Response to Reviewers for "Nitrogen cycling in the subsurface biosphere: Nitrate isotopes in porewaters underlying the oligotrophic North Atlantic" by S.D. Wankel et al.

Anonymous Referee #1

Summary Comment: This is a very interesting paper about N processing and cycling in deep ocean sediments underlying oligotrophic waters. The authors measured nitrate dual isotope profiles next to nitrate concentrations. An inverse reaction diffusion model was used to calculate rates of nitrification and denitrification, which fit the observed profiles. Their model also returned estimates for the isotopic discriminations associated with denitrification and the isotopic composition of nitrate produced by nitrification. Overall I found the text very clear and their discussion and conclusion sound. I consider my comments listed below as minor.

Reply:

We thank the reviewer for the positive opinion of our work and address their comments below.

General considerations

Comment: When integrated over the whole sediment height the model calculated nitrification and denitrification rates closely compensate each other with nitrification slightly exceeding denitrification (case of core 2B). Does this suggest this whole sediment layer is in some kind of steady state for NO_3^-? If so it would imply that areas where oxidants (NO_3^-) are produced are 'connected' to areas where it is consumed in the respiration process. Is this interconnectivity between layers taken into account in the modeling approach (which tackles the oxic and anoxic sediment intervals separately)? How would such a connection work? Is it possible that a microbial system would operate similar to what is known for cable bacteria in coastal environments?

Reply: *As stated by the reviewer, indeed the integrated rates of nitrification and denitrification are closely matched, with higher levels of nitrification leading to the overall accumulation of NO_3^- above bottom seawater in these cores. The connection between nitrification and denitrification (e.g., the supply of NO_3^- by nitrification for respiration by denitrification) is apparent in these sediments (and many others including coastal sediments). Our modeling approach addresses regions of overlapping nitrification and denitrification in the 'transitional' zones – allowing both processes to contribute to the steady-state level of NO_3^- observed in the porewaters. While the idea of cable bacteria activity in these sediments is intriguing – we have no evidence either way of their activity for this study. Indeed, our conceptualization of this system is one driven by diffusional gradients and micro-zonation within the porewaters permitting both aerobic and anaerobic processes to occur in relative proximity to each another – both exerting influence on the dual isotopic composition of porewater NO_3^-. It is not clear that the spatial scales of cable bacteria observed by others (~mm to cm) would play a direct role on much larger gradients observed in our study at North Pond.*

Comment: The sediment column (B2) integrated activities would also imply a whole sediment column integrated chemolithotrophic microbial C production rate of some 1.5 gC/m2/d, balanced by an approx. equal amount of organic C being oxidized by nitrate reduction (1.3 gC/m2/d). To what extent can N_2-fixers contribute to such C production rate?

Reply: *In addressing comments by reviewer #2, we have now included a more comprehensive estimation of the rates of N-fixation required to contribute to the low model-predicted values of $\delta^{15}N_{NTR}$. This analysis is included at the end of section 4.2.2. Specifically we find that on average ~80% of the organic matter being remineralized and oxidized by nitrification must have originated from in situ N fixation. While rates are still low in comparison to other sediment hosted ecosystems, these results point to a relatively large role for N fixation not only in supplying N, but also for the autotrophic fixation of C. Clearly, the additional input of autochthonous carbon requires balancing by oxidation - most likely by aerobic respiration in the O_2 containing intervals.*

Comment: Authors argue that core 4A, which shows little variability in nitrate isotopic composition, is characterized by substantially lower levels of microbial activity due to very low organic carbon levels. For sites which are separated from each other by only a few Km and with similar sediment thickness for 2B and 4A (reflecting similar accumulation rates) this appears as strange. It would be nice to have some idea about the organic C contents of the different cores. Defforey and Paytan 2015, report organic P contents for core 4A, which does not appear as very different from those at 2B and 3D. Can authors comment on whether or not this also could hold for organic C?

Reply: *The reviewer is correct in pointing out the contrasting nature of porewater chemistry between sites 2B/3D and 4A, especially in light of their relative proximity. Specifically, the porewater nitrate isotopes from core 4A appeared to indicate a much lower overall turnover of nitrogen and general microbial activity. We now refer (in section 2.1) to the fact that vigorous subsurface fluid flow appears to move from a recharge zone in the southeast (near 2B and 3D) towards the northwest (near 4A) (Becker et al., 1984; Gable et al., 1992). Given the relatively contrasting intra-basin locations of the boreholes – it seems possible that the proximity of 4A to the discharge side of the basin may play a key role in shaping its biogeochemical milieu.*

While total organic carbon and nitrogen were not measured as part of this study, measurements were made in some of the piston cores (~8.5m depth) during the site survey cruise, revealing average organic carbon and nitrogen content of 0.15% and 0.02%, respectively (Ziebis et al., 2012), and no discernable differences among the sites in that study. Similarly, as noted by the reviewer – we also now reference the measurements of organic P by Defforey and Paytan (IODP 336 Proceedings), which also do not indicate any obvious differences among the three sediment cores – implying possible similarity of organic C and N content as well for the deeper IODP cores studied here.

Additionally, we now reference recent work by Zhao and Jorgensen (in review), which also demonstrates much lower overall cell abundance at site 4A, as well as lower abundance of nitrogen-based functional genes. We now reference this work directly in the text (Section 4.2.1). This work is consistent with our observed NO_3^- concentration and isotope profiles – which we have interpreted as reflecting a much lower overall N cycling activity in the sediments of core 4A. Specifically, the 16S gene abundances observed in core 4A were ~1-2 orders of magnitude lower than those observed in core 3E/D.

Comment: P13551: While available in Edwards et al., it would be nice to reproduce the map locating North Pond and the core sites.

Reply: *We agree with the reviewer and have now introduced a map for reference as Figure 1.*

Comment: P13552: Authors should provide more details about analytical methods for assessment of concentrations and stable isotope composition: In particular the limits of detection for NO, NO2 should be indicated. Apparently sulfamic acid treatment was applied only for cases where NO2 was detected; on from what concentration level was sulfamic acid removal applied? Ammonium is reported (P13557, L10) to be less than 'measurable'; please mention the method and the detection limit for NH_4^+.

Reply: *We agree with the reviewer that too little detail was given regarding some of the analytical methods and have now included more information. Specifically, we now include description of the orthophthaldialdehyde fluorescence method for* NH_4^+ *concentration measurements (Holmes et al., 1999) as well as a more detailed description of when* NO_2^- *was removed by sulfamic acid addition for* NO_3^- *isotopic analyses.*

Minor Comments:

Comment: P13563; L21: micro-aerophilic respiration: please clarify

Reply: *Here we were simply using this term in reference to aerobic microorganisms that may be explicitly adapted to low-oxygen conditions. Typically, this term can be loosely meant to indicate any condition lower than atmospheric equilibrium. Here this term is meant to refer to organisms respiring oxygen, yet requiring very little* O_2 *as the result of low metabolic rates. We have now added the parenthetical "(e.g., organisms adapted to respiration under low* O_2 *conditions)" for clarity.*

Comment: P13564; L23 and P13565; L16: "extremely low levels of organic material " please specify the POC concentration

Reply: *We now reference work done by Ziebis et al., (2012) during the site survey piston coring of North Pond – measuring an average of 0.15% and 0.02% organic C and N, respectively.*

Comment: P13570; L4: please clarify what is meant by biologically catalyzed equilibration

Reply: *We have now included reference to the fact that some researchers have shown that some bacteria may accelerate oxygen isotope equilibration between nitrite and water (Buchwald et al., 2012) beyond what would be expected for abiotic isotopic equilibration.*

Comment: P13571; L8: "incorporation of dual nitrite isotopes" - would such incubation experiments be feasible? Decompression effects may cause a major problem

Reply: *As nitrite is a dissolved ion, no problems would be anticipated due to decompression. Here we were simply referring to the added information that might be gleaned on microbially catalyzed transformations from isotopic measurements in a second contemporaneous inorganic nitrogen pool.*