of this sediment was transferred into replicate vials (5.9 mL, 93 replicate vials per site or 31
163 replicate vials per microcosm replicate) to conduct parallel incubations at different temperatures.
164 The headspace of the vials was made anoxic by purging the headspace with helium, and vials
165 were pre-incubated overnight at the associated *in situ* or experimental microcosm temperature to
166 remove ambient porewater NO_x^-. For the microcosm experiment, replicates were maintained
167 within the thermoblock, yielding 3 measurements of E_a for each treatment.
168 Temperature responses were measured using a thermal gradient incubator (thermoblock)
169 similar to Rysgaard et al. (2004). The thermoblock consisted of a 1.8 m long piece of aluminum
170 with a silicone rubber heater on one side, a Peltier cooler at the other, and 31 parallel rows of 3
171 holes (vial wells) along its length to fit the vials. This created a stable linear temperature gradient
172 with endpoints at 2.8 ± 0.7°C and 58.9 ± 0.8°C (mean ± s.d.), as determined by measurement of
173 temperatures in vial wells before and after experiments, as well as with temperature probes
174 embedded in the thermoblock during all incubations. The vials were transferred from their pre-
175 incubation temperature into the thermoblock for approximately 90 minutes to allow for complete
176 temperature equilibration of sediments. Potential rate measurements were commenced after the

90 min equilibration period by adding 50 μL of $^{15}NO_3^-$+$^{14}NH_4^+$ (100 nmol N mL^{-1} sediment) to the vials. After 5-50 min incubations in the presence of added $^{15}NO_3^-$+$^{14}NH_4^+$, all reactions were completely stopped by adding 100 μL 7M $ZnCl_2$. The amount of ^{15}N-N_2 that accumulated in vials during the incubation was used to determine rates of denitrification and anammox. Shorter incubations were conducted for sediments with higher inherent rates, such as estuarine sediments. Rates were plotted as a function of temperature in the thermoblock, which by definition is referred to as a thermal profile in this study.

^{15}N-N_2 production in the vials was measured with an isotope ratio mass spectrophotometer (Isoprime CF-IRMS interfaced with Multiflow-Bio Unit) and rates were calculated as described in Thamdrup and Dalsgaard (2002). By convention, the percent of N_2 production accounted for by anammox is abbreviated as *ra* (relative anammox), and calculated as 100 X (anammox) / (anammox + denitrification).

In addition to thermoblock experiments, parallel sets of potential rate measurements in triplicate vials were run to serve as different controls, as follows. One set of vials received unlabeled NO_3^- and NH_4^+ and was incubated at *in situ* temperature in the seasonal study, or 17°C for the microcosm experiment, in order to assess NO_3^- concentrations remaining in the vials after time intervals that were used in thermoblock incubation. This confirmed that NO_3^- was not depleted during incubations. Three additional ^{15}N isotope additions were run for samples collected on the different sampling dates at the estuarine and shelf sites. These incubations were done at *in situ* temperature, in parallel to thermoblock incubations. One incubation received the same $^{15}NO_3^-$+ $^{14}NH_4^+$ addition as in the thermoblock incubation, with four equally spaced measurement time points starting immediately after N addition, confirming linear production of $^{29}N_2$ and $^{30}N_2$ during the incubation. An additional incubation received $^{15}NH_4^+$ + $^{14}NO_3^-$, and

another received $^{15}NH_4^+$ alone, confirming the presence or absence of anammox and that N_2 was not produced by some other process independent of NO_3^- reduction (Yang *et al.*, 2012). The rates from incubations with added $^{15}NH_4^+$+$^{14}NO_3^-$ or $^{15}NH_4^+$ alone were reported previously (Brin *et al.*, 2014), and those results are consistent with the relative rates of anammox reported in this study. Vials with no added N were also included at the beginning of the experiment to correct for any residual $^{14}NO_3^-$ that might have remained after the pre-incubation. The fraction of ^{15}N-labelled NO_3^- in the incubations, accounting for the fraction of ^{15}N in added $^{15}NO_3^-$ (i.e., 0.99), was >0.96 across all incubations.

Statistical analysis

Statistical analyses were conducted using R version 2.15.0 (R Development Core Team). For all analyses, statistical tests were considered significant at the $p<0.05$ level.

To statistically define T_{opt}, a general additive model was fit to each profile using the R package *mgcv* (Wood, 2006, 2011; Zuur *et al.*, 2009), using cubic regression splines and cross-validation. Temperatures with modeled rates that fell within the 95% confidence interval of the maximum rate were all considered to be T_{opt}. Therefore, the T_{opt} values reported below reflect this statistically defined range. One exception was made for denitrification in PRE sediments in January 2012, for which there was a double peak; both peaks were subject to this analysis and considered to be part of T_{opt}. If the range in T_{opt} overlapped between any given comparison of samples, we considered T_{opt} to be not significantly different. Whether relationships between T_{opt} and temperature in the seasonal study or microcosm experiment were significant were determined with linear regression ($p<0.05$).

Nitrate reduction temperature responses

Temperature-rate relationships were examined with the linearized form of the Arrhenius equation with a standardized temperature (Rysgaard *et al.*, 2004; Yvon-durocher *et al.*, 2010):

$$\ln[\text{Rate}(T)] = -E_a*(1/kT - 1/kT_c) + \ln[\text{Rate}(T_c)] \quad (1)$$

where T_c is the standardized temperature of 15°C (Perkins *et al.*, 2012); $\ln[\text{Rate}(T_c)]$ is the Arrhenius constant in the traditional derivation; E_a is the apparent activation energy for the measured process; k is the Boltzmann constant ($8.62 * 10^{-5}$ eV K^{-1}); and T is the measurement temperature in Kelvin. E_a is calculated as the negative slope of the linear regression through the linear range of the thermal profile below T_{opt}. E_a values in eV and kJ mol^{-1} are presented here to compare directly with previous work on both nitrogen cycling (kJ mol^{-1}) and ecosystem respiration (eV). In the seasonal study, the standard error in E_a was estimated from regression lines in Arrenhius plots, whereas in the microcosm experiment, standard error was determined across microcosm replicates. Relationships between the linear intercept (rate at 15ºC) and *in situ* temperature were assessed with linear regression.

We used similar linear mixed effects models using the function *lme* within the R package *nlme* (Pinheiro *et al.*, 2016) to test for differences in E_a between processes, sampling sites, or microcosm treatments (Zuur *et al.*, 2009). Three datasets were analyzed corresponding to the seasonal study, microcosm experiment, or the combined data. For each analysis, models included the following main effects: measurement temperature, site or treatment, process, and interactions between temperature and both site/treatment and process. For each analysis, we used Akaike information criterion (AIC) scores to compare three models with all main effects to determine the random effects structure of the data: with no random effects; with random intercepts; and with random slopes and intercepts. Random effects assessed variation at the level of individual and distinct thermal profiles. As such, sampling date (for the seasonal study) and

245 treatment replicate (for the microcosm study) were treated as random effects on the slope and
246 intercept. Comparisons between these three models indicated whether the random effects term
247 varied in slope (E_a) as well as intercept (magnitude of rates). We continued with the model with
248 the lowest AIC score to test for significance of main effects. For all microcosm analyses, models
249 with random slopes and intercepts had lowest AIC scores and were selected further analysis. E_a
250 was considered to vary significantly for main effects if their interaction with temperature was
251 significant. For example, to assess differences in E_a across sites, we assessed whether there was a
252 significant interaction between site and temperature, which would indicate that the relationship
253 of rate with temperature varied by site.

254 In the seasonal study, within each site and process, we further explored which sampling
255 dates contributed to differences in apparent activation energies (i.e., denitrification or anammox)
256 using a similar linear mixed modelling approach in which temperature was the sole main effect.
257 Select dates were removed to determine their effect on random effects structures.

Results

Temperature responses by site and season

Rates of denitrification and anammox increased with temperature up to 20-35°C, with declining rates thereafter (Fig. 1a-d). There were strong seasonal differences in absolute rates within a site (sampling date $p<0.001$), particularly for denitrification in shelf sediments, with the lowest rates in January 2011 and highest rates in March 2012 (Fig. 1a). Potential denitrification reached a higher maximum rate in estuarine (PRE) compared to shelf (RIS2) sediments, but the range in maximum rates between the two sites overlapped, indicating strong potential for denitrification at both sites during the sampling period (Fig. 1a vs. b). In shelf sediments, potential anammox rates were 2-6 times lower than denitrification rates (Fig 1a vs. c). Rates were not related to *in situ* temperatures for either site or process. In estuarine sediments, anammox rates were undetectable or close to the detection limit (<1 nmol N h^{-1} mL^{-1} sediment) (Fig. 1d). We therefore did not calculate T_{opt} and E_a values for anammox at the estuarine site.

The range in T_{opt} was 18-35ºC for denitrification and 22-33ºC for anammox (Table S1, Fig. 2). T_{opt} overlapped for anammox and denitrification on each sampling date. There was no relationship between T_{opt} and *in situ* temperature, nor was there a consistent pattern in T_{opt} across sites or seasons. Within each site and process, T_{opt} overlapped for all sampling dates, with the exception of denitrification in January 2011 in shelf sediments, which had a narrower profile and higher T_{opt} than September and March (Fig. 2). The thermal profile for denitrification in estuarine sediments in January 2012 had a double peak that bracketed those for other seasonal measurements.

Apparent E_a values were between 0.40 and 0.63 eV (38.5 and 60.4 kJ mol^{-1}) for denitrification in shelf sediments, 0.36 and 0.69 eV (34.3 and 66.9 kJ mol^{-1}) for anammox in

shelf sediments, and 0.37 and 0.55 eV (35.8 and 53.0 kJ mol^{-1}) for denitrification in estuarine sediments (Table S1, Fig. 1e-g). Apparent E_a did not differ significantly between sites for denitrification nor between denitrification and anammox (linear mixed effects model, $p>0.05$). The mixed model with the lowest AIC score included both random slope and intercept, indicating that E_a differed across sampling dates. Differences in denitrification E_a by sampling date were driven by high E_a at the shelf site in January 2011 and low Ea at the estuarine site in June 2011, as models without random slopes became optimal when these dates were omitted. Anammox E_a also differed by date in shelf sediments, driven by higher E_a values in July and September 2011. However, in the full model, the variance was much greater for the intercept (capacity; $d^2=0.40$) than for the slope (E_a; $d^2=0.0085$), indicating that differences among dates were more dependent on overall capacity than temperature dependence.

Across all thermoblock measurements in shelf sediments, *ra* ranged from negligible to 62%. In 3 out of 5 sampling dates, there was no change in *ra* as a function of thermoblock temperature across a range of 3-35°C (Fig. 3). However, in January 2011, *ra* was negatively correlated with temperature ($p<0.001$, $R=-0.89$), decreasing from 62% at 3°C to 28% at 35°C. This switch to a negative correlation was driven not by a change in anammox temperature dependence or capacity across sampling dates, but by a change in the shape of the denitrification thermal profile on this particular date (Fig. 1a, c). In contrast, in September 2011, *ra* was positively correlated with temperature ($p=0.001$, $R=0.70$) (Fig. 3).

Microcosm experiment

Incubating microcosm sediments at 4°C without C addition for 12 weeks did not change denitrification rates compared to t_0 measurements, while anammox rates decreased slightly,

304 relative to the t_0 control (Fig. 4a, b). Contrary to expectations, denitrification rates decreased
305 significantly in 17°C treatments, with or without C, relative to t_0, as well as in the 4°C treatment
306 with C (Fig. 4a; linear mixed effects model with random slope and intercept, $p<0.001$).
307 Anammox rates showed a similar decrease with treatments as denitrification (Fig. 4b; linear
308 mixed effects model, $p<0.001$). T_{opt} overlapped for anammox and denitrification, as well as
309 across treatments for each process (Fig. 2, Table S1). Similarly, T_{opt} in the microcosm
310 experiment did not differ from the seasonal study, although ranges were more consistent in the
311 microcosm experiment (Fig. 2).
312 The sediment that was used in the microcosm experiment was from March 2012, when E_a
313 of anammox was the lowest across sampling dates (Table S1). This lower E_a was reflected in the
314 microcosm experiment, as E_a was significantly lower for anammox than denitrification (linear
315 mixed effects model, process x temperature interaction $p<0.001$). Apparent E_a values were
316 between 0.38 and 0.48 eV (36.5 and 46.4 kJ mol^{-1}) for denitrification and 0.20 and 0.32 eV (19.3
317 and 30.8 kJ mol^{-1}) for anammox. However, E_a was not significantly different between treatments
318 for either process (Table S1, Fig. 4c, d), and as with the seasonal study, variance was much
319 greater for the intercept (capacity; $d^2=0.026$) than for the slope (E_a; $d^2=0.0014$). Furthermore,
320 neither denitrification nor anammox E_a differed significantly between the microcosm experiment
321 and the seasonal study.

Discussion

The denitrification T_{opt} values measured in this study (21 to 35°C) indicate a mesophilic community of denitrifiers in temperate Rhode Island sediments. Given overlapping T_{opt} and mostly similar apparent E_a values, there was no indication of a specifically cold- or warm-adapted population of denitrifiers that developed seasonally, between sites, or in response to experimentally manipulated temperatures. This indicates functionally equivalent denitrifier populations in terms of temperature response, despite variation in rates (Fig. 1e-g, 4 c-d). Furthermore, warmest *in situ* temperatures were within the range of T_{opt}, suggesting that denitrifiers were reasonably well adapted to the annual temperature regime at the sites. Our results agree with the general finding that denitrification rates display a mesophilic T_{opt} and comparable E_a values in a broad range of sediments from temperate to Arctic systems (Dalsgaard & Thamdrup, 2002; Rysgaard *et al.*, 2004; Canion *et al.*, 2014a, 2014b). This implies that relatively large temperature changes from the Arctic to temperate regions do not cause significantly different temperature responses for denitrification. In contrast, denitrification in subtropical sediments has been shown to have distinctly higher T_{opt} and E_a values compared to colder sediments (Canion *et al.*, 2014b). Thus, warmer climates may cause a change in the temperature response of denitrification. However, the degree of warming needed to cause such a change is probably greater than the 2-2.5ºC warming that is predicted to occur in our study region over the next century (Meehl *et al.*, 2007; Taboada & Anadón, 2012; Mills *et al.*, 2013).

Previous studies have suggested that anammox bacteria are more cold-adapted than denitrifiers, due to lower T_{opt} (9-18°C) or E_a in anammox bacteria, and measurements of higher *ra* values at lower temperatures (Dalsgaard & Thamdrup, 2002; Rysgaard *et al.*, 2004; Canion *et al.*, 2014a, 2014b). However, most of these studies have been conducted in permanently cold

345 marine sediments. The present study is one of the few that has been conducted in temperate
346 sediments (Canion *et al.*, 2014b). We found that the range in T_{opt} values of denitrification and
347 anammox were not significantly different in the seasonal study or microcosm experiment (Fig. 2,
348 Table S1). E_a of anammox was significantly lower than E_a of denitrification in the microcosm
349 experiment, which appeared to be driven by initial values of E_a in the sediments used to set up
350 the microcosm experiment rather than any significant influence of experimental treatments. E_a
351 values of anammox and denitrification were not significantly different across the seasonal study,
352 indicating that there was not an overall consistent difference in E_a between the two processes.
353 Cumulatively, we conclude that overall populations of active anammox bacteria are not more
354 cold-adapted than denitrifiers in our study system. Similar to denitrification, the results do not
355 indicate consistent seasonal shifts in temperature responses of anammox. On the one sampling
356 date when *ra* did decrease with increasing temperature (January 2011), this was driven by a shift
357 in the temperature response of denitrification rather than anammox. The correlation between
358 *ra* and temperature across seasons that was previously noted (Brin et al., 2014) may therefore
359 have been due to other factors besides temperature that vary seasonally, rather than relatively
360 faster rates of denitrification compared to anammox at warmer temperatures. As anammox may
361 depend on denitrification for a source of NO_2^- (Trimmer *et al.*, 2003; Risgaard-Petersen *et al.*,
362 2004; Meyer *et al.*, 2005; Brin *et al.*, 2014), similar temperature responses overall might reflect
363 the relationship between the two processes.

364 The capacity for denitrification, as reflected in thermal profiles and in the linear intercept
365 of Arrhenius plots (Fig. 1, 3), changed across sampling dates in the seasonal study as well as
366 with treatment in the microcosm study. These changes in magnitude could be associated with
367 changes in the abundance of denitrifier populations, the amount of enzyme being produced by

368 the denitrifiers present, substrate availability, or a combination of factors. The lack of a
369 correlation between linear intercept and *in situ* temperature in the seasonal study suggests that
370 temperature effects may be indirect, and that potential rates are controlled by other factors in
371 addition to temperature. One potential control of denitrification rates in coastal and marine
372 sediments is organic C availability, with higher rates reflecting greater C availability (Dalsgaard
373 *et al.*, 2005; Brin *et al.*, 2014). Experiments with Arctic sediments demonstrated that addition of
374 organic acids (i.e., acetate, lactate) significantly increased sulfate reduction or denitrification
375 rates in thermoblock experiments (Isaksen & Jørgensen, 1996; Canion *et al.*, 2014a). Similarly,
376 we expected that organic C addition in our microcosm experiment would increase denitrification
377 rates relative to microcosms without C addition. Surprisingly, organic C addition did not yield
378 this result. The lack of response of denitrification rates in our microcosm experiment may have
379 been due to competition for NO_3^- with other processes, as potential dissimilatory nitrate
380 reduction to ammonium (DNRA) rates were stimulated by the organic C addition, while potential
381 denitrification rates were not (Brin *et al.*, 2015). The form of organic C added may also have had
382 an influence on this result, with regular additions of freeze-dried phytoplankton favoring DNRA
383 bacteria over denitrifiers.

384 The aim of this study was to determine how shifts in temperature and C availability
385 through seasonal changes or experimental manipulations influence the temperature responses of
386 anammox or denitrification. We found that temperature responses of anammox and
387 denitrification were more similar to each other than previously reported (Dalsgaard & Thamdrup,
388 2002; Rysgaard *et al.*, 2004; Canion *et al.*, 2014b), and both processes were characterized as
389 mesophilic instead of anammox being more cold-adapted than denitrification. Overall, our
390 results suggest that predicted warming in our study region over the next century will not act

391 through direct temperature effects to decrease the contribution of anammox to N_2 production
392 relative to denitrification. In contrast, strong differences in absolute rates with season suggest
393 that factors other than temperature dependence are important regulators of relative rates of
394 anammox and denitrification.

Acknowledgments

Thank you to Caroline Scanlan, Phil Arevalo, Michaeline Nelson, Amber Hardison and Heather Leslie for help in the lab and the field. Thanks also to Elise Heiss, Lindsey Fields, Shelley Brown, Wally Fulweiler, Scott Nixon, Bethany Jenkins, Jane Tucker, Steve Granger, Rodman Sykes, Rebecca Robinson, Stephen Porder and Aaron McFarlane for help in the project, and to Charlie Vickers, Mike Packer and Brian Corkum for help with thermoblock construction. We are grateful to Capt. Rodman Sykes of the F/V Virginia Marise and his crew for assisting with sampling. We thank two anonymous reviewers whose comments have helped to improve this manuscript. This material is based upon work supported by the National Science Foundation under Grant No. OCE-0852289 to JJR and OCE-0852263, OCE-0927400 and OCE1238212 to AEG, and Rhode Island Sea Grant to JJR.

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549 **Supporting information captions**

550 **Table S1** – Apparent activation energies (E_a) and thermal optima (T_{opt}) of denitrification and

551 anammox in shelf and estuarine sediments and the microcosm experiment. Asterisks denote

552 E_a values that differ significantly from others within the same site and process.

553 **Figure captions**

554 **Figure 1**. Thermal profiles (a-d) and Arrhenius plots (e-h) of denitrification and anammox in
555 shelf and estuarine sediments. Panels are as follows: Denitrification in shelf (a, e) and estuarine
556 (b, f) sediments; anammox in the shelf (c, g) and estuarine (d, h) sediments. Curves in (a)
557 through (d) are general additive models fit to the data, and asterisks on the x-axis denote *in situ*
558 bottom water temperatures at the time of sampling. Lines in (e) through (h) are significant linear
559 regressions, the negative slopes of which are the activation energy (E_a).

560 **Figure 2**. Denitrification and anammox T_{opt} for all seasonal sampling dates and microcosm
561 treatments, and bottom water *in situ* or microcosm incubation temperature. Error bars denote T_{opt}
562 ranges.

563 **Figure 3.** Relative contribution of anammox to N_2 production (*ra*) in shelf sediments as a
564 function of incubation temperature.

565 **Figure 4.** Thermal profiles (a, b) and Arrenhius plots (c, d) of denitrification (a, c) and anammox
566 (b, d) in the microcosm experiment. Curves in (a) and (b) are general additive models fit to the
567 data. Lines in (c) and (d) are significant linear regressions, the negative slopes of which are the
568 activation energy (E_a).

Supporting information

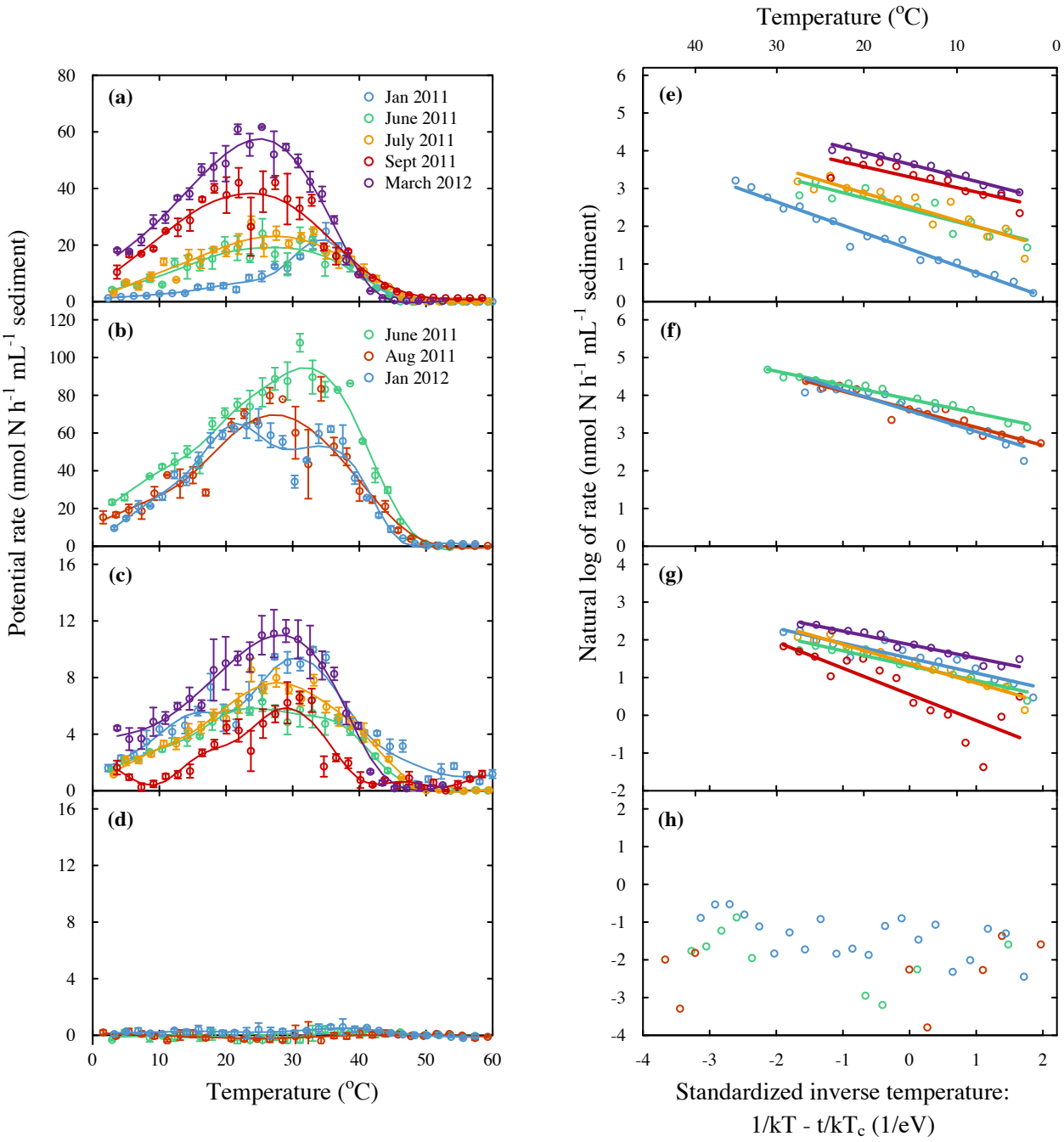
Table S1 – Apparent activation energies (E_a) and thermal optima (T_{opt}) of denitrification and anammox in shelf and estuarine sediments and the microcosm experiment. Asterisks denote E_a values that differ significantly from others within the same site and process.

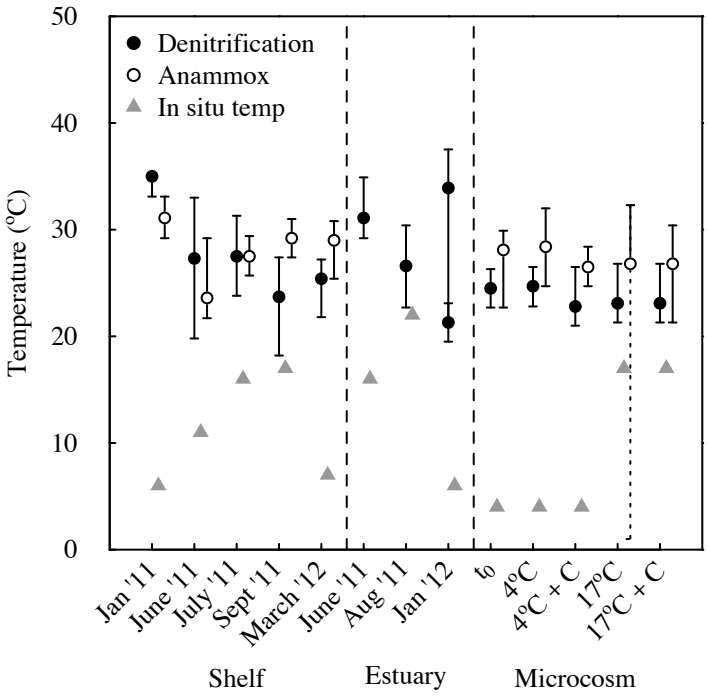
Site and process	Treatment or sampling date	Seasonal or microcosm temperature (ºC)	Activation energy[#] (kJ mol^{-1})	Activation energy[#] (eV)	T_{opt} (°C)	T_{opt} range (°C)
Shelf denitrification	January 2011	6	60.4 ± 2.8*	0.63 ± 0.03*	35.0	33.1 – 35.0
	June 2011	11	43.7 ± 5.6	0.45 ± 0.06	27.3	19.8 – 33.0
	July 2011	16	50.5 ± 6.6	0.52 ± 0.07	27.5	23.8 – 31.3
	September 2011	17	38.5 ± 6.8	0.40 ± 0.07	23.7	18.2 – 27.4
	March 2012	7	43.7 ± 2.9	0.45 ± 0.03	25.4	21.8 – 27.2
Shelf anammox	January 2011	6	38.2 ± 4.1	0.40 ± 0.04	31.1	29.2 – 33.1
	June 2011	11	38.0 ± 3.0	0.39 ± 0.03	23.6	21.7 – 29.2
	July 2011	16	49.4 ± 3.6*	0.51 ± 0.04*	27.5	25.7 – 29.4
	September 2011	17	66.9 ± 12.3*	0.69 ± 0.13*	29.2	27.4 – 31.0
	March 2012	7	34.3 ± 2.6	0.36 ± 0.03	29.0	25.4 – 30.8
Estuary denitrification	June 2011	16	35.8 ± 2.1*	0.37 ± 0.02*	31.1	29.2 – 34.9
	August 2011	22	46.2 ± 4.1	0.48 ± 0.04	26.6	22.7 – 30.4
	January 2012	6	53.0 ± 5.3	0.55 ± 0.05	21.3	19.5 – 23.1
					33.9	23.1 – 37.5
Microcosm denitrification	t_0	4	41.2 ± 2.6	0.43 ± 0.03	24.5	22.7 – 26.3
	4°C	4	40.2 ± 4.1	0.42 ± 0.04	24.7	22.8 – 26.5
	4°C+C	4	36.5 ± 1.7	0.38 ± 0.02	22.8	21.0 – 26.5
	17°C	17	44.4 ± 0.4	0.46 ± 0.005	23.1	21.3 – 26.8
	17°C+C	17	46.4 ± 1.9	0.48 ± 0.02	23.1	21.3 – 26.8
Microcosm anammox	t_0	4	30.5 ± 12.1	0.32 ± 0.13	28.1	22.7 – 29.9
	4°C	4	30.8 ± 6.4	0.32 ± 0.07	28.4	24.7 – 32.0
	4°C+C	4	26.3 ± 4.2	0.27 ± 0.04	26.5	24.7 – 28.4
	17°C	17	19.3 ± 9.9	0.20 ± 0.10	26.8	NA[§] – 32.3
	17°C+C	17	21.5 ± 8.4	0.22 ± 0.09	26.8	21.3 – 30.4

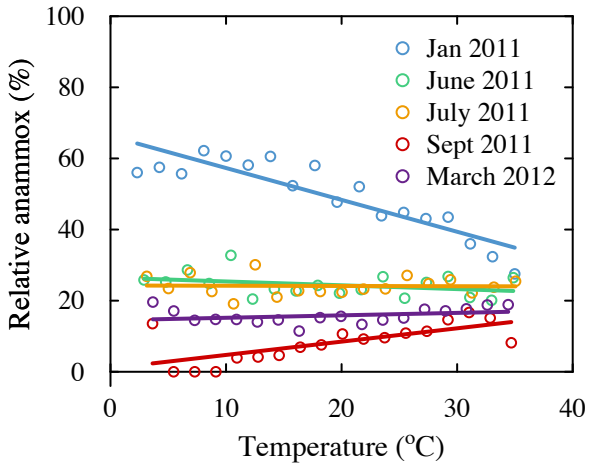
[#]Ea is the negative of the mean slope ± s.e. of the regression line in Arrenhius plots corresponding to shelf and estuarine sediments, while in the microcosm experiment, it corresponds to the negative mean ± s.e. of E_a for three replicate aquaria.

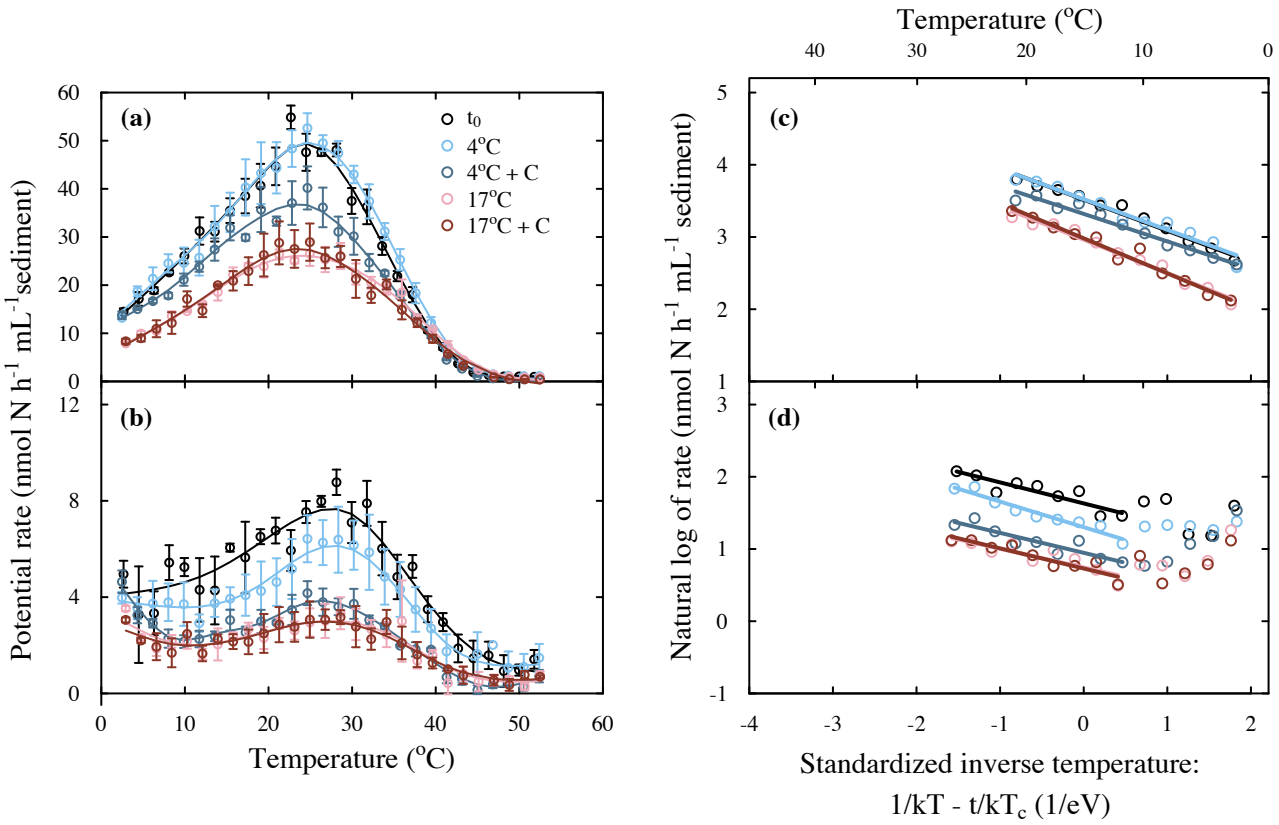
[§]Not able to calculate lower limit as all rates below the maximum rate were within 95%

578 confidence interval of maximum rate.


(a)
Jan 2011
June 2011
July 2011
Sept 2011
March 2012
(b)
June 2011
Aug 2011
Jan 2012
(c)
(d)
Potential rate (nmol N h-1 mL-1 sediment)
Temperature (°C)
(e)
(f)
(g)
(h)
Natural log of rate (nmol N h-1 mL-1 sediment)
Temperature (°C)
Standardized inverse temperature:
1/kT - t/kTc (1/eV)


Denitrification
Anammox
In situ temp
Temperature (°C)
50
40
30
20
10
0
Jan '11
June '11
July '11
Sept '11
March '12
June '11
Aug '11
Jan '12
t0
4°C
4°C + C
17°C
17°C + C
Shelf
Estuary
Microcosm


Relative anammox (%)
100
80
60
40
20
0
0
10
20
30
40
Temperature (°C)
Jan 2011
June 2011
July 2011
Sept 2011
March 2012


(a)
(b)
(c)
(d)
t0
4°C
4°C + C
17°C
17°C + C
Potential rate (nmol N h-1 mL-1 sediment)
Natural log of rate (nmol N h-1 mL-1 sediment)
Temperature (°C)
Standardized inverse temperature:
1/kT - t/kTc (1/eV)