**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 #2:**

**Summary Comment:** The paper of Wankel et al presents a detailed examination of pore water nitrate concentrations and isotopic composition in the pelagic sediments on the flanks of Mid-Atlantic Ridge in the North Atlantic, providing quantitative interpretation of the main contributing processes: nitrification and denitrification. The main novelty of the study (in addition to publishing deep pore water isotopic profiles for nitrate, of which very few have been published to-date), is an inference of a substantial contribution of (in all likelihood, biological) $N_2$ fixation to the pool of sedimentary organic nitrogen in these highly oligotrophic sediments. This conclusion is drawn based on the isotopic mass balance calculations performed as part of the depth-resolved reaction-diffusion model the relevant N and O isotopologues of nitrate. The paper is overall well written, though could benefit from further editing, particularly the first of the manuscript.

**Reply:** *We thank the reviewer for their comments and feedback and address their comments below.*

**General Comments**

**Comment:** More general comments: 1. I have the following comments/suggestions regarding the main conclusion of the paper about $N_2$ fixation. Since it is a rather novel observation, some further supporting discussion seems to be warranted: 1) The low values of $\delta^{15}N_{NTR}$ imply that a large fraction of organic nitrogen oxidized to nitrate originates from $N_2$ fixation, particularly at the sites where the lowest $\delta^{15}N_{NTR}$ is calculated. It would be instructive to provide the readers with some further quantitative assessment of what fraction of N oxidized comes from $N_2$ fixed (assuming the exported $\delta^{15}N$ of PON of 3.7 per mil as reported (in cited references) in this area).

**Reply:** *The reviewer's suggestion is a good one. We have now included a few sentences at the end of section 4.2.3 detailing this quantitative assessment of the relative proportion of N derived from biological N fixation. Indeed, on average 80% of the N oxidized by nitrification appears to be derived from biological N fixation – underscoring the importance of this autotrophic process for sustaining these subsurface microbial communities.*

**Comment:** Alternatively, in the context of $N_2$ fixation discussion, it would be helpful to have at least some idea of what $\delta^{15}N$ of the sedimentary N is in this area. However, this has not been done due to methodological difficulties. The N wt% is described as "extremely low" – Please, specify how low. Were there any estimates made on the N content of these sediments? Could the $\delta^{15}N$ of at least a couple of sediment intervals be measured using POR oxidations?

**Reply:** *We now reference the work by Ziebis et al. (2012) in which organic C and N content was measured on several piston cores across North Pond – revealing and average of 0.15% and 0.02%, respectively.*

**Comment:** Also, on p. 22, there is a statement about "exceedingly low" ammonium. Please, clarify, whether ammonium was measured, and if so, by what method (with Refs).

**Reply:** *We have now included reference to the fact that $NH_4^+$ was below detection using the OPA fluorescence method (detection limit ~20nM).*

**Comment:** 2) The reported rates should be compared to other published rates of $N_2$ fixation in the sediments (mostly coastal), as well as in the euphotic zone of the North Atlantic. Such comparison would put the findings in the more global context, and in fact show that the implied by the mass balance rates of $N_2$ fixation are in fact really high (e.g. Capone et al., 2005 reports the average rate of 0.9 nmol/cm3yr in the euphotic zone of the tropical Atlantic, here conversion made assuming 100 m euphotic zone depth).

**Reply:** *We appreciate and agree with the reviewer's feedback here for putting the inferred nitrogen cycling rates ($N_2$ fixation, nitrification and denitrification) into a more global context. We have now included a new paragraph at the end of section 4.2.2 that uses the model predicted $\delta^{15}N_{NTR}$ as an index of the relative input by N fixation. Further, throughout this section we also now include more direct reference to rates typical of other types of sediments and marine environments.*

*While estimates of N fixation rates were not directly made using the model, as suggested by the reviewer, we use the model-predicted $\delta^{15}N_{NTR}$ values to infer a relative fraction of N fixation contributing to the organic nitrogen pool – ultimately available for nitrification. If we assume that the organic N pool is at steady-state – then the steady-state rate of nitrification must be balanced by steady-state of remineralization of organic matter derived from the water column and in situ N fixation – the proportions of which can be estimated by $\delta^{15}N_{NTR}$. Moving forward with these assumptions – volumetric estimates of N fixation rates are now included in Table 1.*

**Comment:** 3) On the same subject – to get a sense whether these high rates of $N_2$ fixation can be supported by previously reported rates of $H_2$ production, maybe compare at least orders of magnitude of the two processes).

**Reply:** *This is a valid comment by the reviewer; however, we have knowledge of biological $H_2$ production rates for these environments. We refer to the study of the South Pacific Gyre (D'Hondt et al., 2009) in which the authors estimated radiolytic $H_2$ production as a source of electrons supporting the subsurface microbial community. The radiolytic $H_2$ production rates calculated for the SPG subsurface study are several orders of magnitude lower than the $H_2$ production rate that would correspond to our predicted N-fixation rates at NP. While $H_2$ does not generally 'leak out' of N fixing bacteria – a small efflux of $H_2$ could have important implications for other bacteria in the vicinity.*

**Comment:** 2. There is not much information about how well the model actually fits the data. The most straightforward way would be show the model-predicted $\delta^{15}N$ and $\delta^{18}O$, as well as nitrate concentrations directly compared to the data with a specify set of input parameters. Or explain why such comparison is not presented.

**Reply:** *Because our modeling approach designates each sediment interval as a steady-state volume, the solutions to the steady-state mass balance framework outlined in the text are fit absolutely to the measured concentrations and N and O isotopic compositions. Thus, a 'goodness' of fit would not be helpful for revealing strengths/weakness of the model architecture (e.g., the model numerically converges on these compositions by design). Thus, we illustrate the*

*error involved in the numerical convergence on the non-unique solutions, as the standard error of 10 model-run estimates. This error is depicted as the bars in the figures for the predicted model parameters (rates of nitrification and denitrification and isotopic parameters as indicated).*

**Comment:** 3. Specify what type of storage (from frozen sediments, stored at -80C or pore waters stored at -20C) was applied to the samples, which did contain measurable nitrite. This way it would be more clear for the reader whether these samples could be potentially compromised by some of nitrite oxidation during storage)

**Reply:** *We now state that the extracted porewaters were all stored frozen at -20˚C until analysis.*

**Comment:** 4. The $O_2$ concentration is reported down to the "detection limit", but this value is not reported. Please, add the detection limit of $O_2$ measurements.

**Reply:** *We now include mention of the reported detection of $O_2$ by Orcutt et al., (2013) of 5μM.*

**Comment:** 5. The denitrification is assumed to occur in the intervals with $O_2$ up to 40 uM of $O_2$. Please, provide an explanation for this upper limit (e.g. give a reference?)

**Reply:** *This was inadvertently omitted during editing – we now refer to recent thermodynamic calculations on transitions between aerobic respiration and denitrification by Brewer et al., 2014.*

**Minor Comments:**

**Comment:** P. 3, L. 10-15 I would reword the beginning of opening sentence as: "Below the sunlit surface, the dark ocean. . ."

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 4, L. 0-5 re-word to: "Furthermore, in the sediments overlying by relatively young and permeable"

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 4, L. 15-20 reword to ". . . may provide . . . into its role in global marine nitrogen. . ."

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 4, L. 25-30 ". . . sedimentary carbon. . ."

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 5, L. 10-15 a) remove "however" b) Move the sentence which starts with "For example" before the preceding sentence c) replace "generally" with ". . . typically heterotrophic. . . or just "the heterotrophic"

**Reply:** *Agreed – reorganized as suggested.*

**Comment:** P. 5, L. 20-2 Remove "however"

**Reply:** *We choose to keep the original sentence.*

**Comment:** P. 6, L. 5-10 replace with a) ". . . linearly coupled" or "linearly related" b) " . . . in resulting nitrate" instead of "for nitrate"

**Reply:** *We have replaced "tightly" with "linearly" at the reviewer's suggestion. We have also replaced "for nitrate" with "in the resulting nitrate."*

**Comment:** P. 6, L. 15-20 Define here low-energy (this term is used through the text, so here it would be helpful to clarify that you mean "low organic carbon"

**Reply:** *Agreed – we have changed this to say "low-carbon"*

**Comment:** P. 6, L. 20 – remove "constraints" in this line

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 7, L. 0-5 replace with ". . . it was excluded from our study"

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 7, L. 15-20 replace with: ". . . on the shipboard catwalk immediately after"

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 7, L. 20-25 move the sentence starting with "Porewaters were extracted. . ." before the preceding sentence.

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 8, L. 10-20 Wrong reference for nitrite determination method, should be Cox reference

**Reply:** *We now also include reference to Cox, 1980.*

**Comment:** P. 9, L. 5-10 should read "10 mbsf"

**Reply:** *We now use 'mbsf" instead of 'm' to more accurately indicate depth into the seafloor sediments.*

**Comment:** P. 10, L. 5-10 should read: ". . .O2 depleted zone..."

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 11, L. 5 Remove the word "phase"

**Reply:** *We have changed "gas phase products" to "gaseous products" as suggested.*

**Comment:** P. 11, L. 10-15 After "Granger et al., 2008, replace the sentence with something like that for clarity: "the isotopic transformations of N and O are decoupled due to differently sourced N and O atoms in the resulting NO3 molecule"

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 11, L. 20-25 replace "related" with "set by"

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 12, L. 0-5 "canonically" does not fit here

**Reply:** *Agreed – deleted as suggested.*

**Comment:** P. 12, L. 15-20 Label all atoms in the list of nitrate isotopologues.

**Reply:** *Agreed – we now label all isotopologues here.*

**Comment:** P. 20, Line 0-5 Replace "sharper $O_2$ profiles" with "steeper $O_2$ gradients"

**Reply:** *Agreed – reworded as suggested.*

**Comment:** P. 28, L. 20-25, remove a parenthesis after Granger et al., 2008. Also, clarify that study was purely experimental, but cited environmental fractionation factors.

**Reply:** *Agreed – reworded as suggested. We also now use 'experimental' to describe the Granger study.*

**Comment:** P. 30, L. 15-20. Note that Townsend-Small et al (2014) reported co-occurring nitrification/denitrification in the water column.

**Reply:** *We appreciate the reviewer's calling our attention to the recent and interesting paper by Townsend-Small et al. Given the sedimentary context of our study, however, we choose not to include reference to this paper – drawing reference instead to Nunoura et al., 2014, who showed similar overlap of nitrification and denitrification in deep sea sediments.*

**Comment:** P. 30, L. 20 to the end of the page: High relevance for the global ocean models is mentioned in the summary, but not really discussed. Please, elaborate a bit on this.

**Reply:** *This statement is meant to point out that the isotope effects for denitrification ($^{15}\varepsilon_{DNF}$) and the N and O isotopic composition for newly produced nitrate by nitrification ($\delta^{15}N_{NTR}$ and $\delta^{18}O_{NTR}$) are used by many other researchers for constraining global marine N budgets. We now include reference to work by Sigman et al. 2009 as a primary example.*