November 26, 2013

Response to Reviewers

"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"

Canion, et al.
Biogeosciences Discussion 10, 1495 – 14626

We sincerely thank the reviewers for their comments that have helped us improve the content and clarity of the manuscript. Below are our responses to specific comments.

Referee #1: P. Cook

Specific comments:

The paragraph starting on pg 14598 line 14 makes the case that permeable sediments are a significant sink for nitrogen through denitrification. Our work has shown the opposite, and I believe (Cardenas et al. 2008) is misquoted. Cardenas et al actually showed that denitrification rates and efficiencies are very low compared to cohesive sediments. The fundamental control over denitrification in permeable sediments is the amount of nitrate reaching the anoxic zone of the sediment where potential denitrification rates are high. I think this is very low for a number of reasons including: 1. Low concentrations of nitrate in the water column, 2. The flow fields around ripples means that a lot of nitrate advected into the sediment transits through the aerobic zone and is not denitrified. 3. Ammonia produced within the sediment is released through anaerobic chimneys in at the ripple crests resulting in very little nitrification and hence denitrification of ammonia produced within the sediment (Kessler et al. 2012). I am not suggesting these points always apply, however, I suggest this paragraph be tempered against these points.

We acknowledge that the citation of Cardenas et al. 2008 was not entirely accurate. We have modified this paragraph according to the reviewer suggestions, emphasizing the previous findings of multiple research groups concerning the roles of overlying nitrate concentration and nitrification as controls on denitrification.

Following on from this point, I see that the rates are actually very low (with the highest rate equating to 14 mol m-2 h-1) even when you pump nitrate vertically into the core. In these experiments this occurs as a consequence of the low nitrate concentrations in the water column (as acknowledged in the discussion) because the potential rates are very high. I believe the slant of this discussion should be recalibrated to explicitly consider the fact that their results show low integrated rates, but that potential rates are very high.

We agree and we have rewritten the first discussion section to compare our IPT rates to similar previous work. We have also noted that IPT rates with simulated advection are generally lower than direct N_2 flux measurements in cores with continuous advection. We have expanded further on the observation that high rates of N_2 production occur in some exceptional coastal zones with high bottom water nitrate concentrations.

The potential rates of denitrification and the proportion of anammox are remarkably consistent with rates measured by (Evrard et al. 2013) in a warm temperate embayment (the only other study to report potential denitrification and anammox). Given that these results are from very different environments, I think this is very interesting and worthy of discussion.

We agree that this study is interesting in light of our results and have added the Evrard 2013 reference into the discussion.

The extremely high rates reported by Eyre in oligotrophic reef sediments measured using chamber experiments and direct N2 fluxes are mentioned. These high rates are probably artefacts associated with pumping of N2 out of the sediment, see (Cook et al. 2006). Aside from this, I am not sure how this work is relevant to a discussion on nitrate removal in sands under high nitrate loading (as suggested by the heading). Perhaps this section should be a more general discussion of denitrification rates in sands?

We agree. This reference has been removed from the discussion, as it may not be comparable with the present results. The discussion has been modified to be a more general discussion of denitrification rates in quartz sands only.

p14601 l13. What time period did you sacrifice the cores over? Did you allow time for the newly perfused (oxic) water to become anoxic? This could be anywhere between 10 mins and 2 h depending on the rates of metabolism.

The methods have been updated to include information on the length of incubations. We collected an initial T_0 sample and only included the T_0 sample if it was linear with the remaining time points. The assumption was made that equilibration was not yet complete if the T_0 sample was not linear with the remaining time points.

p14604 l14. The symbol for Vanadium is V, not Vn, It is VCl3.

Thank you. Corrected

14609 line 24, I think Sylt is cool temperate?

Corrected

Section 4.3 Very interesting discussion.

Thank you.

p14613. I disagree that these results support this paradigm, the rates are actually some of the lowest in the literature, unless there is a typo in the units or a miscalculation. See main point above.

As per the reviewer's suggestion, the language about an emergent paradigm of permeable sediments as significant sites of N removal has been removed from the manuscript, as significant is a weakly quantitative term. Instead, the dynamic nature of N removal in permeable sediments is now emphasized. Much of the evidence seems to point towards very high rates of N removal only in coastal regions that are impacted by high nitrate concentrations.

Figs 2, 3 and 4 mol L-1 d-1 and nmol cm-3 d-1 are dimensionally the same, use consistent notation

The units of Figure 2 have been corrected to match those of Figure 3.

Referee #2: A. Rao

Moderate revisions are suggested below.

1. p. 14597, Abstract, line 22. Some earlier measurements of denitrification rates in sandy Arctic sediments may be available from Devol et al. (1997), for example.

We are not aware of any other studies that report denitrification rates specifically from sandy sediments. The Devol et al. (1997) study does not report grain size or porosity, however, the oxygen and nitrate profiles suggest that all of the sediments they studied were fine grained. We have removed this statement from the abstract but it is still discussed later in the text.

2. It would be interesting to extend Table 2 with results from previous studies for a better comparison.

We agree that it would be interesting to collate the studies on permeable sediments. However, variation in methods used to measure rates (e.g. IPT vs. N_2:Ar, advective vs. diffusive conditions) make it difficult to compare directly between studies. Also, the primary focus of the paper is on temperature adaptation, so we do not feel it is necessary to include this information in tabular form. We now report ranges from previous studies in the first section of the discussion for comparison.

It seems that some of the denitrification rates measured in this study are quite a bit lower than previously reported rates in permeable marine sediments, some of which are cited in section 4.1. Particularly in the cores that were not perfused, what is the effect of non-homogeneous mixing of the added 15NO3 (Ferguson and Eyre 2007) on the measured denitrification rates? To what extent might this have led to lower denitrification rates with the isotope pairing technique relative to other studies using the N2:Ar method? Might a difference in methodology be responsible for some of the difference?

We have updated the discussion to clarify that rates measured by IPT are generally lower than other methods that simulate continuous advection. Please refer to the response to reviewer #1 (Cook) for more detail.

3. p. 14601. Very little detail was given on the core incubations. Particularly given that these are permeable sediments in which advection is important, it's important to provide some details on hydrodynamics in the overlying water (stirring rate? Boundary layer thickness? porewater flushing rate due to stirring and perfusion?) As porewater advection is induced in permeable sediment cores even in response to a ship's motion or flow over very small (< 1 mm) mounds (Huettel and Webster 2001), then it's certain that there was some degree of flushing due to the stirring in these incubations, which needs to be better characterized.

The stirring devices in these core incubations were used to maintain diffusive conditions in the overlying water. A more elaborate stirring chamber apparatus would be required to keep the cores under constant advective conditions and was not used in this study as the focus was on temperature response. The perfusion volume was chosen to flush a depth of 5cm in the cores. Previous work suggests the majority of denitrification potential is found within this layer.

line 15. "time points" – at what intervals?

The text has been updated to include more details on the timescale of core sampling.

4. Rates of D_{14} (denitrification of $^{14}NO_3^-$) coupled to in situ nitrification are included in Table 2 and discussed in the text. It would be nice to include a comparison with rates of D_{15}, or direct denitrification of overlying water $^{15}NO_3^-$. Does advection favor D_{14} or D_{15} more?

To clarify, the rates reported in Table 2 represent the "genuine" rate of total N_2 production as defined by Risgaard-Peterson et al. (2003). We did not attempt to parse nitrate sources (i.e., overlying water and nitrification) for denitrification according to the classical IPT equation (Nielsen 1992) because of the presence of anammox. The question of whether advection enhances or reduces coupled nitrification-denitrification has been addressed by other studies (see Cook et al 2006, Gihring et al 2010) and was beyond the scope of this work because ideally it requires that measurements be made under continuous advection.

Referee: Anonymous Referee #2

The main general issue is that the temperature dependence results are discussed as if they only reflect enzymatic temperature dependencies in different populations with fixed temperature characteristics, which is an over-simplification. The temperature dependence of microbial respiration depends not only on enzyme kinetics, but also on factors such as the fluid state of lipid membranes, which clearly could affect the functioning of respiration chains and might well contribute to the distinct temperature characteristics of anammox bacteria and nitrifiers known to be particularly dependent on intracellular membrane systems. The ability of bacteria to adjust membrane composition (acclimate) to temperature is well-known (I believe there are even studies with anammox bacteria), which implies that respiratory rates may also show an acclimation response on the time scale required for lipid synthesis. Acclimation could also include differential enzyme expression. Altogether this means that a different temperature response might have been observed even without population change had the organisms been allowed to acclimate. The similarity between seasons for denitrification at the Sylt site argues against this as an huge issue, but nonetheless it needs to be discussed.

We agree with the reviewer that observed temperature dependency results from the combined effects of enzymatic/structural adaptations, acclimation, and community structure. For this reason, we measured temperature dependency over short time periods (< 24 hr) to isolate adaptational effects from acclimation and community structure shifts, and we explicitly state this in the discussion (p. 14609, L19). While we cannot completely rule out acclimation during the temperature gradient measurements, we did observe linear rates at all incubation temperatures, which suggests incubations were short enough to exclude acclimation. We refer readers to the study of Canion, et al. (2013), for more information on membrane lipid acclimation and growth versus respiration optima in psychrophilic denitrifying isolates.

Another issue that there is too much focus on the optimal temperature relative to the temperature response in the environmentally relevant range (e.g., only T-opt and not Ea is mentioned in the abstract). The authors cite Feller and Gerday who correctly note the irrelevance of T-opt particularly for organisms in colder environments, but nonetheless continue to focus on T-opt. T-opt is an easily recognized and understood parameter, but it really only indicates the point where organisms begin to malfunction. I suggest to focus the discussion more on the relevant range.

We respectfully disagree that T_{opt} is an irrelevant parameter in the present analysis. Pyschrophilic enzymes are more heat labile (i.e., they have a lower T_{opt}) than their mesophilic counterparts because their high catalytic activity at low temperatures is achieved by a weak conformational stability (Feller and Gerday 2003). A comparison of activation Energy (E_a) should theoretically be enough to distinguish low temperature adaptation. However, temperature gradient incubations with natural sediments measure the combined effects of multiple enzymes from a diverse community, and E_a in this case has no physical meaning. Therefore, we used T_{opt}, E_a, and the rate at 5°C relative to T_{opt} as multiple indicators of temperature adaptation.

Specific comments: 14602, 4: I believe that it is statistically more correct to make a non-linear for of the Arrhenius equation directly to the data, but regardless, it is ot clear how a ”linear range” can be defined BEFORE the linear regression is made. For clarity the fitted function should be included in Fig. 3 and 4. I further recommend to list Q(10) values together with Ea. Although this is in principle redundant, Q(10) values are much easier to understand and use for quick estimates of temperature effects.

A non-linear fit of the Arrhenius function would require modification to account for the inflection and T_{opt} of the response curve, and thus we decided to use the well accepted method of fitting a linear form of the equation. Linear ranges were visibly determined by abrupt changes in the direction or slope on linear Arrhenius plots (see Supplemental Figure 1). We also observed decreases in R^2 of at least 0.1 when the linear range was exceeded. For clarity, we have added the Arrhenius plots to the supplemental information and have amended Table 3 to include Q10. However, it should be noted that the range of temperatures chosen for Q10 calculation affects the Q10 value.

Table 3 and throughout the text: Given the uncertainties in rates, it makes little sense to report single optimum temperatures for most experiments. Ranges as those given in parentheses in Table 3 should be used throughout. You might consider if a statistical definition of the T-opt range (i.e. range of values not significantly different from the highest value) would better than the 90% definition, though it would not make a big difference.

We previously considered a statistical significance test to define a range for T_{opt}, but there was still a subjective aspect to this approach. For example, would the visibly chosen T_{opt} be used as a reference for comparison or the mean of a pre-determined

temperature range? Furthermore, errors were not of equal magnitude between sites, making the T_{opt} range selection even less consistent. Many similar studies only define T_{opt} visibly, and we feel that the method used to define the range retains information about the peak width of the response.

14610, 25: It is also relevant to compare to values for other benthic respiratory processes - could temperature changes switch the partitioning between pathways?

The incubations were performed under non-limiting nitrate concentrations, therefore, we did not expect that other respiration pathways would become dominant over the timescales of the incubations. This question is pertinent, though, and has been investigated in pure cultures of nitrate reducing bacteria (see Ogilvie et al 1997).

14611, 4: I am not convinced that there is significant difference between summer and winter results.

We agree that there is not an abundance of evidence to support this conclusion. The increase in rate at 36 °C (Figure 3c) is significant and consistent with the T_{opt} observed for the temperate site. We have modified the discussion text to clarify that the results are not entirely conclusive.

Fig. 5: Mention that curves are hand fitted (?) and avoid the initial decrease in rates at the Arctic site.

Corrected.

References

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